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TECHNO-FUNCTIONAL PROPERTIES OF CHICKPEA PROTEIN ISOLATE-TREATED ACIDIC AND BASIC PH-CYCLING

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ABSTRACT
This study examined the impact of extreme pH-cycling treatments on chickpea protein isolate (CPI). Untreated CPI, along with samples shifted to pH 2 (pH2) and pH 12 (pH12), displayed solubilities of 60.25%, 25.01%, and 75.48%, respectively. Both treatments significantly improved water and oil absorption capacities. Emulsion activity and stability for CPI at pH2 and pH12 were 125 m^2/g and 110 m^2/g, respectively, versus 75 m^2/g for the untreated sample. Notably, the foaming capacity and stability of pH12-treated CPI increased by 3.5 and 8.8 times, respectively, compared to the untreated protein. pH12-treated CPI also demonstrated the lowest gelling concentration at 10%, compared to 14% and 18% for untreated and pH2-treated CPI, respectively. Microstructural analysis revealed partial disintegration of CPI under pH-cycling, underscoring that alkaline pH12-shifting notably enhances functional properties of CPI.
Keywords: Chickpea, pH-cycling, modification, plant protein, functionality

ASİDİK VE BAZİK PH-DEĞİŞTİRME UYGULANMIŞ NOHUT PROTEİNLERİNİN TEKNOFONKSİYONEL ÖZELLİKLERİ

ÖZ
Bu çalışma, nohut protein izolatı (CPI) üzerindeki pH değiştirme uygulamasının etkisini incelemiştir. Uygulama yapılmamış CPI ile pH 2'ye (pH2) ve pH 12'ye (pH12) değiştirme uygulanmış örnekler sırasıyla %60.25, %25.01 ve %75.48 çözünürlük göstermiştir. Her iki uygulamada su ve yağ bağlama kapasitelerini önemli ölçüde artırmıştır. pH2 ve pH12'de uygulanmış CPI için emülsiyon aktivitesi ve stabilitesi sırasıyla 125 m^2/g ve 110 m^2/g iken, uygulama yapılmamış örnek için bu değer 75 m^2/g'dır. Özellikle, pH12 değiştirme uygulanmış CPI'nin köpük kapasitesi ve stabilitesi, uygulama yapılmamış proteine kıyasla sırasıyla 3.5 ve 8.8 kat artmıştır. pH12 uygulanan CPI ayrıca, uygulama yapılmamış ve pH2 uygulanan CPI'ye göre sırasıyla %14 ve %18 olan jel oluşum konsantrasyonunda en düşük değer olan %10'u göstermiştir. Mikroyapısal analiz, CPI'nin pH değiştirme ile kısmi olarak denatüre olduğunu ortaya koymuş ve pH12 değiştirme uygulamasının CPI'nin fonksiyonel özelliklerini belirgin şekilde artırdığını vurgulamıştır.
Anahtar kelimeler: Nohut, pH-değiştirme, modifikasyon, bitkisel protein, fonksiyonel

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INTRODUCTION

The increasing demand for alternatives to animal protein has grown the rapid expansion of plant-based proteins within the food sector. Traditional sources like wheat and soy face problems over allergenic concerns, leading a shift towards pulses like peas, fava beans, chickpeas, lupins, and lentils, which are emerging as key dietary components globally due to their farming benefits and nutritional value (Bessada et al., 2019). Notably, chickpea protein stands out for its higher levels of essential amino acids (lysine and arginine), superior digestibility, and lower allergen potential, attracting attention from food industry (Wang et al., 2021).

Nonetheless, recent research has indicated that solubility issues of chickpea protein (Chang et al., 2022; Tang et al., 2021) present challenges for its broader application in the food industry. These solubility issues are primarily attributed to the inherent structural properties of protein and its interaction with other food components, which can affect its functionality in different food matrices. For instance, the globulin and albumin fractions of chickpea protein tend to aggregate under certain processing conditions, leading to reduced solubility and functionality (Tang et al., 2021).

This reduced solubility not only hinder the ability of protein to be efficiently incorporated into various food products but also impacts its sensory attributes and consumer acceptance. Furthermore, the processing methods employed to isolate and purify chickpea protein can increase these solubility issues. For example, high-temperature treatments, enzymatic hydrolysis, and pH adjustments, commonly used in protein extraction, can lead to protein denaturation and aggregation, further decreasing solubility (Chang et al., 2022). Enzymatic hydrolysis can break down protein aggregates into smaller peptides, enhancing solubility and bioavailability (Wang et al., 2021). Ultrasonication, which uses high-frequency sound waves, can disrupt protein aggregates and improve dispersion in aqueous solutions (Tang et al., 2021). Chemical modifications, such as phosphorylation and glycosylation, can alter the surface properties of chickpea protein, reducing hydrophobic interactions and enhancing solubility (Chang et al., 2022).

