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Effect of Heat Treatment, Water and Vinegar Soaking on Protein and Phytic Acid Levels in Hemp Seed Meal

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ABSTRACT

Hemp plants are notable for their climate resilience, and hempseed meal (HSM) is a potential high-protein feed for poultry. However, HSM has high levels of the antinutritional factor phytic acid (PA). This study aimed to evaluate the effects of heat and soaking treatments on the protein and PA content of HSM. HSM was obtained through cold pressing of whole hempseed and then subjected to heat treatment at 70°C for 24 hours. Soaking treatments involved water, water-vinegar mix, and vinegar for 1, 7, and 24 hours, followed by drying and analysis of PA and protein content. Results indicated that heating increased PA content without affecting protein levels. Soaking duration did not significantly alter protein content but did affect PA levels, with 24-hour soaking significantly increasing PA compared to 1-hour and 7-hour durations. The soaking material also influenced PA content: water soaking increased PA, while a 1-hour vinegar-water mix and 7-hour vinegar soaking significantly reduced PA. The highest PA concentration occurred with 24-hour water soaking. The protein content was highest with 7-hour vinegar soaking. In conclusion, acidic soaking solutions, particularly vinegar and vinegar-water mix, effectively reduced PA in HSM without protein loss.

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Introduction

Cannabis sativa L. has been cultivated in Anatolia since ancient times, with commercial hemp fiber and seed production fluctuating throughout the twentieth century due to regulatory and market forces, leading to a decline in the 1980s. Today, hemp production in Turkey persists in specialized markets, and has seen a recent increase (Clarke, 2023). Hemp (*Cannabis sativa L.*) is a yearly herbaceous plant that belongs to the *Cannabaceae* family, and seeds from industrial hemp are typically known as 'hemp seeds. (Xu et al., 2021). When oil is removed from the seeds using chemical or mechanical techniques, a high-protein hempseed meal (HSM) is left behind (Karlsson et al., 2012). On average, HSM consists of 30 to 35% protein, and 80% of the fat present in whole seeds comprises polyunsaturated fatty acids, which could act as an energy source while enhancing the health of nonruminant animals (Abrahamsen et al., 2021; Hessle et al., 2008; Mustafa et al., 1999). Due to its high nutritional value, HSM seems an alternative feed material for poultry, and recently research has been dedicated to investigating the effects of HSM replacement soybean meal. Nonetheless, hempseed items (including hempseed, hempseed meal, and hempseed oil) are not recognized as 'safe' feed ingredients for poultry because they may negatively impact animal health and productivity (Shariatmadari, 2023). Moreover, even though there is a high potential to take place in diets of monogastric animals, some studies have reported negative effects on poultry performance with the usage of HSM. For example, Ondrej et al. (2015) reported that incorporating hempseed cakes into the diet adversely impacted the growth of chickens. At 37 days of age, chickens fed with a portion of hempseed cakes had a significantly lower final body weight. Moreover, a higher inclusion rate (15%) also deteriorated the feed conversion ratio. In another study, diets containing 25 g/kg HS caused a reduction in both the average daily feed intake and the average daily gain in broiler chickens (Mahmoudi et al., 2015), moreover, some studies reported that even low-level dietary hempseed (3%) inclusion decreased weekly weight gain, and increased FCR in the broiler (Bahari et al., 2014). The results of nutritional experiments indicate the presence of antinutritional factors in HSM. Evaluations of anti-nutritional compounds in various HSM types revealed extremely high levels of phytic acid. Monitoring phytate content in monogastric animals is critical, as long-term exposure to high phytate levels could lead to significant nutritional deficiencies. (Russo & Reggiani, 2015). Phytic acid (PA) is the primary way phosphorus is stored in seeds,

