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Debittering Process of Lupin (*Lupinus albus* l.) by Ultrasound Pre-treatment

Cem Baltacıoğlu[1,a,*], Asiye Özcan Tarım[1,b]

[1]*Niğde Ömer Halisdemir University, Engineering Faculty, Food Engineering Department, Niğde, Türkiye*
[*]*Corresponding author*

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ABSTRACT

This study aims to investigate the application of ultrasound as a pretreatment method in the debittering process of lupin (Lupinus albus L.) and its effectiveness. Lupin seeds are rich in protein and other essential nutrients, but they contain bitter alkaloids, primarily lupinidin, which creates difficulties in their use as food and feed. The traditional debittering method is soaking in water, which takes a long time and causes nutrient losses. Ultrasound application has been tried as a promising alternative due to its ability to accelerate debittering without compromising nutritional value. This study pretreated lupin seeds with ultrasound at different temperatures, powers and durations during the debittering process. The pretreated lupin samples were then analyzed regarding changes in alkaloid amounts, protein content and other nutritional properties. The applied ultrasound-assisted extraction method brought a new perspective and the debittering process was carried out in a shorter time by adapting the heating process with ultrasound. The results show the effectiveness of ultrasound pretreatment in reducing the concentration of bittering alkaloids in lupin seeds. Moreover, the process preserves the essential nutrients found in lupins, thus increasing their potential as a valuable protein source for human consumption and animal feed. This study contributes to the development of sustainable and efficient debittering methods for lupins, paving the way for their wider use in food, feed and various industrial applications.

[a] *cembaltacioglu@gmail.com* *https://orcid.org/0000-0001-8308-5991* | [b] *asiye12395@gmail.com* *https://orcid.org/0009-0004-2340-5569*

Introduction

Among the reasons for planting lupin (*Lupinus albus*), which has an annual herbaceous stem, it is emphasized that it contributes significantly to human nutrition and to improving the structure of the soil where it is grown, due to the high amounts of protein and fat it contains. In addition, it is cultivated as raw material for the pharmaceutical industry to evaluate the high alkaloid and natural pesticide effects it contains. Due to the increase in the world population, the use of protein sources in human nutrition is gaining importance. The protein contained in lupine increases its value in this regard. It is stated that the possibility of use as a functional food in human nutrition increases its value even more (Ferchichi et al., 2021; Osorio & Till, 2022).

Lupine has 2-3 times more protein than grains. Although soybean ranks first in terms of vegetable protein production, it can compete with mature soybean with its high protein amount (28-47.6%) if its yield and production are increased. In the world food industry; It is used as a raw material in products such as bread, cake, biscuits, pasta, and soy sauce, as a soy alternative, confectionery, vegetable oil with high antioxidant content, emulsifying agent, gluten-free flour, snacks and milk alternative products. It also plays an important role in the pharmaceutical industry (Yaver & Bilgiçli, 2021;Goula et al., 2017; M. van de Noort, 2016).

Ultrasound-assisted extraction methodis known as mechanical waves propagating in a medium degradation of plant cell walls occur under the effect of those waves. With this technique and by accelerating mass transfer, the desired bioactive ingredients are compared to traditional techniques. to be obtained in a short time and with higher efficiency can be provided (Topdaş & Şengül, 2019).

Ultrasound-assisted extraction offers several advantages over conventional extraction methods, including higher extraction yields, shorter extraction times, and lower solvent consumption. It is particularly useful for extracting heat-sensitive compounds or compounds from hard-to-reach matrices. Additionally, it is considered a more environmentally friendly extraction technique due to reduced solvent usage and energy consumption. Ultrasound-assisted extraction (UAE) is a technique used to extract compounds from various materials using ultrasound as a means to enhance the extraction process. This method is commonly applied in fields such as chemistry, biochemistry, pharmacology, and food science (Karacaoğlu et al., 2016; Kamberoğlu et al., 2024).