Among the modification approaches, pH-shifting is a simple, adaptable, and safe method for modifying protein structures. This method involves subjecting proteins to extremely acidic or alkaline conditions, well away from their isoelectric points, causing an increase in repulsive forces among protein molecules, which leads to their partial unfolding (Karabulut et al., 2024). The extreme pH conditions disrupt the electrostatic interactions and hydrogen bonds within the protein, destabilizing its native structure. Subsequently, when the pH is neutralized to 7, proteins refold into a more flexible configuration referred to as the "molten state" (Wang et al., 2020). This state is characterized by a less ordered, more flexible conformation that enhances the protein's functional properties, such as solubility, emulsification, and gelation capabilities. This unfolding and refolding cycle profoundly modifies the proteins' structure and functionality, enabling their application in various food formulations. For instance, treating soybean protein with pH shifting at pH 2 selectively unfolds the 11S globulin, enhancing its surface hydrophobicity and significantly boosting its foaming capacity and stability (Jiang et al., 2018). Another researchers demonstrated the efficacy of pH shifting in enhancing the gelling and emulsifying traits of various plant proteins (Li and Wu et al., 2020; Wang et al., 2018). Furthermore, combining ultrasound with pH shifting has been identified as particularly effective in enhancing protein functionalities, as proteins unfolded at extreme pH levels are more susceptible to ultrasound. For example, this synergistic approach markedly enhanced the solubility of proteins from soybeans (Lee et al., 2016), peas (Jiang et al., 2017), rice (Zhang et al., 2018), and rapeseed (Li et al., 2020) beyond what either pH shifting or ultrasound could achieve independently. Yet, investigations into using pH shifting to improve functional attributes of plant proteins, particularly CPI, remain scarce.

The current study is aimed to: (i) explore the effects of acidic and basic pH cycling on chickpea proteins, and (ii) investigate the functional and morphological properties of these proteins. Our research seeks to offer an accessible and efficient approach to enhance the functionality of chickpea protein, thereby widening its application in the food industry.

MATERIALS AND METHODS

Chickpea (*Cicer arietinum* L.) was purchased by the local market (Sakarya, Türkiye). All chemicals were purchased from Sigma (Steinheim, Germany) with technical and higher grades. Distilled water was used in all experiments.

Extraction of Chickpea Protein Isolate

Chickpeas were first ground using a blender (Waring, USA) and then deoiled using hexane for 8 hours in a Soxhlet apparatus. The deoiled chickpea powders were air-dried overnight and subsequently ground into flour using a grain mill. To ensure uniformity, the ground flour was sifted through a 40-mesh sieve to produce consistent chickpea flour. This flour was then used to obtain CPI following the alkali extraction-isoelectric point precipitation method described by Malomo et al. (2014).

In summary, the deoiled flour was mixed with distilled water at a 1:20 w/v ratio, and the pH of the suspension was adjusted to 10.0 using 2 N NaOH. This suspension was stirred at a constant speed of 450 rpm at a steady pH of 10.0 for 2 hours at 37°C. After stirring, the mixture was centrifuged at 13000 x*g* for 25 minutes at 4°C using a centrifuge (Hettich Universal 320R, Germany) to separate the supernatant. The pH of the supernatant was then adjusted to 4.5 (the isoelectric point) using 2 N HCl, and centrifuged again under the same conditions to collect the pellet. This pellet was redissolved in distilled water and neutralized to a pH of 7.0 using 2 N NaOH. The resulting solution was freeze-dried using a lyophilizer (Labconco Freezone 6, USA), labeled as "Untreated", and stored at 4°C for further analysis.

The proximate composition of CPI was analyzed and found to consist of 90.2 ± 0.5% protein, 0.3 ± 0.0% lipid, 5.6 ± 0.0% ash, and 3.9 ± 0.2% carbohydrates, calculated on a dry weight basis in accordance with the guidelines set by the Association of Official Analytical Chemists (AOAC, 1990). Protein content, calculated as nitrogen multiplied by 6.25, was measured using a Nitrogen Analyzer (Velp NDA 701, Italy), using the Dumas method.

pH-cycling Prosess

The pH-cycling treatments, involving pH shifts to pH 2 and pH 12, were referenced from Jiang et al. (2018).

pH2-shifting treatment (pH2): A 3% (w/v) suspension of CPI was acidified to a pH of 2 using 2 N HCl, aiming to unfold the protein structures at room temperature. This acidified suspension was held at a stable pH of 2.0 for 1 hour, then neutralized back to pH 7.0 using 2 N NaOH. The neutral protein solutions were freeze-dried, labeled as "pH2", and stored at 4°C for further analysis.

pH12-shifting treatment (pH12): In a similar procedure, a 3% (w/v) CPI suspension was adjusted to an alkaline pH of 12 using 2 N NaOH to facilitate protein unfolding at room temperature. The solution was maintained at a constant pH of 12.0 for 1 hour before being neutralized back to pH 7.0 using 2 N HCl. The neutralized protein solutions were then freeze-dried and stored at 4°C, labeled as "pH12", for further analysis.

Solubility

Protein solubility was assessed using a modified method of Bradford (1976). In this procedure, 150 µL of a protein solution (3 mg/mL) was mixed with 3 mL of commercial Bradford dye reagent (Bio-Rad, USA). The mixture was incubated for 10 minutes to allow the reaction, followed by measuring the absorbance at 595 nm using a spectrophotometer (Shimadzu UV-Mini 1240, Japan). Bovine serum albumin was used as the standard reference protein solution

(correlation coefficient (R^2): 0.992). Protein solubility was calculated using the Equation (1).

$$Solubility\ (\%) = \frac{Psupernatant}{Pisolate}\ x\ 100 \qquad (1)$$

where $Psupernatant$ is the protein content in soluble part (mg/mL), $Pisolate$ is the protein content of protein isolate (mg/mL).

