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Foaming and emulsifying properties of aquafaba powders as affected by saponin and amino acid content

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ABSTRACT

Aquafaba, the viscous liquid by product from legume cooking, has gained significant attention due to its ability to replicate the functional properties of egg albumen. This is attributed to the presence of proteins and saponins, which provide aquafaba with superior foaming and emulsifying capacities. In this study, aquafaba was first prepared using different microwave-assisted cooking techniques, then spray dried, and the effects of these treatments on the functional properties of the resulting powders were investigated. Specifically, the effects of microwave and microwave-infrared treatments on the stability and performance (with an emphasis on foaming and emulsifying properties) of aquafaba were analyzed in comparison to conventional pressure cooking. In addition to protein and saponin content, amino acid composition was also measured to explore its potential role in functional behavior, particularly foaming. The results showed that although the concentrations of proteins and saponins were reduced relative to the conventional method, microwave applications exhibited enhanced foaming and emulsifying capabilities. Saponin content, quantified via LC-MS/MS, was found to predominantly influence emulsification, whereas both protein and saponin levels contributed to foaming behavior.

1. Introduction

Aquafaba is the viscous liquid produced after cooking or canning legumes like chickpeas or white beans (Mustafa et al., 2018). During soaking and cooking, chickpeas release organic compounds, mainly carbohydrates and proteins, into the aquafaba (Cabrita et al., 2023; Stasiak et al., 2023). This by-product, which was previously considered waste, has suddenly become well-known as a highly adaptable and sustainable substitute for egg whites (Raikos et al., 2019; Stasiak et al., 2023). The composition of aquafaba might change based on the type of bean, the amount of cooking time, the temperature and the method of production (Crawford et al., 2023). Aquafaba, like egg whites, has exceptional foaming qualities (He et al., 2019; Mustafa & Reaney, 2020). It could create stable foams when whipped, resulting in structures that could trap air bubbles. Aquafaba contains approximately 1.5 % of proteins which are especially globulins and albumins, and they have a major contribution to its foaming capacity (Boye et al., 2010; He et al., 2021). These proteins unfold and denature, resulting in the formation of a protein network capable of stabilizing air bubbles. Aquafaba also has emulsifying ability which is attributed to its saponin content which helps to stabilize oil-in-water emulsions (Hernández-López et al., 2022). Saponins are naturally occurring glycosidic, amphiphilic, and heat-stable substances found in plant foods (Sagratini et al., 2009). Saponins are composed of triterpenoid or steroidal aglycone moieties linked to a mono- or oligosaccharide structure. The aglycone moiety is strongly hydrophobic (non-polar), whereas the oligosaccharide portion is hydrophilic (polar) (Savarino et al., 2023). The occurrence of polar and non-polar end groups gives saponin a strong surface-active property which enables saponin to act as an emulsifier and foaming agent. Legumes primarily contain soya saponin βg which belongs to group B soyasaponins (Sagratini et al., 2009) and chickpeas mainly contain soya saponin βg and soya saponin I (Srivastava & Vasishtha, 2013). Soyasaponin β group is characterized by the presence of a reducing sugar moiety on C-22 known as 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (Price et al., 1988). DDMP conjugated soyasaponins are named as soyasaponin αg, βa, βg, γa and γg while non-DDMP conjugated saponins are called soyasaponin I (Bb), II (Bc), III (Bb′), IV (Bc′) and V (Ba) (Serventi et al., 2013). The heat-sensitive residue, DDMP, gives the

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saponin its unique characteristics, such as its antioxidant capability, unique absorption spectrum, and sweet flavor (Kerem et al., 2005; Kudou et al., 1993).

