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OPTIMIZATION OF A SOAKING AND ULTRASOUND METHOD FOR THE REDUCTION OF NON-NUTRITIONAL COMPOUNDS IN CHICKPEAS (*CICER ARENTIUM* L.)

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ABSTRACT

Background. Chickpeas are known for their nutritional value, especially their carbohydrate and protein content. However, they also contain certain non-nutritional compounds that can interfere with the absorption of nutrients. Therefore, it is necessary to optimize extraction methods to effectively remove these compounds.
Materials and methods. The objective of this study was to optimize the extraction of saponins and trypsin inhibitors in the chickpea variety 'El Patrón' by ultrasonication and maceration using response surface methodology. Chickpea samples were treated with two extraction methods under different conditions: maceration at 25°C and 50°C for 24 and 48 h and ultrasonication at 25°C and 50°C for 30 and 60 minutes. The saponin, trypsin inhibitor and protein content in both the residual soaking water and the chickpeas were evaluated after the treatments.
Results. Ultrasonication was more efficient in eliminating undesirable saponins and trypsin inhibitors, with a reduction of 30°C to 60%. In addition, a minor loss of less than 1% of soluble protein was observed. These findings suggest that ultrasonication is a promising alternative method for processing this legume.
Conclusion. The results indicate that ultrasonication is the most effective method for the removal of non-nutritional compounds. It is a low-cost and environmentally friendly technology that facilitates the soaking of legumes.

Keywords: legumes, non-nutritional compounds, maceration, ultrasonication, soaking

INTRODUCTION

Legumes are nutrient-rich foods and are an important source of various types of proteins, including enzymes, trypsin inhibitors, and lectins, which are considered non-nutritional compounds (Sánchez-Mendoza et al., 2016). Saponins have a complex structure with a hydrophobic steroid nucleus and a hydrophilic part composed of monosaccharide units. These structures can be considered non-nutritional compounds. In previous studies, saponins have reduced the absorption capacity of metal ions in the gastrointestinal tract,

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which is fundamental to the enzymatic activity of the digestion of various nutrients affecting the growth of laboratory animals. In the case of lectins, they can produce toxic effects in biological models (Valadez-Vega et al., 2021). Trypsin inhibitors can block the absorption and digestion of proteins by inhibiting the pancreatic enzymes trypsin and chymotrypsin, leading to digestive problems such as pancreatic enlargement and growth deficiency (Avilés-Gaxiola et al., 2018).

The chickpea is considered a significant legume due to its economic accessibility, resilience to drought, and nutritional value. It is a notable source of protein, which comprises up to 20% of its weight, as well as carbohydrates and dietary fiber. Additionally, it contains other compounds such as saponins (56 $mg \cdot g^{-1}$) and trypsin inhibitors (21.7 $U \cdot mg^{-1}$), which are classified as non-nutritional compounds (Avilés-Gaxiola et al., 2018; Kaur and Prasad, 2021; Serventi, 2023). Therefore, it is necessary to apply a treatment that allows these compounds to be removed in the processing of chickpeas.

Traditionally, legumes are subjected to a soaking process, often at elevated temperatures, depending on the type of legume (Coffigniez et al., 2018). Soaking is an effective method for reducing the presence of undesirable compounds and enhancing protein digestibility (Coffigniez et al., 2019). Moreover, it is utilized as a pre-treatment for chickpea processing and can take up to 48 hours. This procedure upgrades the digestion of proteins and the starch present in the legume (Yegrem, 2021). Reducing the soaking time via new technologies can increase the efficiency of this process and provide a more effective means of removing undesirable compounds from the product.

Ultrasound-assisted extraction enhances the extraction of compounds by reducing the processing time, improving compound extraction, and minimizing energy consumption (Navarro et al., 2018).

Ultrasound waves are acoustic waves above the human auditory threshold and can be classified as low intensity-high frequency or high intensity-low frequency (Zhu and Li, 2019). The phenomenon of cavitation induced by ultrasound leads to localized temperature elevation, high shearing forces, and membrane damage in the sonicated matter, thereby enhancing the extraction of bioactive compounds (Vela et al., 2021). Additionally, ultrasound application can induce lipid oxidation and protein breakdown, leading to increased enzymatic interaction with substrates (Caballero-Figueroa et al., 2022).