Emulsion Activity and Stability

The emulsion activity index (EAI) and emulsion stability index (ESI) of CPI were assessed using a slightly modified version of Pearce and Kinsella (1978) method. 4 mL of CPI solution (10 mg/mL) was mixed with 1 mL of sunflower oil in a beaker. The mixture was homogenized using an ultraturrax mixer (IKA, T20, Germany) at 10000 rpm for 2 minutes. After homogenization, a 25 µL aliquot of the emulsion was taken from the bottom of the beaker and diluted with 10 mL of a 0.1% (w/v) sodium dodecyl sulfate (SDS) solution. The EAI and ESI were determined by measuring the absorbance of the diluted emulsion at 500 nm using a spectrophotometer. Absorbance measurements were taken immediately and again after 10 minutes to calculate the EAI and ESI, respectively, using the given Equations (2) and (3).

$$EAI\ (m^2/g) = \frac{2x2.303xA0xDF}{CxØx\partial x10000} \qquad (2)$$

$$ESI\ (min) = \frac{A0x\Delta t}{A0-A10} \qquad (3)$$

where DF is the dilution factor, 2.303 is converts the absorbance value (which is typically measured as an optical density) to the natural logarithm scale, aligning with the Lambert-Beer law for absorbance measurements., 10000 is converts the g of protein concentration and cm of optical path, C is the concentration of protein (g/mL), $Ø$ is the optical path (1 cm), ∂ is an oil fraction in the emulsion (v/v, 1/5=.0.20), and A0 and A10 are the absorbances of emulsions after 0 min and 10 min, respectively.

Foaming Capacity and Stability

Foaming capacity (FC) and stability (FS) were evaluated with slight modifications from the method of Karabulut et al. (2022). Protein suspensions (20 mL) at a concentration of 10 mg/mL in distilled water, adjusted to pH 7.0, were homogenized at 10000 rpm for 2 minutes using a homogenizer (IKA T20, Germany) to obtain foam. FC and FS were then determined using Equation (4) for FC and Equation (5) for FS, respectively.

$$FC\ (\%) = \frac{V0-20}{20}\ x\ 100 \qquad (4)$$

$$FS\ (\%) = \frac{V10-20}{V0-20}\ x\ 100 \qquad (5)$$

where V_0 is the volume after whipping at 0 min, V_{10} is the volume after 10 min. of the CPI suspension

The Least Gelling Concentration

Protein solutions (2% to 20%, w/v) were subjected to thermal treatment in a water bath maintained at 90°C for one hour. After heating, the samples were quickly cooled to room temperature and then stored at 4°C for two hours to facilitate gelation. Following the gelation period, the flow properties of the protein gels were assessed by inverting the test tubes containing the gels. The lowest concentration at which the protein formed a gel that did not move or slide down the inner surface of the inverted tube was identified as the least gelling concentration (LGC, % w/v). This determination of LGC for the protein isolates follows the methodology described by Karabulut and Yemiş (2022).

Water and Oil Absorption Capacity

The water and oil absorption capacities of CPI were evaluated using a modified procedure of Karabulut et al. (2022). A 50 mg of CPI was mixed with 1.5 mL of distilled water (pH 7.0) or 1.5 mL of sunflower oil in separate experiments. Each mixture was shaken for 20 seconds and then allowed to stand for 30 minutes at room temperature to enable thorough absorption. Following the resting period, the mixtures were centrifuged at 7000 xg for 20 minutes at 4°C. After centrifugation, the supernatant was gently decanted, and the tubes were left undisturbed for an additional 20 minutes to facilitate complete

drainage of any residual supernatant. The water absorption capacity (WAC) and oil absorption capacity (OAC) of CPI were quantified by recording the change in net weight of the samples after the absorption and centrifugation processes, as described in Equations 6 and 7.

$$WAC\ (\%) = \frac{m2w - m1w}{m1w}\ x\ 100 \quad (6)$$

$$OAC\ (\%) = \frac{m2o - m1o}{m1o}\ x\ 100 \quad (7)$$

where m_{1w}, m_{1o} are initial weight (mg) and m_{2w}, m_{2o} final weight (mg) of protein isolates after water/oil addition, respectively.

Morphology

The microstructural properties of CPI powders were analyzed using a scanning electron microscope (SEM, S-4700, Hitachi, Ltd., Tokyo, Japan). For examination, samples were mounted on conductive carbon adhesive tape and sputter-coated with a gold-platinum alloy for 60 seconds to prevent charging under the electron beam. Imaging was conducted at an accelerating voltage of 10 kV, covering a range of magnifications from 100x to 1000x.

Statistical Analysis

All experimental treatments and analyses were performed in triplicate, with results reported as the mean ± standard deviation. Statistical evaluations were conducted using the SPSS 20.0 software package (SPSS Inc., Chicago, USA). The data were subjected to a one-way ANOVA to test for significant differences, with a significance level set at a 95% confidence interval ($P < 0.05$).

RESULTS AND DISCUSSIONS

Solubility

Protein solubility is a crucial functional attribute that influences emulsifying and digestive qualities, as well as various other functional characteristics (Tontul et al., 2018). The changes in CPI solubility following the pH-shifting procedure are shown in Figure 1a.

