



Cloud Point Extraction for the Recovery of Bioactive Compounds from Peanut Shells

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ABSTRACT

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This study investigated the effectiveness of the cloud point extraction (CPE) method for the recovery of bioactive phenolic and flavonoid compounds from peanut shells. Key parameters affecting extraction efficiency, including salt concentration, pH, temperature, and sample amount, were systematically evaluated. The findings revealed that the optimal salt concentration for higher total phenolic content was 10%, whereas the maximum yield of total flavonoid compounds was obtained at a 12% salt concentration. Beyond these concentrations, a decline in extraction efficiency was observed for both compound groups. In terms of pH, the highest recovery of total phenolics occurred at pH 4, while total flavonoids reached their peak at pH 4.5. Overall, acidic conditions were found to enhance extraction performance. Regarding temperature, both phenolic and flavonoid yields were maximized at 90 °C. For the sample amount, the optimum recovery was achieved with 0.05 grams, while higher sample quantities led to decreased efficiency. These results underscore the critical importance of precisely optimizing CPE conditions to ensure efficient extraction of phenolic and flavonoid compounds from peanut shell biomass.

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Introduction

Peanut (*Arachis hypogaea* L.) is a legume crop cultivated worldwide primarily for its seeds and oil (Bertioli et al., 2011). Due to its versatile applications, it is considered a crucial industrial product on a global scale and is widely consumed due to its high nutritional value, health benefits, and unique flavor (Sorita et al., 2020). In 2019, 49 million tons of in-shell peanuts were produced worldwide across 30 million hectares, with the majority of production coming from China, India, and Nigeria. Over the past 60 years, Türkiye has seen a significant increase in both its peanut cultivation area and productivity (FAOSTAT, 2020). In 2020, Türkiye produced 55,000 tons on 215,000 hectares, with most of this production coming from the Çukurova region, particularly in the provinces of Adana and Osmaniye. Türkiye generally meets its domestic demand and occasionally imports small quantities (FAOSTAT, 2020). In Türkiye, Virginia type peanuts (*Arachis hypogaea* L.) are preferred in production due to their large seed size and they are mostly consumed as snacks (Şahin, 2014). Additionally, Virginia-type peanut (*Arachis hypogaea* L.) cultivated in Osmaniye was granted geographical indication status in 2002 (Turkish Patent and Trademark Office, 2002).

A peanut pod consists of three main parts: the shell, the skin, and the kernel (Win et al., 2011). Accordingly, processing 1 kg of in-shell peanuts yields approximately 776 g of kernel, 200 g of peanut shell and 24 g of peanut skin. Peanut shells account for approximately one-third of the pod's weight. It has been reported that these waste products from the peanut industry amount to approximately 11,000,000 tons annually worldwide (Mandala et al., 2023). A large portion of peanut shells (PS) is currently either incinerated for fuel or discarded as waste, leading to environmental pollution and the loss of valuable biomass (Niu et al., 2025).

Peanuts are notable functional foods due to their richness in bioactive compounds such as phenolic compounds, stilbenes, lignans, isoflavonoids, and phytosterols, which offer various health benefits through their antioxidant properties (Jung et al., 2020). The type and amount of bioactive compounds vary among different parts of the peanut pod (Zhao et al., 2012). Raw peanut kernels are a rich source of diverse polyphenols, including *p*-hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, quercetin, and kaempferol. Conversely, the peanut skin is notably abundant in procyanidine compounds, while

luteolin is the predominant compound found in the shell (Win et al., 2011; Liu et al., 2022). Early work by Pendse et al. (1973) identified 5,7-dihydroxychromone, eriodictyol, and luteolin in extracts of peanut shells. Daigle et al. (1988) further established luteolin as the dominant flavonoid in mature peanut shells, while eriodictyol prevailed in immature shells. Comprehensive studies (Duh et al., 1992; Duh et al., 1995) investigated the antioxidant properties of peanut shells. Subsequent findings by Wee et al. (2007) and Qiu et al. (2012) confirmed the antioxidant activity of various peanut shell compounds, consistently reporting luteolin as the primary antioxidative component (Peng et al., 2021). Also, it has been reported to exhibit significant antibacterial activity against various microorganisms, including *Escherichia coli*, *Enterobacter gergoviae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Trueperella pyogenes* (Guo et al., 2020; Mishra et al., 2021). Accordingly, peanut shells hold significant potential as a source of luteolin for use in various industries, including natural cosmetics, food, pharmaceuticals, chemicals, and agriculture.