accounting for up to 80% of the total phosphorus and comprising as much as 1.5% of the seed's dry weight (Bohn et al., 2008). Monogastric animals are unable to digest phytate because phytate possesses six negative ions and binds divalent cations (Kumar et al., 2021). PA may bind to macronutrients and minerals and alter some features of these compounds like solubility, functionality, digestion, and absorption of these compounds (Feizollahi et al., 2021). To deal with P's lack of poultry diets inorganic forms are used with high dosage and excess P and other nutrients are excreted by the animal; 65–75% of the total P in formulated diets with supplemental inorganic P is excreted in the manure and this creates environmental problem when animal waste is left on farmland (Oatway et al., 2001). Another way to increase the usability of plant source P is to add phytase enzymes in poultry diets. However, some processes may also be used to remove antinutritional factors including phytic acid from the seed and increase nutritional value. Soaking, autoclaving, heating, germination, microwave cooking, extrusion, fermentation, irradiation, and enzymatic treatments are widely utilized chemical and physical techniques for eliminating or diminishing antinutritional factors (Idate et al., 2021). For the soaking treatment generally, water is used as a solution and results showed that water soaking for different duration may cause a reduction in the phytic acid content of seeds (Egli et al., 2002), on the other hand, previous studies reported that pH of soaking solution and duration have effective of phytic acid reduction from the seeds (Luo et al., 2009). There is not sufficient research on the removal processes of phytic acid from the hempseed meal and therefore based on previous studies' results, the current study has been conducted to investigate the effects of heating and soaking on protein and phytic acid content of HSM. Therefore, in this study, 24h heating at 70C, water, water-vinegar (50-50), and vinegar soaking treatment with durations 1h,7h, and 24 h were applied to HSM and phytic acid and protein content were determined.

Material and Method

Hempseed Meal Soaking and Drying Process

Whole Hempseed was supplied by a local seller, and hempseed meal was achieved through the cold pressing of hempseed using an expeller (Karaerler model NF 100, Ankara, Türkiye) at 45-50°C to remove the oil. For the soaking process, three different solutions were used: tap water, a 50-50% mix of water and grape vinegar, and 100% grape vinegar. The tap water, sourced from the city water network, had a pH value of 8.00. A commercial grape vinegar, purchased from the local market, was used for the soaking, with both the grape vinegar and the water-vinegar mix having a pH of 4. For soaking treatments, 100 g HSM was weighed into glass beakers, and 500 ml soaking liquid was added for each solution. Soaking durations of 1 hour, 7 hours, and 24 hours were applied for each soaking liquid at 25°C. After soaking, the hempseed was drained using a plastic sieve for 1 hour and then dried at 70°C for 24 hours. Hempseed meal without any soaking and heating was used as the control group. To observe the effects of the heating process of hempseed meal without any soaking process was only heated in the oven at 70°C for 24 hours. The Hemp Seed soaking and drying process, experimental design, and naming of the groups are given in Table 1.

Crude Protein and Phytate Content Analysis

After the soaking and drying process crude protein and phytic acid content of all samples were determined as 3 replicates. Crude protein content was determined with the carbon-nitrogen analyzer instrument (N-C LECO, Michigan, US). Phytate content was determined and calculated according to Haug and Lantzsch (1983). For the phytate phosphate content determination, 0.30 g hempseed samples were weighed into an Erlenmeyer flask, and 0.2 N 25 mL HCL solution was added and shaken for 2h by stirring water bath. After sedimentation, 0.5 mL of the extract was transferred to a glass tube. Next, 1 mL of ferric solution was added, and the mixture was heated in a boiling water-bath for 30 minutes then cooled off in ice 15 minutes. Once the samples reached room temperature, 2 mL of bipyridine solution was added and the mixture was vortexed for 30 seconds. Finally, the samples were analyzed using a spectrometer at a wavelength of 519 nm.

Statistical Analysis

Statistical analysis of the data was performed using a two-sample t-tests for the heating treatment. To evaluate the effects of soaking material and duration, a completely randomized design was employed. Data analysis was conducted using the factorial procedure within the General Linear Model in SPSS. The Tukey test was applied to identify significant differences between treatments ($P < 0.05$).

Results and Discussion

The effect of 24-hour heating on HSM phytate content is presented in Figure 1. and on protein content in Figure 2. The heating process significantly increased the phytic acid content of HSM and the phytic acid concentration increased by 138.89 mg per 100 g HSM ($P \leq 0.01$). The protein content of HSM showed a slight decrease with 24-hour heating but was not significant statistically and, in the C group the protein was determined as 36.81% and in the Y group 35.49%. It has been reported that Phytate is resistant to heat and does not break down during cooking; however, early stages of cooking may activate endogenously phytases or phosphatases, which can reduce the levels of phytic acid (Feizollahi et al., 2021).

Table 1. Soaking and drying process and naming of the groups.