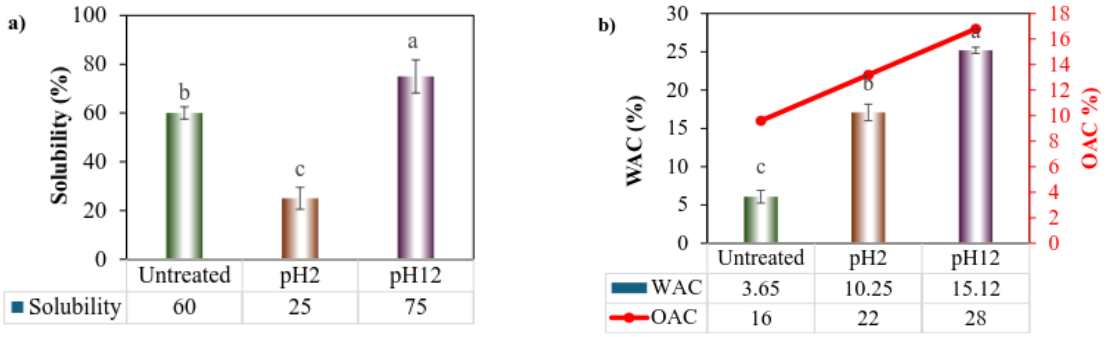

Figure 1. a) Solubility (%) and b) water/oil absorption capacity (%, WAC, %, OAC) of chickpea protein isolates (CPI). Untreated: control CPI, pH2: pH2-shifted CPI, and pH12: pH12-shifted CPI.

The maximum protein solubility, recorded at 75%, was observed with pH 12 shifting, while the solubility with pH 2 shifting was 25%, which was lower than that of untreated protein. The pH 12-shifting procedure enhanced CPI solubility, aligning with the outcomes of prior research. However, solubility was reduced with pH 2-shifting compared to untreated CPI, likely due to extensive aggregation at pH 2, approximating the isoelectric point of CPI, leading to increased sedimentation (Wang et al., 2022).

Previous studies have reported significant improvements in chickpea protein solubility following pH cycling. For example, Wang et al. (2024) observed that chickpea protein isolate solubility increased from 65% to 73% post pH 12 treatment. Similarly, Jiang et al. (2017) reported that pH 12 treatment boosted pea protein isolate

(PPI) solubility from 8.17% in the control to 54.94%. Factors such as pH, temperature, and ionic strength are known to influence protein solubility (Bolontrande et al., 2013).

This pH shifting technique has also been shown to elevate protein solubility in other plant proteins. Yildiz et al. (2017) reported that pH 12 treatment significantly increased the solubility of soy protein to 57.0%, compared to 9.1% in untreated protein samples. Additionally, exposing proteins to extreme pH levels, such as pH 12 or pH 2, can cause partial unfolding, with subsequent readjustment to pH 7 aiding in their refolding. This process, known for altering protein properties, may enhance CPI solubility due to increased protein-water ionic interactions (Jiang et al., 2010; Yildiz et al., 2017).

Water and Oil Absorption Capacity

The capacity of protein isolates to absorb water or oil measures their ability to bind with these substances, quantified by the volume of water or oil a gram of protein isolate can incorporate. These properties, reflecting the balance between hydrophilic and hydrophobic attributes, significantly impact storage-related quality aspects like texture and brittleness. In Figure 1b, the absorption capacities for CPI and pH-shifted CPIs are detailed. Both pH-shifted proteins exhibited better water and oil absorption capacities than the untreated chickpea protein.

Studies by Malik et al. (2018) and Resendiz-Vazquez et al. (2017) have highlighted that an increase in surface hydrophobicity can enhance interactions between proteins and oil, a correlation less evident in water absorption. The dynamics of water and oil absorption depend on the interplay between hydrophilic and hydrophobic forces within the protein, influenced by factors such as ionic strength, protein-water interactions, and overall protein solubility (Malomo et al., 2014).

Figure 1b illustrates that pH-shifting treatment enriched the protein surface with hydrophobic groups, which naturally gravitate towards oil, enhancing oil absorption. This enhanced oil absorption capability can positively impact the shelf life of food products by minimizing oil separation and preserving flavor compounds.

Emulsion Activity and Stability

The EAI and ESI are crucial metrics that demonstrate a protein's emulsifying capability. Proteins facilitate emulsification by encasing oil droplets within a film, which are then dispersed in water, thereby inhibiting emulsion destabilization processes such as coalescence, creaming, or sedimentation. The EAI quantifies the volume of oil that can be emulsified by a specific amount of protein, whereas the ESI measures how well the emulsion maintains its integrity over time (Boye et al., 2010).