While the significant antioxidative potential of peanut shells is well-documented, current extraction methodologies often face limitations in terms of efficiency, sustainability, and economic viability (Imran et al. 2022). Traditional extraction methods (e.g., Soxhlet, solid-liquid, or liquid-liquid extractions) typically use solvents like methanol, ethanol, and acetone. Although these methods are efficient and help preserve bioactive properties, they often involve lengthy processes, high solvent consumption, and the risk of toxic residues, making them environmentally unfriendly. In recent years, there has been a growing shift toward environmentally friendly and sustainable extraction techniques (Imran et al., 2022). Cloud point extraction (CPE) is an innovative method used to effectively isolate and concentrate bioactive compounds from complex matrices (Motikar et al., 2021). This technique relies on the selective separation of target molecules using surfactants (More et al., 2022). When a solution containing surfactants and target molecules is heated above the cloud point temperature, the surfactants form micelles that encapsulate the target molecules. Due to density differences, a micelle-rich phase and an aqueous phase are formed (More et al., 2019). CPE offers advantages such as low solvent usage, cost-effectiveness, environmental friendliness, and ease of application (Katsoyannos et al., 2006). The efficiency of this method depends on various factors, including the type and amount of surfactant used (Melnyk et al., 2015), incubation temperature, pH of the medium (Sun et al., 2006), incubation time (Gökkaya, 2014), and salt concentration (Ulusoy et al., 2013).

This study aims to effectively recover bioactive compounds from peanut shells using the CPE method by optimizing parameters like surfactant concentration, pH, salt addition, and temperature to get the best possible extraction efficiency. The recovery of bioactive compounds from agricultural wastes such as peanut shells using environmentally friendly methods like CPE represents a significant area of research in terms of

sustainability and the utilization of functional compounds. To the best of our knowledge, this is the first study to investigate the CPE of bioactive compounds from peanut shells.

Materials and Methods

Peanut Shell Material

The peanut shells, from Virginia market type peanuts (*Arachis hypogaea* L.), utilized in this study were sourced in 2024 from one of the leading peanut processing facilities (Okur Peanut) located in Osmaniye, Türkiye. These shells represent the by-products generated following the wet-shelling process. In this method, the peanuts are moistened with water before mechanical shelling to soften the shells, thereby facilitating the separation of kernels from the shells. The waste material was collected immediately upon exiting the production line and stabilized by air-drying under ambient conditions for two days to minimize potential microbial deterioration. Subsequently, the shells were transported to the laboratory, vacuum-packed in 500 g portions, and stored at room temperature to preserve their integrity and physicochemical properties.

Experimental Work for Cloud Point Extraction

The experimental design employed a one-factor-at-a-time (OFAT) approach to assess the individual effects of key parameters on the total phenolic content (TPC). In this design, each variable was systematically varied while the remaining parameters were maintained constant. For the extraction process, peanut shell samples were ground using a coffee grinder (Fakir, 80GRN01, China), and the resulting powder (0.05, 0.1, and 0.15 g) was suspended in 50 mL of distilled water within falcon tubes. The mixtures were allowed to stand for 1 h at room temperature in the dark. Subsequently, the suspensions were centrifuged (Nüve, NF 1200R, Türkiye) at 10,000 rpm for 10 min at room temperature to separate the solid and liquid fractions. The supernatants were filtered using a Büchner funnel with coarse filter paper to remove residual particulates. As a surfactant, lecithin (10% w/v) was then added to the clarified extracts, and the mixtures were homogenized using a vortex mixer. The pH was adjusted to predetermined levels (2, 4, and 6) using a pH meter (Hanna Instruments, HI Edge, USA). Sodium chloride (NaCl) was subsequently added at concentrations of 8%, 10%, and 15%, ensuring complete dissolution before incubation. The samples were heated in a water bath at 70°C, 80°C, or 90°C to induce phase separation, followed by centrifugation at 7,500 rpm for 10 min. This process yielded two distinct phases: a surfactant-rich, gelatinous phase presumed to contain the target phenolic and flavonoid compounds, and a residual aqueous phase (Arya et al., 2016).