This study aimed to investigate the use of the ultrasound-assisted extraction method as an alternative to the traditional method for the removal of bitter alkaloids that prevent the consumption of lupin known for its high protein, mineral, and fiber content. Ultrasound power, application time, and temperature parameters were evaluated as variable parameters in ultrasound-assisted extraction applied as a pretreatment for the debittering process. Total phenolic matter, total alkaloid, and protein contents were evaluated and how they were affected by the variables. In addition, the results obtained with conventional bitterness removal were compared with ultrasound-assisted extraction of pretreated lupins.

Material and Methods

Lupin (*Lupinus albus* L.) used in the study was supplied dry (at 5% moisture) from a local producer in the Doğanhisar district of Konya, Türkiye.

Debittering Process

As a traditional method to remove bitterness from lupin, 100 g of the sample was kept in hot water at 60±3°C for 90 min (100 g sample/3 L water) and then kept in water at room temperature for 2, 3, and 4 days. During the waiting process to remove alkaloids, the water was renewed every day (Yorgancılar et al., 2009). The debittered lupin seeds were kept in a suitable size strainer for 20 min to remove the water and made them consumable.

In the ultrasound application as an alternative to the traditional method, a 3 L capacity ultrasound bath operating at a frequency of 53 kHz was used (Kudos HP, China). A hundred g of sample was placed in the bath. In order to remove bitterness, three different parameters (60-80-100%) were applied as the ultrasound power to be applied, which is the first of the ultrasound process parameters. Ultrasound application time was chosen as 1-2-3h. The effect of temperatures applied in the bitterness removal process was evaluated at three levels being as 20, 40, and 60°C, and the holding period after heating was applied as 1 days. For control purposes, it was compared with the samples obtained using the traditional method.

Total Alkaloid Analysis

Total alkaloid analysis was performed according to the INEN method (INEN-2-390, 2005). Accordingly, 0.6 g of Al_2O_3 was added to 0.2 g of lupin powder was obtained. 0.2 mL KOH (150.4 g/l) is added to the powder mixture and mixed until it reaches a homogeneous consistency. This viscous mixture was transferred to a centrifuge tube and 6mL chloroform was added and centrifuged at 3000g for 5 min (Nüve NF800, Turkey). The filtrate was poured into a cotton filter and collected in a glass bottle. The chloroform, centrifugation, and filtrate collection steps wererepeated at least 10 times until no alkaloids remained in the extract (Nerin and Garnica, 1986) and evaporated until 1 mL remained at 30°C. To analyse the amount of alkaloids, 5 mL sulfuric acid (0.49 g/l) and 2 drops of methyl red indicator were added and titrated with NaOH (0.40 g/l). The total amount of alkaloid was calculated as lupin (g/100g).

Protein Analysis

The amount of protein was determined by modifying the Lowry method (Lowry et al., 1951). Lowry method; It was extracted for 16 -17 hby adding 40 mL NaCl solution to 0.25 g of sample. Then, it was centrifuged at 8000g for 15 min at 4 °C. At the end of the process, the samples were filtered with filter paper. Standard solutions with concentrations of 0, 20, 40, 60, 80, and 100 mg/l were prepared from BSA (bovine serum albumin) stock solution. Solution B was prepared as a solution of 4 g Cu_2SO_4.5H2O in 100 mL of distilled water and solution C as a mixture of solution A: solution B in a ratio of 100:1 (v/v). OnemL of the sample to be analyzed was taken into the test tube. 3 mL of solution C was added and left at room temperature for 15 min. At the end of this period, 0.3 mL of Folin-phenol chemical was added, vortexed and incubated at room temperature for 45 min. Protein amounts were determined by reading the absorbance values of the solutions at 660 nm on a UV spectrophotometer. To create the standard curve, 1 mL of the standard solution (BSA) of known concentration was added to the tubes instead of the sample, and the above procedure was applied. The absorbance was read at 660 nm and a graph of absorbance versus concentration was drawn. For the blank solution, the same procedure as for the sample was applied by adding 1 mL of pure water instead of the sample.