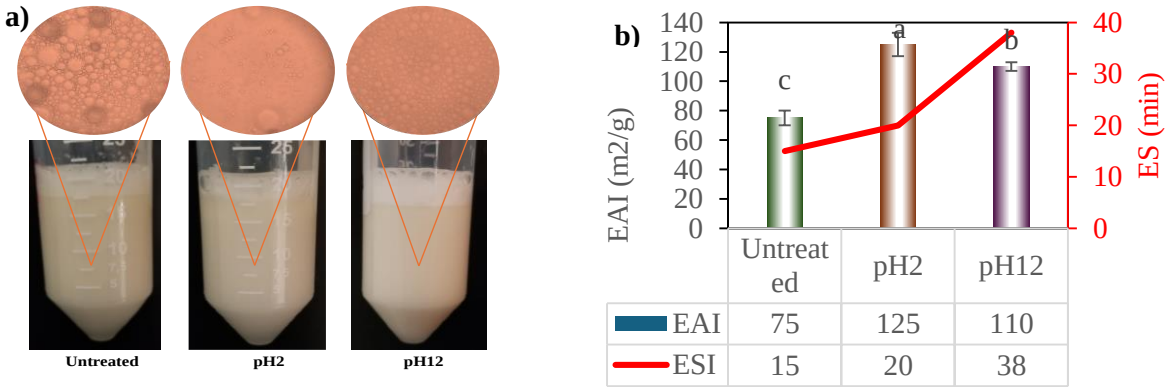

Figure 2. a) Emulsion morphology and b) emulsion activity index (EAI) and emulsion stability (ES) of chickpea protein isolates (CPI). Untreated: control CPI, pH2: pH2-shifted CPI, and pH12: pH12-shifted CPI.

Micrographs presented in Figure 2a depict the morphological characteristics of emulsion droplets for CPI treated at pH 12 and pH 2, alongside untreated CPI. Both pH-treated emulsions displayed uniformly small droplet sizes, in contrast to the untreated isolate, which exhibited larger and more variably distributed droplet sizes. Figure 2b illustrates the EAI and ESI for chickpea protein isolate (CPI) under various conditions: untreated, and subjected to pH adjustments to 2 and 12. The CPI treated at pH 12 demonstrated an enhanced EAI of 110 m^2/g, surpassing that of the untreated CPI (75 m^2/g), but lower than the pH 2-shifted CPI (125 m^2/g). Similar trends were observed in ESI measurements, where pH 12-shifted CPI (38 min) showed better stability than untreated CPI (15 min).

This evidence supports the hypothesis that treatment at pH 12 augments both the formation and stability of emulsions. The augmentation in EAI and ESI attributable to the pH 12 shift may be related to the unveiling of internal hydrophobic domains within the protein molecules (Cabra et al., 2007; Arzeni et al., 2012). Alkaline pH-shifting treatments induce protein denaturation, facilitating the exposure of hydrophobic regions to the surrounding aqueous environment (Lee et al., 2016; Wang et al., 2018). Such structural modifications improve the hydrophilic-lipophilic balance of the proteins, enhancing their efficacy at the oil-water interface.

This phenomenon was supported by research showing that pH-shifting treatments induce protein conformation changes into a molten globule state through unfolding and partial refolding, making the proteins more susceptible to physical modifications (Choe et al., 2022; Dijkstra et al., 2018). In this state, proteins exhibit a reduction in tertiary structural integrity and a loosening of tightly packed amino acid side chains, resulting in a disordered tertiary conformation with an expanded hydrophobic surface area. The enhanced emulsification properties may also stem from the generation of flexible random coil structures during treatment and a notable increase in protein solubility, which boosts the protein concentration in suspension and promotes more effective oil droplet adsorption.

Foaming Capacity and Stability

The foaming characteristic of proteins is associated with their ability to create a sticky, elastic film at the air-water interactions (Wang & Damodaran, 1991). Figures 3a and 3b show the foaming properties of CPI. The FC% and FS% values increased by more than 3 and 8 times, respectively, after extreme acidic and basic pH-cycling treatments compared to untreated CPI. This phenomenon likely results from CPI undergoing partial unfolding at extremely high or low pH levels, followed by partial refolding at neutral pH, leading to a molten globule configuration (Jiang et al., 2018). This process enhanced surface hydrophobicity and reduces particle size.

Additionally, the cavitation and turbulence effects from intense ultrasound exposure promote the translocation of hydrophobic groups from the core of protein to its surface, increasing surface hydrophobicity and further reducing particle size. The significance of the hydrophobic area is crucial for protein adhesion at the air-water interface (Jiang et al., 2018), facilitating easier adsorption and yielding more stable foams.

At pH 12, which is further from the isoelectric point of CPI than pH 2, protein repulsion is heightened, causing more dynamic unfolding and refolding of CPI. This results in more pronounced structural and physicochemical modifications, thereby improving the foaming qualities of pH 12-shifted CPI over pH 2-shifted CPI.

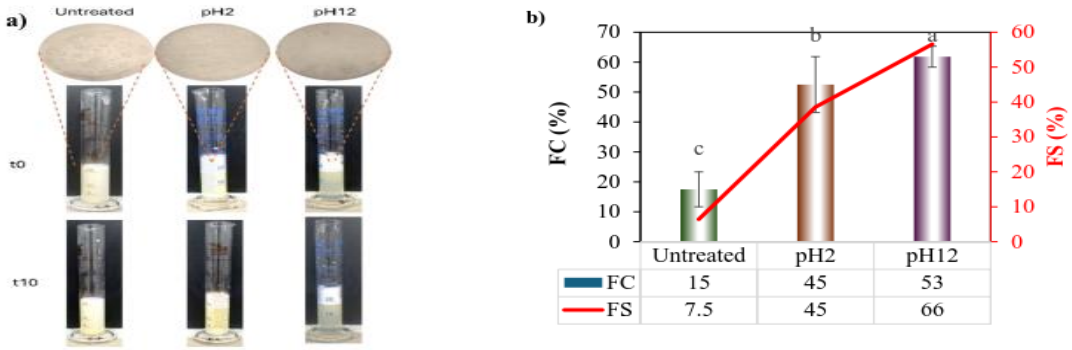

Figure 3. a) Foam morphology and b) foam capacity (FC) and foam stability (FS) of chickpea protein isolates (CPI). Untreated: control CPI, pH2: pH2-shifted CPI, and pH12: pH12-shifted CPI.