However, subsequent determination of the total flavonoid content (TFC) required modifications to the parameter ranges due to the surfactant-induced turbidity observed after cloud point extraction, which interfered with accurate flavonoid quantification. To address this limitation, the experimental conditions for TFC analysis were refined by employing higher salt concentrations (10%, 12%, and 18%) and modified pH values (1.5, 4.5, and 7.5).

Total Phenolic Content Analysis

The total phenolic content of the extracts was determined according to the method of Shahidi and Ambigaipalan (2015). To a test tube, 0.2 mL of appropriately diluted sample and 1.5 mL of 10-fold diluted 2 N Folin-Ciocalteu reagent were added. The mixture was vortexed thoroughly and allowed to stand for 5 minutes. Then, 1.5 mL of sodium carbonate solution (6%, w/v) was added and the tubes were vortexed again. The blank solution was prepared by adding the same amount of distilled water instead of the extract, following the same procedure. The samples were incubated for 1.5 hours at room temperature in the dark and then absorbance was measured at 765 nm using a spectrophotometer against the blank. Results were expressed as gallic acid equivalents (GAE) based on a standard calibration curve.

Total Flavonoid Content Analysis

The total flavonoid content of the extracts was determined based on the method described by Zhishen et al. (1999). To a test tube, 2 mL of appropriately diluted sample and 150 μ L of 5% (w/v) sodium nitrite solution were added, and the mixture was vortexed and left to stand for 5 minutes. Then, 150 μ L of 6% (w/v) aluminum chloride solution was added and vortexed again. Subsequently, 1 mL of sodium hydroxide was added, followed by 1.2 mL of distilled water, and the final solution was mixed. The blank solution was prepared by replacing the extract with the same volume of distilled water and performing the same steps. After incubating for 10 minutes at room temperature in the dark, absorbance was measured at 510 nm using a spectrophotometer against the blank. Results were expressed as luteolin equivalents using a standard calibration curve.

Results and Discussion

The effects of pH, salt concentration, temperature, and sample amount on CPE were investigated in this study. Experiments were conducted to evaluate how these parameters influence the recovery efficiency of bioactive compounds. In this context, efforts were made to determine the optimal conditions that yield the highest amount of bioactive compounds.

Effect of Salt Concentration

Figure 1 presents the effect of different salt concentrations (8%, 10%, and 15%) on the total phenolic content, while maintaining pH constant at 2. The experimental findings indicated that the highest recovery of total phenolic compounds was achieved at 10% salt concentration. However, when the salt concentration exceeded 10%, a significant decrease in total phenolic content was observed. Similarly, Ji et al. (2021) reported that increasing the salt concentration from 5% to 10% improved the CPE yield but further increases beyond 10% did not result in meaningful changes in efficiency. This enhancement was attributed to the ability of electrolytes such as NaCl to increase the number of micelles in the system, thereby facilitating phase separation (El-Abbasi et al., 2014; Ji et al., 2021). More and Arya (2019) also reported that salt enhances yield at low concentrations (4–6%) through a salting-out effect, whereas higher salt

concentrations (>8%) may reduce the critical phase separation temperature, leading to bioactive degradation and yield loss. These researchers recommended a NaCl concentration range of 6–8% for optimal performance.