Total Phenolic Substance Analysis

The method suggested by Alothman et al. (2009) was used to. According to this method, 100 µl, 50 µl (50 µl pure water), 75 µl (25 µl pure water) samples were prepared, 10% Folin-Ciocalteu reagent was added and vortexed. After waiting for five min, it was mixed with 750 µl NaCO3 (75g/l), and 60 min later, measurements were made on a UV-VIS spectrophotometer at 725 nm. Gallic acid was used as a reference and results are expressed as mg gallic acid equivalents (GAE) per kg sample (mg GAE/kg sample)antioxidant activity analysis

Antioxidant Activity Analysis

The method of Dorman et al. (2003) was used to determine antioxidant activities. After the obtained extracts were mixed homogeneously, 20, 40, 60, 80, and 100 µl representative samples were mixed with freshly prepared DPPH and vortexed. The resulting mixture was left for inhibition at room temperature and in the dark for 30 min. After the incubation process, the absorbance of the samples was read with a spectrophotometer at a wavelength of 517 nm. The same procedures were applied for witnesses and controls. The difference in the control solution is the solution used in extraction instead of the sample. In blank preparation, the same amounts of methanol were used instead of the sample and DPPH. (A: Absorbance value of the control sample, B: Absorbance value of the sample extract)

$$\% \text{ Inhibition} = \left[\frac{A-B}{A}\right] \times 100$$

Colour, Moisture, Fat, and Ash Content Analysis

Colour values L^*, a^*, b^* values were measured according to the CIE Lab colour parameter scale (Konica Minolta CR-400, Japan). Moisture determination was

carried according to ICC Standard Method No: 110/1 (Anonymous, 2002). Fat content determination in lupin was performed by Soxhlet according to AOCS Am 2-93 (Anonymous, 2010). The amount of ash was determined according to ICC Standard Method No: 104/1 (Anonymous, 2002).

Statistical Analysis

The data were analyzed using the Minitab.18 (Minitab Inc., State College, PA, USA) package program with a 95% confidence interval and one-way ANOVA was used to analyze the data. Multiple comparison Tukey's test was also performed to determine the differences between treatments. Each experiment was repeated at least three times.

Results and Discussion

The results obtained according to the method used as the traditional method for the removal of bitterness in lupin are given in Table 1. Here, it is seen that total alkaloids decreased with soaking in water. Total alkaloid content decreased by 21.25% with four days soaking in water. When the color values were examined, an increase in the *b** value was determined despite the decrease in the *L** value. This shows that lupins have a darker yellow color with soaking in water. The *a** value is not important for lupins since it has an expression between red and green. When the fat values were analyzed, no statistically significant result was obtained with the soaking time. In terms of total phenolic matter content, a decrease of 22.37% was observed when water storage was examined. Soaking time was the main factor in this decrease. According to the results of the comparison test, the amount of total phenolic substances differed on the 3rd and 4th days with the soaking time in water (at room temperature, 20°C). Antioxidant activity values were expressed as % inhibition. When the antioxidant activity was examined, a decrease of 18.99% was observed, which is expected to be in a parallel behavior with the total phenolic matter. In addition, when the antioxidant values obtained from dry lupins were compared with the values obtained during soaking in water after boiling, the boiling process was effective on the antioxidant value. However, it was determined that the change in antioxidant activity values in lupins kept in water for 2, 3, and 4 days after boiling was not statistically significant ($p>0.05$). When the previous studies are examined, it is seen that similar color values were obtained by to keep in water in the studies (Ertaş & Bilgiçli, 2014; Yaver & Bilgiçli, 2021). In a study conducted by Yarpuz (2011), statistically insignificant results were obtained in oil value with soaking in water and parallel results were obtained with this study.