As seen in Figure 3a, the microstructural analysis of foam over various durations was analyzed to better understand the FA and FS of CPI. Specifically, smaller bubbles tend to form denser packings, which are less susceptible to structural changes, thereby enhancing the resistance of foam to liquid drainage (Xiong et al., 2018). Conversely, larger bubbles exhibit a greater tendency toward rupture and instability, leading to inferior foaming qualities (Zhao et al., 2021).

Foam inherently lacks thermodynamic stability; as time progresses, gravity induces liquid drainage, diminishing the volume of foam and altering its structure, as seen in the reduced film thickness and enlarged bubble size (Martinez-Velasco et al., 2018). These findings underscore that pH shifting significantly enhanced foaming capability of CPI, particularly when CPI was treated at pH 12.

The Least Gelling Concentration

Protein gelation plays a vital role in the creation of various food products, including puddings, jellies, desserts, and meat-based items. The LGC is a measure used to determine the concentration at which proteins form a stable, self-supporting gel through a network of covalent and non-covalent linkages. The gelation process is influenced by multiple factors, including the size of protein, structural conformation, flexibility, and interactions among molecules, as well as the hydrophobicity and spatial arrangement of amino acids (Wang & Damodaran, 1991).

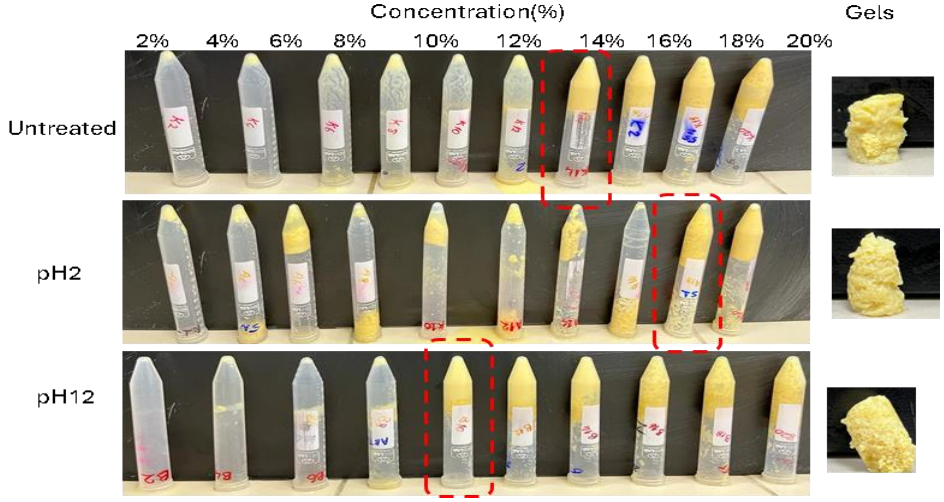

Figure 4. The gelling performance of chickpea protein isolates (CPI) at different concentrations (2-20%, w/w). Untreated: control CPI, pH2: pH2-shifted CPI, and pH12: pH12-shifted CPI.

As indicated in Figure 4, the application of pH-cycling enhanced the gelation abilities of pH 12-shifted CPI, reducing the LGC from 14% to 10%. Conversely, pH 2-shifted CPI showed the highest gelling concentration at 18%. Enhanced solubility aids in establishing the three-dimensional gel network proteins (Arzeni et al., 2012). The accessibility of these groups induce gelation through the creation of disulfide, hydrogen, hydrophobic, and van der Waals bonds. The partial unfolding and unordered particle size of CPI enhance protein flexibility, crucial for constructing the three-dimensional gel matrix, thereby achieving a reduced LGC.

Morphology

Scanning electron microscopy analyses were performed at magnifications ranging from 100× to 1000× to elucidate the morphological impacts of various treatments on chickpea protein isolate powders, as detailed in Figure 5.