The objective of this work was to apply two extraction methods during the chickpea soaking process to evaluate their effectiveness in eliminating non-nutritional compounds, as measured in both the legumes and the residual soaking water collected after treatment. It is speculated that if elevated temperatures during soaking are combined with ultrasonication, more non-nutritional compounds can be removed from the chickpeas.

MATERIALS AND METHODS

Materials

Evaluation of the extraction conditions for the target compounds from chickpeas

In this research, chickpeas of the variety 'El Patrón' provided by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) and obtained from Celaya, Guanajuato, from the 2019 crop cycle were used. The chickpeas were stored in hermetically sealed jars at room temperature until use.

The maceration conditions for the extraction of compounds from chickpeas of the variety 'El Patrón' were 25°C and 50°C for 24 and 48 h. The chickpea seeds were placed in containers with water in a 1:5 ratio (chickpea:water) (Coffigniez et al., 2018). This process was carried out without agitation, and the temperature was controlled. Ultrasonication was carried out at 25°C and 50°C for 30 and 60 min. The chickpeas were placed in water in a 1:5 ratio (chickpea:water) in a Cole-Palmer ultrasound bath (Model 8891) (Navarro et al., 2018; Yen and Quoc, 2020). The analyses were performed on the soaked chickpeas and the residual water was obtained after the process.

Analytical techniques

Protein quantification. The protein extract was obtained by weighing 100 mg of chickpeas. Subsequently, 100 µL of 72% trichloacetic acid and 900 µL of distilled water were added, followed by stirring for 1 min and centrifugation for 30 min at 3000 rpm. The supernatant was discarded, and the pellet was washed three times with distilled water. Finally, the sample was resuspended in water for subsequent analysis. The

protein content was determined according to the method established by Lowry (Lowry et al., 1951) with Folin-Ciocalteau reagent (Sigma) and a bovine albumin (Sigma) standard curve at concentrations ranging from 0.02 to 0.10 mg·mL^{-1}. The results are expressed in mg·g^{-1}.

Quantification of saponins. The saponin extracts were obtained by taking 100 mg of the sample and subsequently adding 1 mL of distilled water. The mixture was stirred for 1 min, then centrifuged at 10 000 rpm for 15 min, and the supernatant was separated from the pellet. This process was repeated a second time to improve the extraction, resulting in a final volume of 2 mL. The determination was carried out using the obtained supernatant, while the soaking water was taken directly. Saponin content was determined by placing a ground sample in water with a mixture of acetic anhydride (Meyer) and concentrated sulfuric acid (Meyer) in a 1:5 mixture, after which the mixture was allowed to rest for 30 minutes. The absorbance was measured at 528 nm in a UV–Vis spectrophotometer (Model VE-5600UV, Velab). The quantification was conducted using a standard curve for saponin (Sigma) with concentrations ranging from 0 to 5 mg·g^{-1} (Guzmán et al., 2013). The results were derived from the standard curve, factoring in the weight of the chickpeas used, and expressed in mg·g^{-1}.

Trypsin inhibitors. For this assay, 5 g of the sample were suspended in 25 mL of distilled water and mixed for 90 min at 300 rpm. The mixture was centrifuged at 15 000 rpm for 20 min. The soaking water was taken directly. Inhibitory activity was evaluated by spectrophotometry in an enzymatic assay with benzoyl-DL-arginine-p-nitroanilide (BApNA, Sigma) as the substrate and the enzyme trypsin (Sigma). The variation in absorbance at 400 nm due to the formation of *p*-nitroaniline was recorded every 15 seconds for 3 minutes (Erlanger et al., 1961; Nagl et al., 2023). The results are presented as U·mg^{-1}, where "U" is the amount of inhibitor that will inhibit one unit of trypsin activity.