In traditionalextraction, total alkaloids, total phenolic substances, and antioxidant activity values were calculated as 4.38 g/100 g, 335.62 mgGAE/kg, and 2.5% respectively. In a study, phenolic contents of different lupin seeds were investigated. The total phenolic content obtained varied between 374.4 and 2660.4 mgGAE/100 g. Total phenolic content of *L. albus* species was determined as444 mgGAE/100g (Wang & Clements, 2008). The reasons for the difference between the values given in the literature and the values found within the scope of the study were the differences in growing conditions, seed variety, climate, soil types, storage conditions and bitterness removal process. In this study, the results were expressed as % inhibition and a decrease in the values was observed. The fact that a decrease occurred during extraction reveals the importance of time.A study on shortening the time for the removal of bitterness will be useful in order to prevent the decrease of valuable components. The loss of nutritional components in the traditionalmethod of bitterness removal has revealed the necessity of investigating the ultrasound application, which is the fast method applied in this study.

Table 1. Results for dry and soaked lupin samples

Material	Total alkaloid (g/100g)	Color		Oil (%)	Total phenolic content (mg GAE/kg)	Antioxidant acitivity (% inhition)
		*L**	*b**			
Dry lupin	7.62±0.424^{A}	85.25±0.51^{A}	33.03±0.64^{B}	18.82±0.15^{A}	3303.56±37.31^{A}	42.69±0.95^{A}
2 day soaked	6.42±0.254^{C}	81.80±0.47^{B}	42.40±0.22^{A}	16.39±0.03^{A}	3203.58±65.67^{A}	36.94±0.94^{B}
3 daysoaked	6.18±0.254^{B}	82.42±0.25^{B}	42.55±0.11^{A}	17.47±0.11^{A}	2901.09±64.18^{B}	35.28±0.90^{B}
4 day soaked	6.00±0.169^{B}	81.52±0.50^{B}	43.17±0.49^{A}	18.39±0.13^{A}	2564.77±56.71^{C}	34.58±0.88^{B}

Different capital letters in the same column indicate that the difference between the results is statistically significant ($p\leq0.05$).

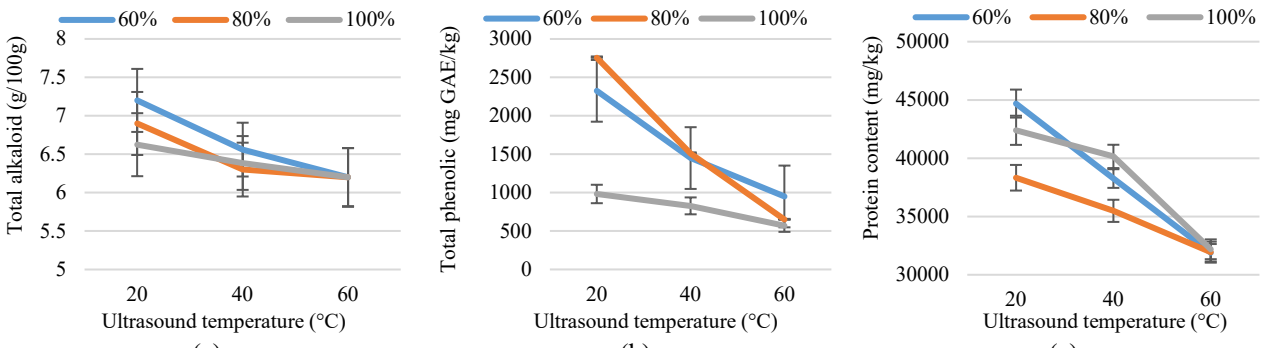
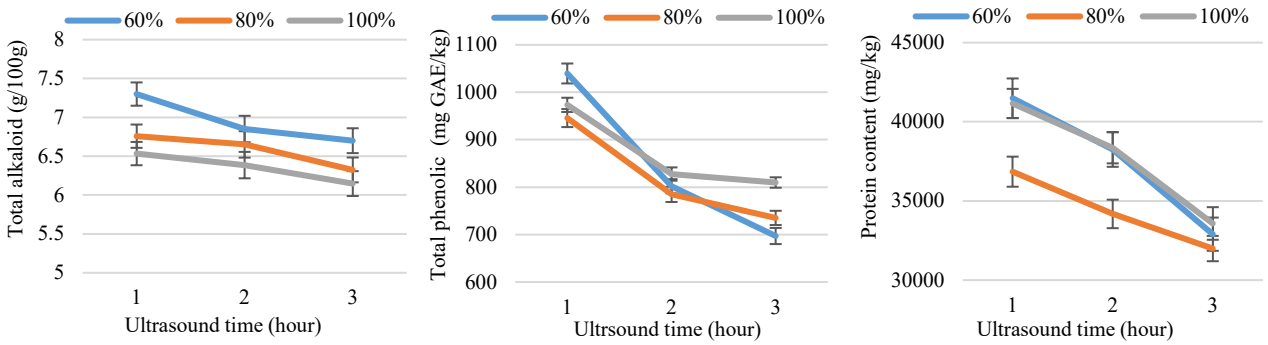