Microwave technology can offer a rapid and effective approach to concentrating aquafaba by enhancing the release of soluble solids, including proteins and carbohydrates, from chickpeas into the cooking water. Compared to conventional thermal processing, microwave and microwave-infrared (IR) technologies have been reported to reduce processing time and improve the extraction efficiency of functional components, such as proteins, from plant-based materials (Yildiz et al., 2018). Microwave-assisted extraction involves the interaction of microwaves with water molecules within the plant matrix, causing rapid localized heating and structural disruption, thereby facilitating the release of intracellular compounds (Q. Hu et al., 2021; Routray & Orsat, 2012). Previous studies have demonstrated that microwave can improve the extraction efficiency and preserve the functional and structural integrity of target compounds. For example, Mirzadeh et al. (2020) showed that polysaccharides extracted by microwave from various sources exhibited higher yields and improved bioactivities compared to those extracted by conventional techniques (Mirzadeh et al., 2020). Similarly, Wen et al. (2021) demonstrated that microwave led to a 5.8- to 7-fold increase in protein recovery from coffee silverskin (Wen et al., 2021). Similar principles were applied in this study to enhance the concentration of soluble matter in aquafaba. Following extraction, spray drying, which is a widely used industrial method for converting liquid materials into powders, was employed to produce aquafaba powder while preserving its functional properties, such as emulsifying, foaming, and gelling abilities (Begliyev et al., 2023; He et al., 2021). This process aims to preserve aquafaba's functional properties, such as its emulsifying, foaming and gelling abilities, while also obtaining a product that is more stable, portable and has a longer shelf life.

The purpose of this study was to comprehensively evaluate the effects of various cooking methods and post-cooking treatments on the physicochemical and functional properties of spray dried powder aquafaba. This study aimed to investigate how these processing methods influence the interfacial stability of aquafaba, particularly its ability to form and maintain stable foams and emulsions. Moreover, the study focused on the comparison of conventional pressure cooking, microwave heating, and microwave infrared to assess their efficiency in extracting saponins and soluble proteins from chickpeas. It was hypothesized that microwave processing techniques would show higher protein and saponin content thus higher foaming and emulsifying properties.

2. Materials and methods

2.1. Materials

Chickpea (*Cicer Arientum* L.) (Reis Gida, Kastamonu, Türkiye) was purchased from a local market in Ankara, Türkiye. All chemicals including an HPLC Grade Soyasaponin I were purchased from Sigma Aldrich Co. (St Louis, MO, USA).

2.2. Preparation of aquafaba

Initially, the chickpeas were washed, and excess water was removed; then, the chickpeas were soaked in distilled water at a ratio of 1:3 at 4 °C for 16 h (Meurer et al., 2020). Two different methods (microwave and microwave-infrared techniques) were used to increase the solid content of aquafaba along with the conventional pressure cooker.

1. *Conventional Pressure Cooker (PC):* Chickpeas were cooked in an electric pressure cooker for 20 min (control process).
2. *Microwave Heating (MW-C):* Chickpeas were cooked in a microwave oven (Arçelik, Istanbul, Türkiye) for 50 min at maximum power (900 W) corresponding to 720 W effective output based on the 2-L IMPI test.
3. *Microwave-Infrared (MW-IR):* Chickpeas were cooked in a microwave-infrared oven (GE Advantium, Louisville, KY, USA) for 50 min using microwave power at 50 % and upper and lower Infrared (IR) power at 100 % (1500 Watts). Microwave power was determined as 635 W by a 2L IMPI test (Aydogdu et al., 2015).

The chickpea-to-water ratio was kept constant across all trials, with parameters optimized for maximum dry matter yield. Treatment time was fixed at 50 min for all methods except the conventional pressure cooker (PC), which followed standard cooking practice. The microwave heating methods required 50 min to achieve sufficient dry matter for further spray drying processing.

Preliminary Trials: In preliminary experiments for both microwave techniques, chickpea-to-water ratio, power, and time were optimized. Initially, cooking time was fixed while varying power (80 % and 100 %) and ratios (1:3 and 1:5). The highest dry matter yield was achieved at a 1:3 ratio under 100 % power. Subsequently, cooking times between 20 and 60 min were tested, and 50 min was selected as optimal, balancing yield and minimizing water loss that hindered spray drying.

Due to differences in behavior between microwave treatments (MW-C and MW-IR), the same power settings could not be applied. MW-IR consistently caused overflow, even in the largest container, so the microwave power was reduced while upper and lower IR powers remained at 100 % to ensure safe and comparable conditions.