Statistical analysis

For this experiment, a full factorial design with two factors at two levels (2^2) was employed with three replications of the central point; for the maceration treatment, temperatures of 25°C and 50°C were applied for 24 h and 48 h, while for the ultrasonication treatment, temperatures of 25°C and 50°C were applied for 30 and 60 min. The proposed design was assessed using Design-Expert software, version 7.0 (DX7) (Stat-Ease, Minneapolis, MN, USA) The response variables for both treatments were the saponin content and trypsin inhibitor content in the soaking water and in the chickpeas, and the results were evaluated by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Effect of maceration and ultrasonication extraction conditions on saponin content in chickpeas of the variety 'El Patron'

Chickpea seeds treated by maceration (Fig. 1A) for 48 h had a lower concentration of saponins, with an average of 71.58 mg·g^{-1}, than chickpeas treated for 24 h, with a value of 76.45 mg·g^{-1}, and the difference was significant ($p \leq 0.05$). Chickpeas treated by maceration at 50°C lost an average of 21% of their saponins relative to untreated chickpeas, while those treated at 25°C lost 16% of their saponins, showing a significant difference ($p \leq 0.05$) with respect to soaking temperature. The amount of saponins in the residual soaking water after treatment reached 12.01 mg·mL^{-1}. The removal of as much as 36% of the initial saponin content of chickpeas has been reported in other studies (Antoine et al., 2022). Additionally, saponins are glycosides with polar and nonpolar components, which allows their partial extraction in water or more complete extraction in ethanol:water mixtures (Yen and Quoc, 2020). Among the ultrasonicated chickpea seeds (Fig. 1B), no significant difference ($p \geq 0.05$) was found between samples treated for different times or at different temperatures; the average saponin content in the chickpeas was 85.91 mg·g^{-1}, corresponding to a reduction of up to 6%. Maceration resulted in 5% greater extraction of saponins compared to that obtained by ultrasonication, which was due to the retention of the chickpeas in water. Although saponins have a nonpolar part within their chemical structure, they also contain sugars, which allows them to be solubilized in water (Zhou et al., 2023). However, it is important to consider that the treatment time for ultrasonication is shorter than that for maceration, which can significantly reduce the processing time (Wang