	Soaking solution		
Soaking and heating at 70°C duration	Water (pH:8)	Vinegar-Water (50-50) (pH:4)	Vinegar (pH:4)
1-h soaking- 24 h heating	W1	A1	B1
7-h soaking- 24 h heating	W7	A7	B7
24-h soaking -24 h heating	W24	A24	B24

Control group (C): No soaking and heating Y: 24 hours heated at 70°C hempseed meal

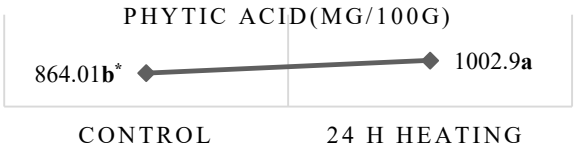

Figure 1. Effect of 24 h heating on the phytic acid concentration of hempseed meal
*a, b, c letters mean differences statistically significant as p≤0.01

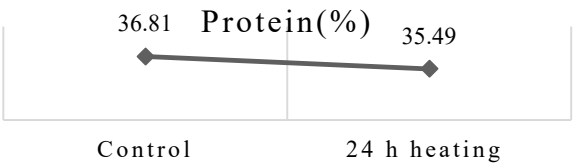

Figure 2. Effect of 24 h heating on the crude protein content of hempseed meal

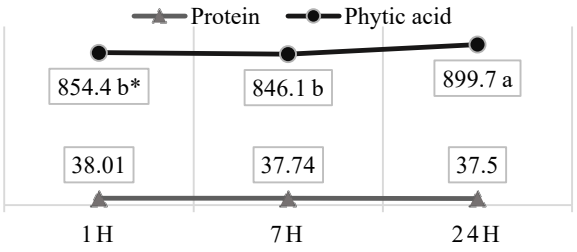

Figure 3. The effects of soaking durations on the protein content (%) and phytic acid concentration (mg/100g) of hemp seed meal
*a, b, c letters mean differences statistically significant as p≤0.01

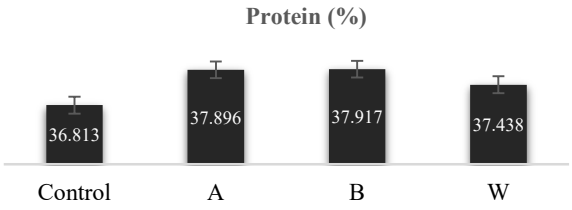

Figure 4. The effects of soaking material on protein contents of hempseed meal
A: 50-50% grape vinegar and water, B: 100% Grape vinegar, W: 100% water.

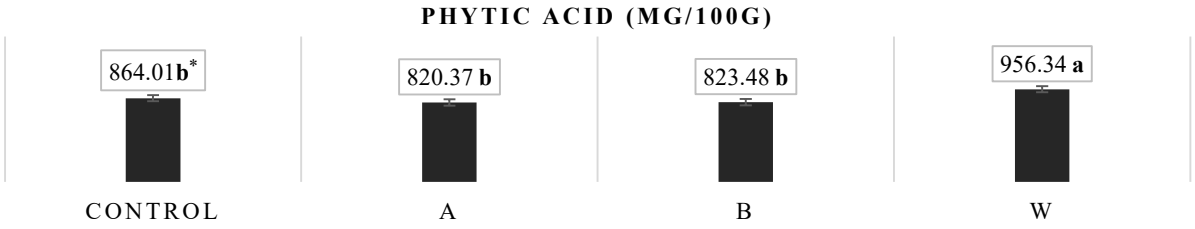

Figure 5. The effects of soaking material on the phytic acid concentration of hempseed meal
A: 50-50% grape vinegar and water, B: 100% Grape vinegar, W: 100% water. *a, b, c letters mean differences statistically significant as p≤0.01

Furthermore, it has been suggested that warm incubation conditions can enhance phytase activity, facilitating the breakdown of phytates in grains during food processing (Egli et al., 2003). However, evidence has not been sufficient that warm temperatures may decrease phytic acid concentration in plant seeds. In this study, long-term heating (24h) at 70 ˚C increased the determined phytate content of HSM. Results showed that long-term heating was ineffective on protein degradation.