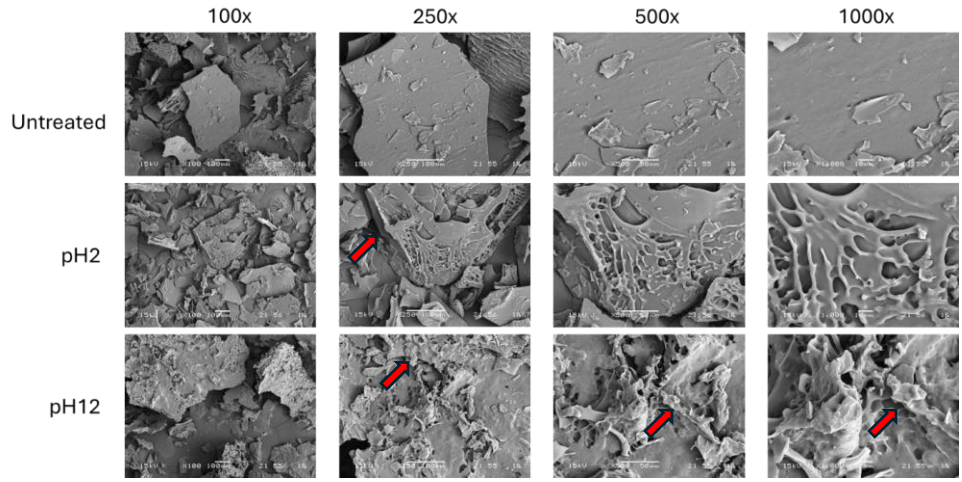

Figure 5. Morphological images of chickpea protein isolates (CPI) by Scanning Electron Microscopy (SEM). Untreated: control CPI, pH2: pH2-shifted CPI, and pH12: pH12-shifted CPI.

pH-cycling processes induced partial disintegration of the CPI, as evidenced by rough, flaky morphologies with pronounced edges in the SEM micrographs. These observations suggest the induction of molten globule states, characterized by the partial unfolding of protein structures, as reported by Zhang et al. (2019). The formation of cracked, irregular, and small fragments significantly increases the surface area of the treated proteins. This increase in surface area may show the observed enhancements in solubility and emulsion characteristics. Furthermore, the enhanced surface activity attributed to pH-shifting treatments enhances the interactions between oil and water with protein aggregates, thereby improving interfacial adsorption capabilities. The sheet-like structures following the integration of physical and chemical treatments was hypothesized to bolster hydrophobic interactions among protein surfaces, as found by Yang et al. (2017). This change in protein morphology and surface properties highlighted the multifaceted effects of treatment methods on the functional properties of CPI, especially solubility and emulsion stability.

CONCLUSIONS

This study aimed to assess the efficacy of a pH-shifting technique as a chemical method for altering and improving the functional properties of CPI. Among the different pH levels tested, applying a pH 12 condition notably enhanced the solubility, emulsifying properties, and foaming characteristics of CPI. These results highlight the effectiveness of pH 12 treatment as a promising method for protein modification, significantly enhancing essential functional properties of chickpea proteins. The pH-shifting technique utilized in this research demonstrates potential in

meeting the food industry's growing demand for broader applications of chickpea proteins.

CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Gulsah Karabulut: Writing – review – editing, Writing – original draft, Methodology, Investigation, Conceptualization, Funding acquisition.

REFERENCES

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O. E., Pilosof, A. M. R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering*, 108(3), 463-472.

Bessada, S. M., Barreira, J. C., Oliveira, M. B. P. (2019). Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. *Trends in Food Science & Technology,* 93, 53-68.

Bolontrade, A. J., Scilingo, A. A., Anon, M.C. (2013). Amaranth proteins foaming properties: Adsorption kinetics and foam formation—Part 1. *Colloids and Surfaces B: Biointerfaces*, 105, 319–327.

Boye, J., Zare, F., Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, 43(2), 414-431.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254.

Cabra, V., Arreguin, R., Vazquez-Duhalt, R., Farres, A. (2007). Effect of alkaline deamidation on the structure, surface hydrophobicity, and emulsifying properties of the Z19 α-zein. *Journal of Agricultural and Food Chemistry*, 55(2), 439-445.

Chang, L., Lan, Y., Bandillo, N., Ohm, J. B., Chen, B., Rao, J. (2022). Plant proteins from green pea and chickpea: Extraction, fractionation, structural characterization and functional properties. Food Hydrocolloids, 123, 107165.

Choe, U., Chang, L., Ohm, J. B., Chen, B., Rao, J. (2022). Structure modification, functionality and interfacial properties of kidney bean (*Phaseolus vulgaris* L.) protein concentrate as affected by post-extraction treatments. *Food Hydrocolloids*, 133, 108000.

Dijkstra, M. J. J., Fokkink, W. J., Heringa, J., van Dijk, E., Abeln, S. (2018). The characteristics of molten globule states and folding pathways strongly depend on the sequence of a protein. *Molecular Physics*, 116(21-22), 3173-3180.

Jiang, J., Xiong, Y. L., Chen, J. (2010). pH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions. *Journal of Agricultural and Food Chemistry*, 58(13), 8035-8042.

Jiang, J., Wang, Q., Xiong, Y. L. (2018). A pH shift approach to the improvement of interfacial properties of plant seed proteins. *Current Opinion in Food Science*, 19, 50-56.

Jiang, S., Ding, J., Andrade, J., Rababah, T. M., Almajwal, A., Abulmeaty, M. M., Feng, H. (2017). Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics Sonochemistry*, 38, 835-842.

Karabulut, G., Feng, H., Yemiş, O. (2022). Physicochemical and antioxidant properties of industrial hemp seed protein isolate treated by high-intensity ultrasound. *Plant Foods for Human Nutrition*, 77(4), 577-583.

Karabulut, G., Yemiş, O. (2022). Modification of hemp seed protein isolate (*Cannabis sativa* L.) by high-intensity ultrasound treatment. Part 1: Functional properties. *Food Chemistry*, 375, 131843.

Lee, H., Yildiz, G., Dos Santos, L. C., Jiang, S., Andrade, J. E., Engeseth, N. J., Feng, H. (2016). Soy protein nano-aggregates with improved functional properties prepared by sequential pH

treatment and ultrasonication. *Food Hydrocolloids*, 55, 200-209.