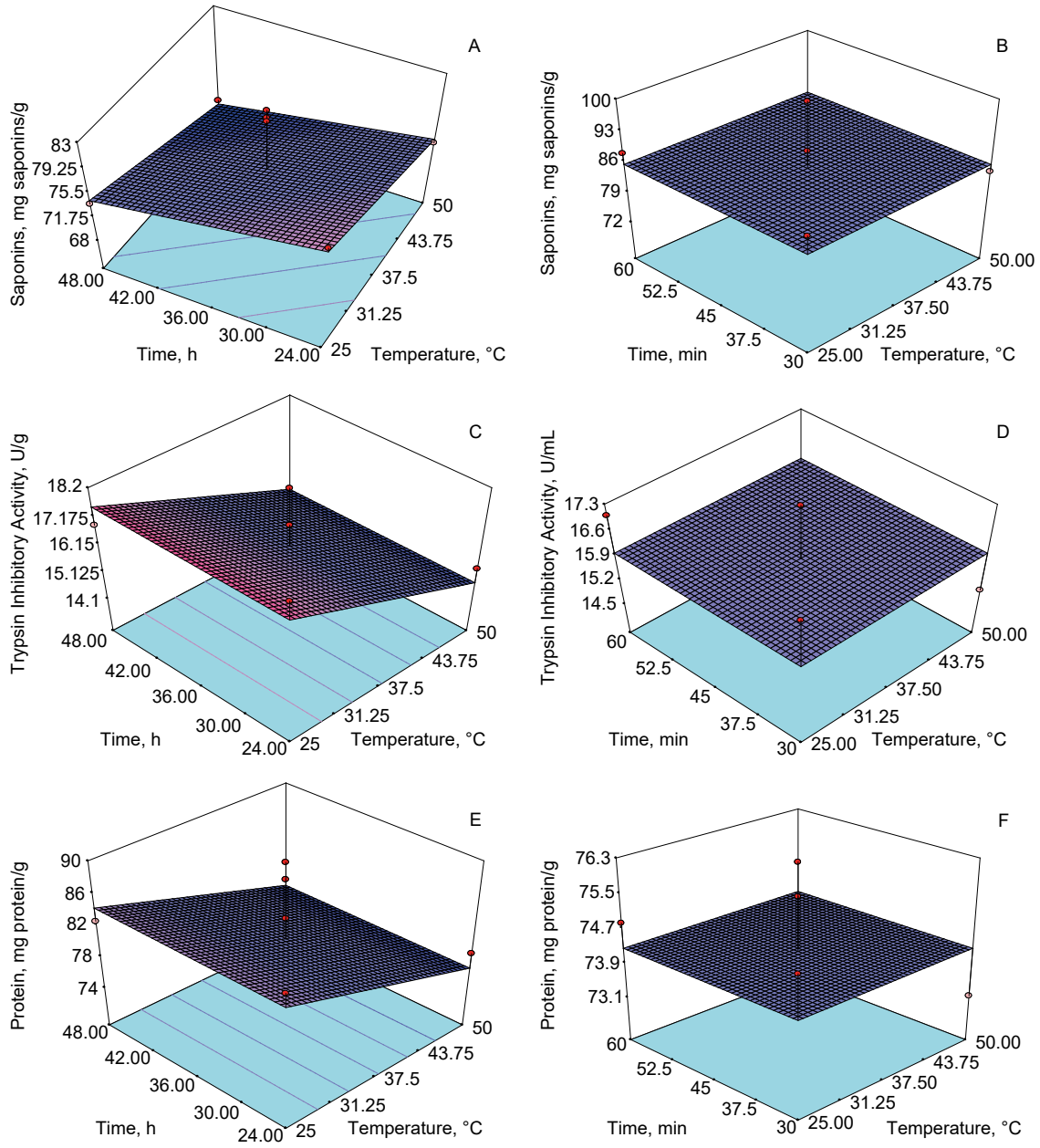

Fig. 1. Effects of maceration (A) and ultrasonication (B) on the saponin content in chickpeas; effects of maceration (C) and ultrasonication (D) on the activity of trypsin inhibitors in chickpeas; and effects of maceration (E) and ultrasonication (F) on the protein content in chickpeas

et al., 2022). Although the most commonly used saponin extraction method is maceration (approximately 37%), ultrasonication is also used in 14% of cases, which suggests that it may be a feasible alternative (Cheok et al., 2014). At present, ultrasound treatment is utilized to eliminate non-nutritional compounds from cereals and legumes, demonstrating its efficacy (Caballero-Figueroa et al., 2022). The integration of temperature with this treatment enhances the soaking process, resulting in the softening of chickpea seeds

and an improvement in the digestibility of proteins and starches (Godrich et al., 2023; Mazı et al., 2023; Youshanlouei et al., 2022).

Effect of maceration and ultrasonication extraction conditions on the content of trypsin inhibitors in chickpeas of the variety 'El Patrón'

Regarding trypsin inhibitors in chickpeas treated by maceration (Fig. 1C), no significant difference was observed ($p \geq 0.05$) as a function of the processing time. However, temperature did affect trypsin inhibitor activity, with a 32% reduction in activity in the chickpeas treated at 50°C, while those treated at 25°C showed a 20% decrease in activity. In the residual soaking water, the average activity of trypsin inhibitors was 6.72 $U \cdot mg^{-1}$, indicating that these compounds were present in the soaking water.

In this study, temperature, but not soaking time, affected trypsin inhibitors. This phenomenon is related to the sensitivity of the inhibitors to high temperatures since, as proteins, they are susceptible to degradation. A study conducted on autoclaved chickpeas revealed that temperature elevation could suppress about 83.87% of the activity of trypsin inhibitors (Ruiz-Zambrano et al., 2023).

In this study, inhibitory activity was diminished by up to 35% compared to unsoaked chickpeas. The maceration-induced soaking process effectively leaches trypsin inhibitors, as the water utilized disperses the protein fraction, thereby aiding in the denaturation of the proteins (Yegrem, 2021). In a separate study examining the impact of maceration on green and red lentils, it was observed that the soaking process and subsequent drying led to a reduction in trypsin inhibitory activity by 52.2–80.1% (Mazı et al., 2023).