The effects of soaking durations 1, 7, and 24 hours on phytic acid concentration and protein content of hempseed meal have been given in Figure 3. As can be seen in the figure, the protein content of HSM was not affected by soaking time and did not observe a significant protein content loss. On the other hand, interestingly soaking duration affected the phytic acid content, between 1-h and 7-h soaking durations differences were insignificant, but the highest phytic acid concentration was determined in 24 hours soaking duration (P≤0.01). Previous studies have suggested that soaking duration may increase phytic acid content in legumes. Rasha Mohamed et al. (2011) reported that soaking of soybeans for 12, 18, and 24 hours increased the phytic acid content by 5.8, 6.5, and 7.2 %, respectively and this result indicates the concentration of phytic acid may increase as the soaking time increases. Similarly, Egounlety and Aworh (2003) found that soaking Soybean for 12-14 h increased PA content by 1.71%. Oppositely, some studies stated that soaking for 24 hours but at 30˚C reduces the phytic acid content of some legumes (soybean) and grains (millet, rice, maize) (Lestienne et al., 2005a; Liang et al., 2008; Rasha Mohamed et al., 2011). Soaking duration term and seed species may affect phytic acid concentration, however, the current study showed that long-term soaking (24h) may increase the detectable phytic acid concentration of HSM.

The effect of soaking materials vinegar, vinegar-water mix, and water on protein content is presented in Figure 4., and on phytic acid concentration in Figure 5. The lowest protein content has been found with water soaking but differences were insignificant. On the other hand, soaking materials were effective on phytic acid concentration, results were similar between C, A, and B groups but water soaking significantly increased the detectable phytic acid content of HSM (p≤0.01). No study uses soaking to detect the phytic acid concentration change of hempseed. However, some studies that use water, vinegar, or acidic solutions as soaking material to determine the effects on legumes phytic acid are available.

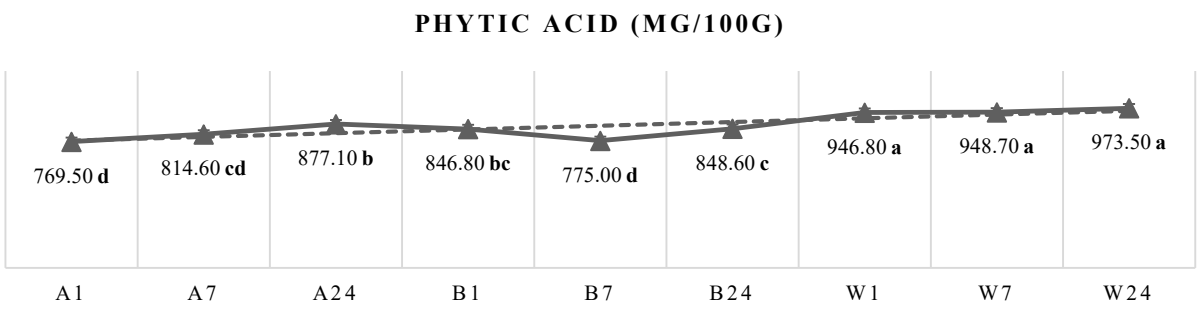

Figure 6. The effects of soaking material and duration interactions on the phytic acid contents of hempseed meal
A: 50-50% grape vinegar and water, B: 100% Grape vinegar, W: 100% water.1,7 and 24 indicates soaking hours. *a, b, c letters mean differences statistically significant as p≤0.01.

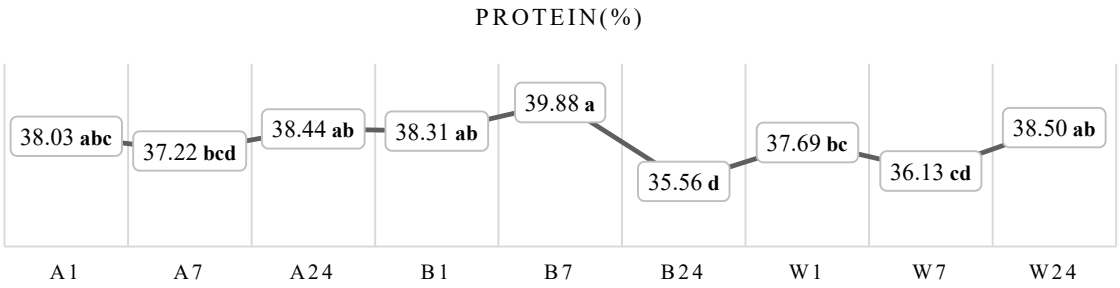

Figure 7. The effects of soaking material and duration interactions on the protein content of hempseed meal
A: 50-50% grape vinegar and water, B: 100% Grape vinegar, W: 100% water.1,7 and 24 indicates soaking hours. *a, b, c letters mean differences statistically significant as p≤0.01.