For clarity, throughout this manuscript, "MW-C," "MW-IR," and "PC" refer to aquafaba powders produced by Microwave-Conventional, Microwave-Infrared, and Pressure Cooker methods, respectively, unless otherwise stated when describing the processing techniques.

2.2.1. Further processing techniques

After cooking, the obtained aquafaba was treated in two different ways: immediately drained and separated and kept with water overnight at 4 °C and then filtered which were labelled as ***'S' (Non-soaked)*** and ***'O' (Overnight)***, respectively.

A summary of experimental design regarding the production of aquafaba by different methods was given in Table 1 (Ates et al., 2025).

2.3. Preparation of powder aquafaba

Aquafaba powder was obtained by following the method (Ates et al., 2025). A pilot-scale spray dryer (UNOPEX, İzmir, Turkey) was used for powder production. The spray drying parameters were set as 8–9 ml/min, 150 °C, 83 °C and 80 % for the feed flow rate, inlet temperature, outlet temperature and aspiration rate, respectively. The aquafaba powders were stored at room temperature in a glass container wrapped

Table 1
Summary of Experimental Design for Aquafaba Production.

Sample Code	Cooking Method	Soaking Treatment	Microwave Power (W) (Efficiency)	Infrared Power (W)	Time (min)
PC-S	Pressure Cooker	Non-soaked ('S')	–	–	20
PC-O	Pressure Cooker	Overnight ('O')	–	–	20
MW-C-S	Microwave	Non-soaked ('S')	900 (100 %)	–	50
MW-C-O	Microwave	Overnight ('O')	900 (100 %)	–	50
MW-IR-S	Microwave-Infrared	Non-soaked ('S')	635 (50 %)	1500 (upper & lower)	50
MW-IR-O	Microwave-Infrared	Overnight ('O')	635 (50 %)	1500 (upper & lower)	50

with parafilm for later use.

2.4. Characterization of powder aquafaba

2.4.1. Soluble protein content

The amount of soluble protein was determined by using the Lowry method (Kusunoki et al., 2012; Tas et al., 2021). 0.5 ml of sample was mixed with 0.7 ml of ACR reagent which was incubated for 10 min. Then, 0.25 ml of Folin reagent (diluted 1:1) was added and mixed by using a vortex. This solution was kept in the dark and incubated for 30 min. The absorbance was measured at 680 nm using a UV-spectrophotometer. The calibration curve was obtained by using BSA (Bovine Serum Albumin) solution prepared at different concentrations.

2.4.2. Determination of saponin content by the LC-MS/MS method

The amount of saponin in aquafaba processed in different methods (PC, MW-C and MW-IR) was measured by LC-MS/MS (Pilar et al., 2014). LC-MS/MS analysis was carried out using an HPLC system (Shimadzu Sci., Kyoto, Japan) connected to a 3200 QTRAP (AB SCIEX, Foster City, CA, USA) equipped with an electrospray ionization (ESI) and Turbo VTM Ion Source. An electrospray source (ESI) operating at 300 °C and 4 kV capillary temperature and voltage, nebulizer pressure of 50 psi, and nitrogen gas flow rate of 10 mL/min was used to carry out the ionization process.

To extract the saponins, aquafaba powders were mixed with 70 % aqueous ethanol and shaken for 12 h. Then, the samples were centrifuged at 4000 rpm for 5 min. The supernatant was taken and filtered with a 0.45 μm nylon filter and transferred into the vial. 0.1 % Formic Acid in water (A) and pure acetonitrile (B) were used as mobile phase. The gradient elution was performed as follows: 0–10 % B in 8 min, 10 %–35 % (B) from 8 to 16 min, 35 %–90 % from 16 to 26 min, 100 % (B) from 26 to 36 min and finally 36–38 min, concentration B was decreased to 0 % (the beginning concentration). Flow rate, oven temperature and injection volume were selected as 0.4 ml/min, 40 °C and 5 μL, respectively. Data was acquired in negative ionization mode. The saponin peak was observed at around 30 min. MRM for saponins could be found in Table 2. 95 % Soyasaponin I (Sigma Aldrich, USA) was used for the calibration curve (25 ppm–200 ppm). The total saponin content was expressed in mg/g.