In the ultrasonication treatment (Fig. 1D), the soaking temperature and time did not affect trypsin inhibitor activity, with an average value of 14.85 $U \cdot mg^{-1}$ for chickpeas treated at 50°C and 17.1 $U \cdot mg^{-1}$ for chickpeas soaked at 25°C. The results for the residual soaking water indicated that ultrasonication removed an average of 39% of the trypsin inhibitors in the chickpeas. This result may be due to the fact that increasing the temperature during the soaking process increases the transfer of compounds; moreover, inhibitors can degrade at high temperatures, thus decreasing their activity. Another study found that the activity of trypsin inhibitors in white chickpea flour was reduced by up to 59% after heat treatment at 112°C for 15 min (Avilés-Gaxiola et al., 2018).

Ultrasonication may be more effective at inactivating trypsin inhibitors due to the phenomenon of cavitation, which generates shear within the system and can break certain protein structures, as in the case of inhibitors. The combination of ultrasound and temperature has been observed to reduce the inhibitory activity of trypsin. This effect may be attributed to an alteration in the disulfide bonds, leading to changes in the secondary structures of the trypsin inhibitor (Vanga et al., 2020).

Effect of maceration and ultrasonication extraction conditions on the protein content in chickpeas of the variety 'El Patron'

Chickpeas are considered a high-quality food due to their nutritional characteristics, especially their protein content, which can range from 20% to 22% (Kaur and Prasad, 2021). During the soaking process, losses of nutrients, specifically proteins, can occur. The soaking process removes not only components considered undesirable, such as saponins and trypsin inhibitors, but also water-extractable nutrients. Some studies report a decrease in proteins and amino acids such as cysteine, methionine and tyrosine (Ruiz-Zambrano et al., 2023). In this study, the maximum loss of chickpea proteins into the soaking water varied according to the method and conditions.

In the case of soaking by maceration (Fig. 1E), the maximum loss of proteins was 16%, and the effect varied depending on the temperature of the process; for ultrasonication (Fig. 1F), the loss was 15%. It is important to note that this protein fraction corresponds mainly to albumin since albumin is soluble in water and accounts for between 10% and 20% of the protein in chickpeas, consistent with Kaur and Prasad (2021).

Response analysis of soaking experiments of the chickpea variety 'El Patron'

According to the equations in Table 1, the optimized conditions for maximizing the removal of saponins and trypsin inhibitors by maceration were 50°C for 48 h, while for ultrasonication, the optimal conditions were 50°C for 60 min. Espinoza et al. (2021) extracted saponins from quinoa using ultrasound and found

Table 1. Equations obtained from the experimental design by DX7 (in codified terms) applied to the soaking methods of the chickpea variety 'el Patrón'

Maceration	Ultrasound
Saponins in water = 11.29 + 3.74·A	Saponins in water = 8.18 + 2.74·A
Trypsin inhibitors in water = 6.04 + 1.59·A	Trypsin inhibitors in water = 7.22 + 1.23·A
Protein in water = 9.40 + 5.52·A	Protein in water = 9.40 + 1.70·A
Chickpea saponins = 74.02 – 2.92*A – 2.44·B	Chickpea saponins = 85.67
Chickpea trypsin inhibitors = 16.13 – 1.41·A	Chickpea trypsin inhibitors = 16.00
Chickpea protein = 80.48 – 3.87·A	Chickpea protein = 74.27

A – temperature; B – time.

the greatest extraction of compounds at 12 min, while (Yen and Quoc, 2020) observed that an increase in temperature combined with ultrasound facilitated the extraction process. In both methods, increased temperature facilitated the extraction of components into the soaking water, thus reducing their content in the chickpeas.

CONCLUSIONS

The treatment of chickpeas with ultrasonication combined with elevated temperature (50°C) eliminated a higher concentration of trypsin inhibitors, although it did not significantly affect the content of saponins in the chickpea seeds. In contrast, although maceration reduced the saponin content in chickpeas to a greater extent (4% more than ultrasonication), this method has several drawbacks, such as a longer processing time compared to that of ultrasonication. Both methods caused a loss of protein from the chickpeas, which suggests that unwanted components were removed along with important nutrients during the soaking process. It is concluded that maceration may be more effective in eliminating unwanted components in chickpeas, but ultrasonication, as a technology applied in the processing of legumes, could be a viable alternative due to its ability to reduce the processing time.

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DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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