Qureshi and Asmaahamid (2020) reported a drastic reduction in phytic acid concentration in legumes boiling and following overnight soaking in a 1.5% water-vinegar solution. Diouf et al. (2020) reported that acetic acid solution soaking caused a significant reduction of the phytic acid concentration of cowpea than water soaking but the reduction ratio depended on soaking duration and the acetic acid ratio of the solution. Even though previous studies suggested that a more acidic environment can reduce phytic acid concentrations in some food and feed materials, scientific literature indicates that alkaline pH may inhibit the stability of phytate complexes, especially with the influence of sodium. It also states that phytate complexes are more stable in acidic environments (Cheryan & Rackis, 1980). This study showed that soaking material water with alkaline pH (8.0) increased phytic acid concentration and, acidic soaking solutions of vinegar and vinegar-water mix (pH:4.0) caused lower phytic acid concentration than water. Even though the differences were insignificant vinegar and vinegar-water mix soaking treatment caused lower phytic acid concentration in HSM than the control group.

Results of the soaking solution and duration interactions on the phytic acid concentration of HSM are given in Figure 6. and the protein content in Figure 7. The soaking solution and duration interactions were significantly effective on the detected phytic acid concentration of HSM, and the lowest phytic acid contents determined A1 and B7 groups (p≤0.01). The highest phytic acid values were detected in water-soaking groups W1, W7, and W24. As may seen in Figure 6. The phytic acid concentration of HSM significantly changed with the soaking solution and duration effects and, vinegar and vinegar water mixes reduced the phytic acid of HSM dramatically in each of three soaking durations than water soaking. Similarly, the protein content of HSM was affected by soaking solution and duration interactions, with the lowest protein content found in the B24 group and the highest in the B7 group (P≤0.01).

Data from this study agree with some previous studies, for example, Albarracín et al. (2013) reported that acidic lactic acid solution soaking reduced significantly phytic acid in rice but, in the same study the reduction of the amount of the phytic acid changed with duration and temperature. In this study, acidic solutions of vinegar and vinegar-water mix caused maximum reduction of phytic acid in HSM, and this situation can be explained by endogenous phytases having the optimal action at pH 4-5 (Yoshida et al., 1975). It has been stated that both soaking in water and acidic buffer can lead to lower PA levels in faba beans (Luo et al., 2009). Another study revealed that water soaking for 24 hours led to a significant reduction in the phytate content of millet, maize, rice, and soybean (Lestienne et al., 2005b). Alternatively, some studies reported that soaking in water for 4-h in the room temperature did not reduce the PA levels of pulses (Shi et al., 2018). A study examined the impact of soaking broiler feed in two different solutions on the release of free phosphorus and found that soaking with both water and citric acid solution increased the amount of released P, moreover, showed that as the soaking duration increased, the difference in released P between citric acid and deionized water became more noticeable. (Esmaeilipour et al., 2013). A study found that soaking wheat and corn in lactic acid solutions with pH levels of 5.7, 2.2, and 2.0 effectively reduced the phytate phosphorus content, and

the greatest reduction in phytic acid was achieved by using the most acidic lactic acid solution for a soaking duration of 48 hours (Vötterl et al., 2019). In this study, the protein content of HSM changed with soaking material and duration interactions however the highest protein loss was observed in vinegar soaking for 24 hours, and statistical differences between other groups were in the same significance levels. Protein loss can be expected when the soaking solution is removed. In this study, the difference in protein content between the groups with the lowest and highest protein levels was 4.32%, the maximum protein content was found in the B7 group, which also exhibited the highest reduction in PA. Similarly, Agume et al. (2017) reported that soaking caused significant protein loss in soybean flour and long soaking duration (72h) increased protein removal from the seed.

Conclusion

The results of this study demonstrated that both the soaking solution and duration significantly affected the phytic acid concentration in HSM, with acidic solutions being more effective in reducing PA levels. Vinegar, a common and organic substance, can improve the nutritional value of HSM through soaking treatment. However, additional research is required to determine the optimal vinegar concentration for effectively decreasing PA levels in HSM.

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