2.4.3. Foaming properties

For foaming properties, a 1.5-g powder sample was mixed with 30 ml of distilled water at 15,000 rpm for 2 min in a 250 ml graduated cylinder, and the foam capacity (FC) and stability (FS) were measured (Koç et al., 2011). V_f, V_i and V_{10}; show the volume value at the final, initial and 10 min later.

$$FC = \frac{v_{f-}v_i}{v_i} * 100$$

$$FS = \frac{v_{10}}{v_i} * 100$$

2.4.4. Emulsifying properties

A mixture was prepared by dissolving 0.3 g of powder in 30 ml of water, to which 10 g of sunflower oil were added. The mixture was then homogenized at 10,000 rpm with a High Shear Homogenizer for 2 min. Then, a fifty μL of emulsion was taken from the bottom part and diluted with 5 mL of 0.1 % sodium dodecyl sulphate (SDS) solution and vortexed for 10 s. Absorbance measurements were taken using a UV spectrophotometer at 500 nm at the initial time and at the 10-min mark. The emulsion activity index and stability index were calculated as follows (Buhl et al., 2019; Cheung et al., 2014).

$$EAI = \frac{2*2.203*A_0*N}{c*\phi*10000}$$

$$ESI = \frac{A_0}{\Delta A} * \Delta t$$

A_0 represents the absorbance value of the diluted emulsion immediately after homogenization, N is the dilution factor, c is the protein weight per volume (g/ml), ϕ is the oil content in the emulsion, ΔA is the absorbance difference between initial and 10th minutes, and Δ*t* represents 10 min.

2.4.5. Amino acid composition

99 mL of 0.1 N HCl was added to 1 g of sample, 1 mL of 5-SSA (5-Sulfosalicylic Acid) solution was added, mixed for 15 min and centrifuged at 4000 rpm for 15 min, and the supernatant was taken. Amino acid analysis was performed by using LC-ESI-MS/MS equipment (Lane, 2015). The LC-ESI-MS/MS analysis was conducted using a Shimadzu HPLC (Kyoto, Japan) system coupled with an AB-SCIEX (Framingham, U.S.A.) triple quadrupole mass spectrometer. The mass spectrometer operated in positive ESI mode. The ion spray voltage was set at 4 kV, with nitrogen used as both the sheath gas (25 psi) and the auxiliary gas (1.0 arbitrary units). The ion transfer capillary was to 300 °C. Injections were performed using an autosampler equipped with a 20-μl sample loop. Multiple Reaction Monitoring (MRM) was employed for the specific quantification of amino acids and the internal standard, with a scan time of 0.4 s. These standard solutions were infused into the HPLC system under the following conditions: HPLC system flow rate of 0.94 ml/min. LC separations were performed using a C18 column at 30 °C. For the elution of amino acids, 0.1 % Formic acid in water (solvent A) and 100 % acetonitril (solvent B) was used in a gradient mode. 2–20 % B over 5 min, 20 % to 100 %B 20 min and 100 % to 2 %B in 5 min (total 30 min).

2.4.6. Rheological analysis

The viscosity of the liquid aquafaba samples was measured by using RST-CC Touch Rheometer (Brookfield Ametek, USA). The test used a coaxial cylinder setup with a CCT-25 spindle and DIN 53019 adapter. Before testing, approximately 17 mL of the sample was placed into the cylindrical adapter. Measurements were conducted at room temperature, starting with shear rates from 0.001 s^{-1} to 1600 s^{-1} in a logarithmic increase. Viscosity measurements were conducted to explain the foaming and emulsifying properties in particular.

2.4.7. Fourier transform infrared spectroscopy

Aquafaba powders were analyzed using Fourier transform infrared (FTIR) spectroscopy with an IR Affinity-1 spectrometer (Shimadzu Corporation, Kyoto, Japan). At room temperature, the spectra were recorded at a resolution of 4 cm^{-1}, covering a range of 600–4000 cm^{-1} at a number of scans of 32. The baseline-normalized spectrum was examined using Shimadzu Corporation's (Kyoto, Japan) LabSolutions IR program.