Figure 1. Effect of varying ultrasound temperature and power on total phenolic (a), alkaloid (b) and protein content (c) at constant ultrasound treatment time in debittering process



(a) (b) (c)

Figure 2. Effect of varying ultrasound time and power on total phenolic (a), alkaloid (b) and protein content (c) at constant ultrasound treatment temperature in the debittering process

The extraction studies carried out with the ultrasound technique were analysed as a basis for the possibility that the transition of alkaloids from lupin seeds to water can be carried out with ultrasound.

The values of total alkaloid and total phenolic contents obtained when we used two hours of ultrasound application, which is the pre-treatment performed before the bitterness removal process, and changed the temperature (20, 40, and 60°C) are shown in Figure 1. In the data shown here, the power parameters selected in the device in the ultrasound application also varied (60, 80 and 100%). The amount of bitterness determined at the end of bitterness removal with ultrasound treatment applied as a pre-treatment decreased with the increase in temperature in ultrasound application. In addition, ultrasound power is also effective on alkaloid content. It was observed that the temperature change had a statistically significant effect on the alkaloid and phenolic content obtained on lupin during the ultrasound treatment. It is seen that the effect of the change in the application temperature on the protein amount in all three ultrasound powers was statistically significant ($p \leq 0.05$). The highest protein value was measured as 44688±1221 mg/kg at 60% ultrasound power, 2 h as time and 20°C as temperature.

Figure 2 shows the total alkaloid and total phenolic content obtained as a result of changing the time applied in the ultrasound process if the temperature remains constant at 40°C. The alkaloid value was determined between 7.3 and 6.1 (g/100g). The lowest alkaloid content was determined as 3 hprocessing time at 100% ultrasound power. It is observed that the effect of time application change on the alkaloid amount at all three ultrasound power levels is statistically significant. ($p \leq 0.05$). When the total phenolic matter content is examined, it is seen that the duration is effective on the phenolic matter content at three different power levels. It was determined that the highest amount of phenolic matter was obtained as 1039.65 mg GAE/kg when 1-hour application was selected as the duration at 60% ultrasound power. If the results of protein determination are evaluated, the highest value was determined as 41488±1250 mg/kg in the ultrasound process performed at 40°C and the pretreatment conditions were found as 60% ultrasound power and 1 hour application time. The effect of ultrasound pretreatment at this given temperature on protein content was found to be significant ($p \leq 0.05$).

Karara (1987) reported in a study that the conventional extraction process extracted almost 45% of the alkaloid content in lupin (from 3.46±0.16% to 1.91±0.08%). However, when the grains were subjected to ultrasound-assisted extraction, the alkaloid content was reduced to 1.51±0.09%. In another study, the alkaloid content was reduced to 1.54% (Carvajal-Larenas et al., 2013). If ultrasound is used in the subsequent alkaloid extraction stages, it suggests that water use can be reduced as well as the bitterness removal process time (Miano et al., 2019). Although alkaloid values are not close to each other in the studies in the literature, a decrease in values was observed. In the present study, alkaloid values also decreased. We can say that ultrasound-assisted extraction saves water and is a useful method for removing the bitterness of lupins. In this study, the alkaloid values obtained at the end of one day and the alkaloid values obtained in traditional extraction were approximately the same. However, ultrasound-assisted extraction shortened the time for the bitterness removal process (Bhargava et al., 2021; Estivi et al., 2022).