The effect of different salt concentrations (6%, 12%, and 18%) on the total flavonoid compound content is shown in Figure 2. Experimental findings indicated that the highest total flavonoid yield was obtained at a 12% salt concentration. However, when the salt concentration exceeded 12%, a significant decrease in the total flavonoid content was observed. This can be attributed to enhanced micelle formation and improved phase separation at moderate salt concentrations. However, excessive salt may disrupt micelle integrity and lower the phase separation temperature, leading to degradation of flavonoids and reduced recovery (Sliwa et al., 2021). Similarly, total flavonoid content also declined beyond a certain salt concentration, consistent with the trend observed for total phenolic compounds. Khani et al. (2019) observed increased quercetin extraction with salt concentrations up to 5%. This suggests differing optimal conditions for various compound classes.

Effect of pH

Figure 3 illustrates the effect of pH (2, 4, and 6) on the total phenolic compound content, with the salt concentration maintained at a constant 8%. The analyses revealed that the highest total phenolic compound yield at pH 4.

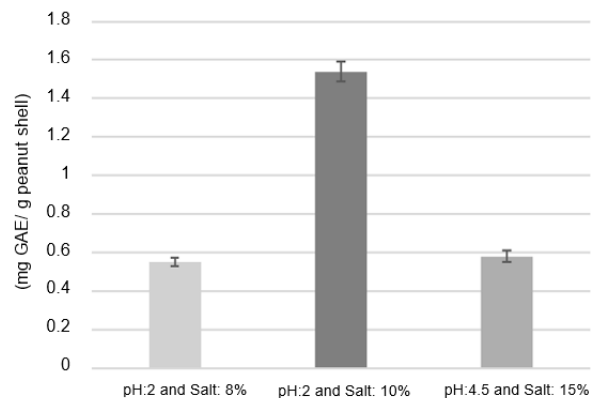


Figure 1. Effect of salt concentration on the total phenolic content at constant pH

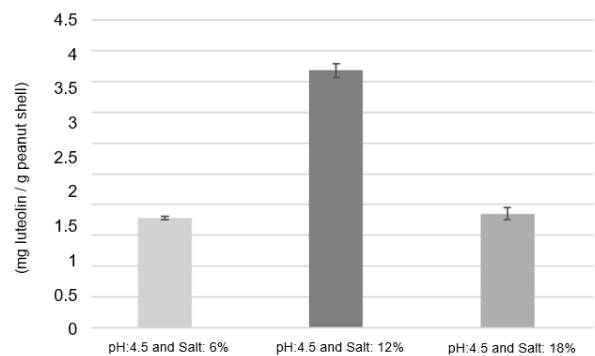


Figure 2. Effect of salt concentration on total flavonoid content under constant pH conditions

Similarly, More and Arya (2019) reported a 95% yield of total phenolic content at pH 4. The pH value plays an important role in the BNE method. Low pH (acidic conditions) helps phenolic and flavonoid compounds remain in their neutral form, facilitating their retention by surfactant micelles. Likewise, More and Arya (2019) achieved high recovery rates for both total phenolic and total flavonoid contents under acidic conditions such as pH 4. Increasing the pH to 6 caused a significant decrease in total phenolic content. This phenomenon can be explained by the findings of Kiai et al. (2018) and El-Abbassi et al. (2014), who reported that increasing the solution pH enhances the dissociation of phenolics from the surfactant phase, leading to loss of bioactive compounds. In conclusion, pH control is critical for the chemical stability of target compounds and their interaction with surfactant micelles, and an acidic pH range was found to be more effective for achieving the highest yield and lowest loss in the CPE of peanut shells.

Figure 4 evaluates the effect of the pH parameter (pH 1.5, 4.5, and 7.5) on the total flavonoid compound content, while keeping the salt concentration constant at 12%. The results revealed that the highest total flavonoid yield was achieved at pH 4.5. Similarly, De Araújo Padilha et al. (2018) applied the CPE method to recover polyphenols from camu-camu fruit waste. Interestingly, authors found that the native pH (3.25) of the camu-camu extract was optimal for polyphenol recovery, with increasing pH values reducing extraction efficiency. In another study, the maximum extraction recovery of the flavonoids (rutin, hyperoside, quercetin-3-O-sophoroside, isoquercitrin, astragaloside and quercetin) was achieved at pH 8 (Zhaou et al. 2015). Ultimately, the optimal pH for bioactive compound extraction is highly specific to the overall extraction system, including the properties of the target analytes and the chosen surfactant. From this, it can be inferred that a universal optimal pH is unlikely, and system-specific optimization is crucial.