2.4.8. Statistical analysis

All data from these experiments were subjected to Analysis of Variance (ANOVA) (Minitab Inc., v19, Coventry, UK) to determine if there were significant differences between the samples. A 95 % confidence interval was used for Tukey's post-hoc test to assess pairwise differences. Experiments were conducted in five replicates. Assumptions of ANOVA were checked before analysis and outliers were removed from the dataset if necessary.

Table 2
Multi Reaction Monitoring (MRM) of Soyasaponin I.

Compound	Precursor ion (m/z)	Product ion (m/z)	Dwell time	Collision energy (eV)	Polarity
Soyasaponin I	941.4	112.9	200	70	Negative

3. Results and discussion

3.1. Determination of soluble protein content

Table 3 shows the protein content of powder aquafaba derived from various production methods. Among the non-soaked production techniques, the conventional method (PC) had higher protein content than the microwave methods. Several aspects of microwave heating could explain these results. Microwaves are recognized for their ability to quickly heat food by penetrating it and generating internal heat. Nonetheless, uneven heating might result from variables like the positioning of the microwave or the food's shape (Wäppling-Raaholt & Ohlsson, 2009). Infrared heating, on the other hand, is especially effective at rapid and uniform heat transfer (Aboud et al., 2019) which is likely to have contributed to the breakdown of cell walls and protein denaturation which leads to lower solubility (Krishnamurthy et al., 2008; Yousefi & Abbasi, 2022). MW-C showed lower protein content than MW-IR for both processing techniques (-S and -O). In the production of liquid aquafaba, 100 % power was used in the MW-C method and 50 % power in the microwave-infrared MW-IR method. This adjustment was necessary due to overflow that was observed in the MW-IR process, regardless of the chickpea to water ratio, container size, or other factors. That is why lower microwave power was used however, 100 % of the lower and upper microwave infrared power was set. Although this introduces a difference in microwave power between MW-C and MW-IR, it was not possible to operate MW-IR at higher microwave power levels due to safety limitations such as persistent overflow, even when larger containers and different ratios were tested. Reducing MW-C power to 50 % was also not feasible, as preliminary tests showed that lower microwave intensity resulted in insufficient dry matter yield for spray drying. Moreover, due to inherent differences in the heating mechanisms of MW-C and MW-IR (i.e., IR elements providing continuous external heat), the total thermal input would differ regardless of microwave power settings. Therefore, some degree of power variation was inevitable. Despite this limitation, the comparison still provides valuable information on how different thermal mechanisms influence the functionality of aquafaba powders, consistent with prior work (Divekar et al., 2017; Kerem et al., 2005). In MW-C, starch gelatinization may have occurred more in the chickpeas due to the use of 100 % power during production (Lamich, 2022). This may have prevented the soluble proteins in the chickpeas from coming out. In a similar study conducted with soybeans, boiling soybeans, which are one of the most protein-rich legumes, unexpectedly lowers their protein concentration. This was explained as since the proteins are probably tightly embedded in the starch matrix in the seed inner core and can therefore be released only in very small pieces (Serventi et al., 2018). In addition, this low protein content may be due to the complex structure formed with the saponins found in aquafaba (Alahverdjieva et al., 2008; Li et al., 2023). The resulting complex structure may have caused the soluble protein content to decrease especially in microwave processing which was further discussed in the saponin content section. Although the initial hypothesis proposed that higher protein and saponin content would enhance functionality, the results indicated improved foaming and emulsifying performance even when these components were reduced in microwave-treated samples.

Moreover, it was observed that the protein content of aquafaba with overnight soaking was significantly higher than the non-soaked method ($p < 0.05$). The extended duration might allow for a continuous diffusion of proteins into the aquafaba. This could contribute to the observed increase in protein content as the proteins have more time to migrate from the chickpeas into the liquid phase. From non-soaked to overnight, the increase in protein content is higher in MW-C (%36) than MW-IR (% 23). This suggests that the internal pressure generated by the microwave was higher in MW-C than in MW-IR since in MW-C, 100 % and 50 % power was used for MW-C and MW-IR, respectively. Internal heating and evaporation create pressure gradients more in MW-C, which leads to fissures and fractures on the wall of the chickpea, and this significantly improves solid content transport (Divekar et al., 2017). During overnight soaking, more solid leached out thanks to enlarged pores.