The bitterness removal process also affects the bioactive composition of the seeds as it can remove or degrade some of the antioxidant compounds and reduce the nutritional value of lupin-based foods, so it is necessary to develop stripping processes that can shorten the processing time, reduce water consumption and minimize the loss of bioactive compounds (Córdova-Ramos et al., 2020; Villacrés et al., 2020). It is thought that factors such as extraction method, lupin variety, and growing conditions are effective. In addition, the observation of a decrease in the amount of total phenolic substances in the lupin subjected to the extraction process is seen as a common point in our study. In a different study, the total phenolic content of lupin extracts of three bitter varieties was found in the range of 491.51±8.95 mg/100 g dry matter - 627.56±5.60 mg/100 g dry matter as vitexin equivalent depending on the traditional extraction conditions (Siger et al., 2012).

If a comparison is made here, we can say that factors such as the application of an ultrasound-assisted extraction method, application temperature, application power, and soaking time in the water after extraction change the total phenolic amount in lupin. In this context, in a study by Hierro et al., the bioaccessibility of saponins from edible seeds and lupin was investigated by an ultrasound-assisted extraction method. Lupin plant was left in three different

solvents (water, methanol, methanol + water) after ultrasound-assisted extraction (Navarro del Hierro et al., 2018). The total phenolic content of lupin kept in water was found to be 2.68±0.13 g GAE/100 kg. Although the results determined here are compatible with the results found in the study, these published results support the data obtained in our study.

Ertaş and Bilgiçli (2014) investigated the effect of soaking medium and soaking time on the protein value of lupin seeds. They found the protein value to be 41.3±0.71% using the Kjehdahl method. When a comparison is made, the value found in the study is close. In addition, it is seen that ultrasound-assisted extraction did not affect the protein value. Only the waiting time produced different values in protein value. In another study, protein analysis of lupin seed was performed by Lowry method and a value of 44.8% was found (Bertoglio et al., 2011). These literature data support the results of this study. Lampart-Szczapa et al. (2003) investigated some functional properties of lupin proteins modified by lactic fermentation and extrusion. They determined the protein value by the Lowry method and expressed it as 29.0 g/100 g dry matter - 34.3 g/100 g dry matter.The values found here and the values found in the treatments in the experimental design are partially compatible.

Conclusion

In this study, the ultrasound-assisted extraction method was used as an alternative to the traditional method for the removal of bitter alkaloids that prevent the consumption of lupin, which is known for its high protein, mineral and fiber content, as it is harvested. Using these two different extraction techniques, total alkaloids, total phenolic substances, and protein content equivalent values were found.

The applied ultrasound-assisted extraction method brought a new perspective and the heating process was adapted with ultrasound and the bitterness removal process was performed in a shorter time. In addition, considering the data obtained, ultrasonic-assisted extraction applications for extraction in foods provide advantages such as reducing the use of solution, cost-effectiveness, and shortening the extraction time. In addition, optimizing the extraction conditions and determining the most favorable extraction conditions for bitterness removal is thought to be a factor that increases the importance of the study.

If the results obtained in this study are evaluated, the waiting time of up to four days in the traditional method of removing bitterness in lupin has been reduced to 1 days with ultrasound pre-treatment. This study has been an important example of the application of ultrasound pre-treatment in the removal of bitterness, which is the biggest obstacle to lupin consumption.

Studies on the use of these methods are increasing day by day and more research is needed on the possibilities of their use in the food sector. By solving the difficulties experienced in the traditional method of removing bitterness, more lupin will be consumed and people will be able to benefit more from this valuable legume.

Declarations

Conflict of Interest

There is no conflict of interest between the authors. Authors' Declaration of Contribution: CB: Design of the study, analysis of the data, writing of the manuscript; AÖT: Collection of data, interpretation.

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