Effect of Temperature

The effect of temperature, varied at 70, 80, and 90 °C, was assessed on TPC as shown in Figure 5. The analyses demonstrated that the highest total phenolic content was obtained at 90 °C. In a study conducted by El-Abbassi et al. (2014), the CPE method was used to recover polyphenols from olive mill wastewater. The effect of temperature on phase separation was investigated using the BNE method under constant extraction conditions (10% surfactant concentration and NaCl, 40 minutes) by varying the water bath temperature between 70°C and 90°C. The optimal temperature for both lutein and β -carotene was found to be 70°C, with a gradual decrease in the amount of extracted carotenoids observed at higher temperatures (Er, 2021). In samples clarified through ultrafiltration, a 30-minute CPE process at 90 °C using 10% Triton X-100 resulted in a yield of 66.5%. The CPE method stands out due to advantages such as low surfactant consumption and its potential for direct integration into food systems.

Figure 6 illustrates the effect of temperature on TFC. The analyses showed that the highest TFC was obtained at 90 °C. Similarly, Mai et al. (2020) investigated the impact of extraction temperature (35–95 °C) and time (5–25 minutes) on flavonoid yield. They found that increasing both parameters, up to 95 °C and 15 minutes respectively,

significantly enhanced flavonoid recovery. However, prolonged extraction times or excessively high temperatures can lead to flavonoid degradation, thus establishing 15 minutes as the optimal extraction time. Considering the technical limitations of the equipment and potential damage due to overheating, the extraction temperature was set to 90 °C for subsequent experiments.

Effect of Sample Amount

In Figure 7, the effect of sample amount (0.05, 0.10, and 0.15 g) on the recovery of bioactive compounds is shown, while salt concentration and pH were kept constant throughout the process. The findings indicated that the highest TPC was achieved with a sample amount of 0.05 grams. This outcome can be attributed to the limitation of solubility in the system and the negative impact on mass transfer as the sample amount increases.

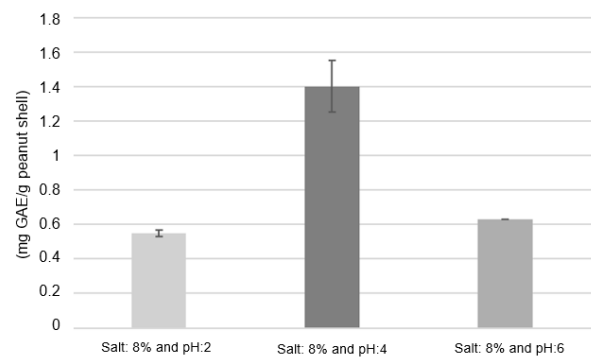


Figure 3. Effect of pH on total phenolic content at constant salt concentration

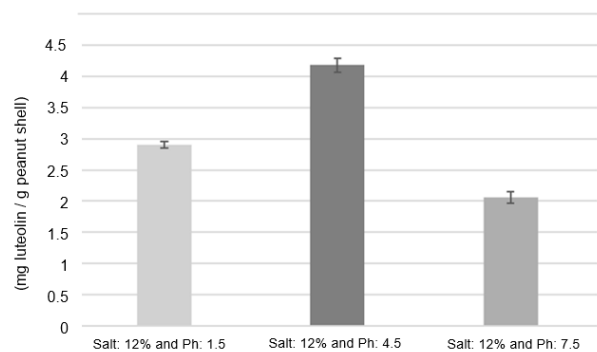


Figure 4. Effect of pH on total flavonoid content under constant salt concentration