Li, J., Wu, M., Wang, Y., Li, K., Du, J., Bai, Y. (2020). Effect of pH-shifting treatment on structural and heat induced gel properties of peanut protein isolate. *Food Chemistry*, 325, 126921.

Li, Y., Cheng, Y., Zhang, Z., Wang, Y., Mintah, B. K., Dabbour, M., ... Ma, H. (2020). Modification of rapeseed protein by ultrasound-assisted pH shift treatment: Ultrasonic mode and frequency screening, changes in protein solubility and structural characteristics. *Ultrasonics Sonochemistry*, 69, 105240.

Malik, M. A., Saini, C. S. (2018). Rheological and structural properties of protein isolates extracted from dephenolized sunflower meal: Effect of high intensity ultrasound. *Food Hydrocolloids*, 81, 229-241.

Malomo, S. A., He, R., Aluko, R. E. (2014). Structural and functional properties of hemp seed protein products. *Journal of Food Science*, 79(8), C1512-C1521.

Martínez-Velasco, A., Lobato-Calleros, C., Hernández-Rodríguez, B. E., Román-Guerrero, A., Alvarez-Ramirez, J., Vernon-Carter, E. J. (2018). High intensity ultrasound treatment of faba bean (*Vicia faba* L.) protein: Effect on surface properties, foaming ability and structural changes. *Ultrasonics Sonochemistry*, 44, 97-105.

Pearce, K. N., Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, 26(3), 716-723.

Resendiz-Vazquez, J. A., Ulloa, J. A., Urías-Silvas, J. E., Bautista-Rosales, P. U., Ramírez-Ramírez, J. C., Rosas-Ulloa, P., González-Torres, L. J. U. S. (2017). Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (*Artocarpus heterophyllus*) seed protein isolate. *Ultrasonics Sonochemistry*, 37, 436-444.

Tang, X., Shen, Y., Zhang, Y., Schilling, M. W., Li, Y. (2021). Parallel comparison of functional and physicochemical properties of common pulse proteins. *LWT*, 146, 111594.

Tontul, İ., Kasimoglu, Z., Asik, S., Atbakan, T., Topuz, A. (2018). Functional properties of chickpea protein isolates dried by refractance window drying. *International Journal of Biological Macromolecules*, 109, 1253-1259.

Wang, C. H., Damodaran, S. (1991). Thermal gelation of globular proteins: influence of protein conformation on gel strength. *Journal of Agricultural and Food Chemistry*, 39(3), 433-438.

Wang, J., Li, Y., Li, A., Liu, R. H., Gao, X., Li, D., ... Xue, Z. (2021). Nutritional constituent and health benefits of chickpea (*Cicer arietinum* L.): A review. *Food Research International*, 150, 110790.

Wang, J., Zhou, X., Li, J., Pan, D., Du, L. (2024). Enhancing the functionalities of chickpea protein isolate through a combined strategy with pH-shifting and cold plasma treatment. *Innovative Food Science & Emerging Technologies*, 103607.

Wang, Q., Jin, Y., Xiong, Y. L. (2018). Heating-aided pH shifting modifies hemp seed protein structure, cross-linking, and emulsifying properties. *Journal of Agricultural and Food Chemistry*, 66(41), 10827-10834.

Wang, Y., Wang, S., Li, R., Wang, Y., Xiang, Q., Li, K., Bai, Y. (2022). Effects of combined treatment with ultrasound and pH shifting on foaming properties of chickpea protein isolate. *Food Hydrocolloids*, 124, 107351.

Wang, Y., Yang, F., Wu, M., Li, J., Bai, Y., Xu, W., Qiu, S. (2020). Synergistic effect of pH shifting and mild heating in improving heat induced gel properties of peanut protein isolate. *LWT*, 131, 109812.

Xiong, T., Xiong, W., Ge, M., Xia, J., Li, B., Chen, Y. (2018). Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. *Food Research International*, 109, 260-267.

Yang, X., Li, Y., Li, S., Oladejo, A. O., Ruan, S., Wang, Y., ... Ma, H. (2017). Effects of ultrasound pretreatment with different frequencies and working modes on the enzymolysis and the structure characterization of rice protein. *Ultrasonics Sonochemistry*, 38, 19-28.

Yildiz, G., Andrade, J., Engeseth, N.C., Feng, H. (2017). Functionalizing soy protein nano-

aggregates with pH-shifting and mano-thermo-sonication. *Journal of Colloid and Interface Science*, 505, 836-846.

Zhang, L., Pan, Z., Shen, K., Cai, X., Zheng, B., Miao, S. (2018). Influence of ultrasound-assisted alkali treatment on the structural properties and functionalities of rice protein. *Journal of Cereal Science*, 79, 204-209.

Zhang, Y., Wang, B., Zhang, W., Xu, W., Hu, Z. (2019). Effects and mechanism of dilute acid soaking with ultrasound pretreatment on rice bran protein extraction. *Journal of Cereal Science*, 87, 318-324.

Zhao, C., Chu, Z., Miao, Z., Liu, J., Liu, J., Xu, X., ... Yan, J. (2021). Ultrasound heat treatment effects on structure and acid-induced cold set gel properties of soybean protein isolate. *Food Bioscience*, 39, 100827.