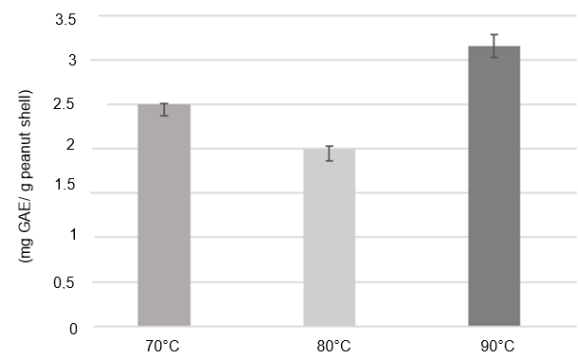


Figure 5. Effect of temperature on total phenolic content

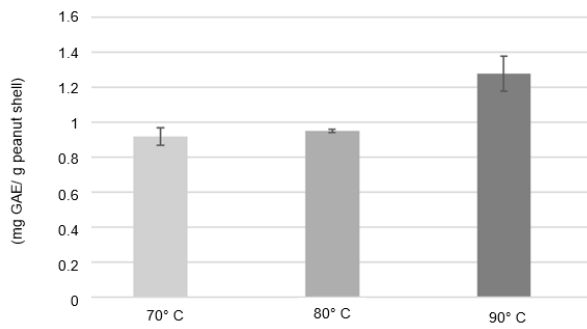


Figure 6. Effect of temperature on total flavonoid content

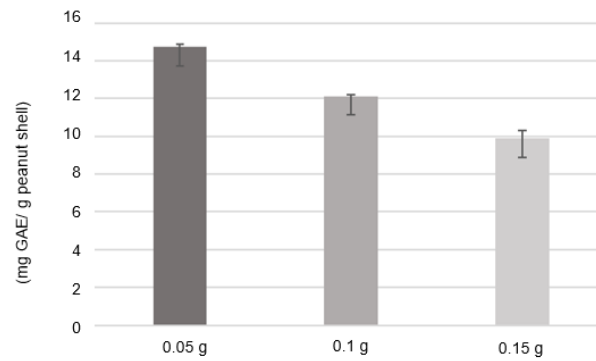


Figure 7. Effect of sample amount on total phenolic content

As the solid-to-liquid ratio increases, the solubility of phenolic compounds from peanut shells into the aqueous phase reaches saturation, leading to a decrease in the extraction yield.

Conclusion

In this study, the highest amount of phenolic compounds extracted from peanut shells using the CPE method was obtained at 10% salt concentration and pH 4, while the highest amount of flavonoid compounds was achieved at 12% salt concentration and pH 4.5. Regarding the temperature parameter, the highest recovery of both phenolic and flavonoid substances was observed at 90 °C. Maintaining salt and pH at optimum levels supports micelle formation and phase separation, thereby increasing yield. However, excessive values of these parameters may cause solubility issues or compound degradation. In terms of sample amount, the greatest yield of bioactive compounds under the applied CPE conditions was obtained with 0.05 grams of sample. These results indicate that the process conditions must be carefully selected for the efficient extraction of phenolic and flavonoid compounds from peanut shells using the CPE method. The CPE stands out as an effective, low-energy, and environmentally friendly method for recovering phenolic and flavonoid compounds from peanut shells. This method can contribute to both creating economic added value by valorising peanut shells and promoting sustainable and eco-friendly production processes.

Declarations

This study was presented at the IV. International Congress of the Turkish Journal of Agriculture - Food Science and Technology (Niğde, TURJAF 2025)

Ethical Approval Certificate

This study did not involve animal subjects or human participants, and therefore, ethical approval was not required.

Author Contribution Statement

Ebru Akgül: Data collection, investigation, formal analysis, and writing the original draft

Tulin Eker: Data collection, investigation, formal analysis, and writing the original draft

Hasim Kelebek: Supervision, conceptualization, methodology, review and editing

Pinar Kadiroglu: Project administration, supervision, conceptualization, methodology, review and editing

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Conflict of Interest

The authors declare no conflict of interest.

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