Table 3
Protein and Saponin Content of Aquafaba Powders and Viscosity of Aquafaba (Liquid) Produced by Different Methods.

PROCESS	Protein Content (mg BSA equivalent/g powder)	Saponin Content (mg Saponin/g powder)	Viscosity (Pa.s)
PC-S	2.76 ± 0.04^{c}	29.79 ± 0.20^{a}	0.003 ± 0.001^{d}
Mw-C-S	1.99 ± 0.08^{e}	28.78 ± 0.05^{a}	0.011 ± 0.002^{a}
Mw-IR-S	2.59 ± 0.08^{d}	16.47 ± 0.01^{c}	0.006 ± 0.001^{bc}
PC-O	3.37 ± 0.09^{a}	18.07 ± 0.12^{b}	0.005 ± 0.001^{c}
Mw-C-O	2.70 ± 0.09^{cd}	13.58 ± 0.13^{d}	0.013 ± 0.003^{a}
Mw-IR-O	3.20 ± 0.10^{b}	9.38 ± 0.18^{e}	0.007 ± 0.002^{b}

*Different letters in a column means significant difference ($p < 0.05$).

3.2. Determination of saponin content

Chickpea contains soyasaponin βg and soyasaponin I (Srivastava & Vasishtha, 2013). However, almost all of the DDMP saponins (soyasaponin βg) are hydrolyzed or converted to B saponins (soyasaponin I) during the food processes (Kerem et al., 2005; Serventi et al., 2013) since DDMP is not a very stable structural unit (Barakat et al., 2015). Therefore, saponin content determination of chickpea is a very hard procedure. Saponin content of chickpea has been reported to be in the range of 0.7–0.9 mg/g (Stasiak et al., 2023) whereas one study reported it as 2.3 mg/g (Price et al., 1988). This means either the saponin content of aquafaba changes from cultivar to cultivar or not all saponin could be measured. In this study, the saponin content of aquafaba powders was measured using LC-MS/MS methods. The results were given in Table 3 and the peaks are shown in Fig. 1. The LC-MS/MS results also showed that PC showed higher saponin content when compared to microwave methods (Table 3). Conversely, the use of microwave infrared (MW-IR), particularly with overnight soaking (MW-IR-O), drastically reduced the saponin content. Microwave treatment may alter protein content by decomposing or reducing saponin, likely due to the formation of complex structures with other components of aquafaba, thereby decreasing its availability. Furthermore, in this study, overnight soaking has been shown to decrease the saponin content. Another possible explanation is that a saponin-protein complex may have formed, considering the decrease in both protein and saponin content during microwave treatment (Alahverdjieva et al., 2008; Böttcher et al., 2017; Li et al., 2023; Timilsena et al., 2023; Yan et al., 2022). The interaction between proteins and surfactants, specifically saponins, in solutions is governed by the nature and concentration of the surfactant (Fainerman et al., 2004). With non-ionic surfactants, there exists a competitive adsorption from the onset, leading to the gradual displacement of proteins from the interface as surfactant concentration increases. This continues until the surfactant's behavior resembles that of its pure form, covering the surface mainly with surfactant molecules. Moreover, the dynamics with ionic surfactants like saponins begin with electrostatic interactions due to charged structures at lower concentrations, where saponins bind to proteins, enhancing their hydrophobicity and improving their surface activity, thus potentially increasing the foaming properties of the solution (Alahverdjieva et al., 2008; Saint-Jalmes et al., 2005). As the concentration of saponins increases, hydrophobic interactions become more dominant, making the protein-surfactant complex more hydrophilic and less effective at the air-water interface, ultimately reducing its foaming ability. Beyond certain concentrations, particularly at or near the critical micelle concentration (CMC) of the surfactant, saponin molecules tend to form micelles and dominate the interface, displacing protein complexes and thereby reducing the overall stability and effectiveness of the foam (Alahverdjieva et al., 2008). Especially considering that the overnight soaking reduces the saponin content more significantly ($p <$

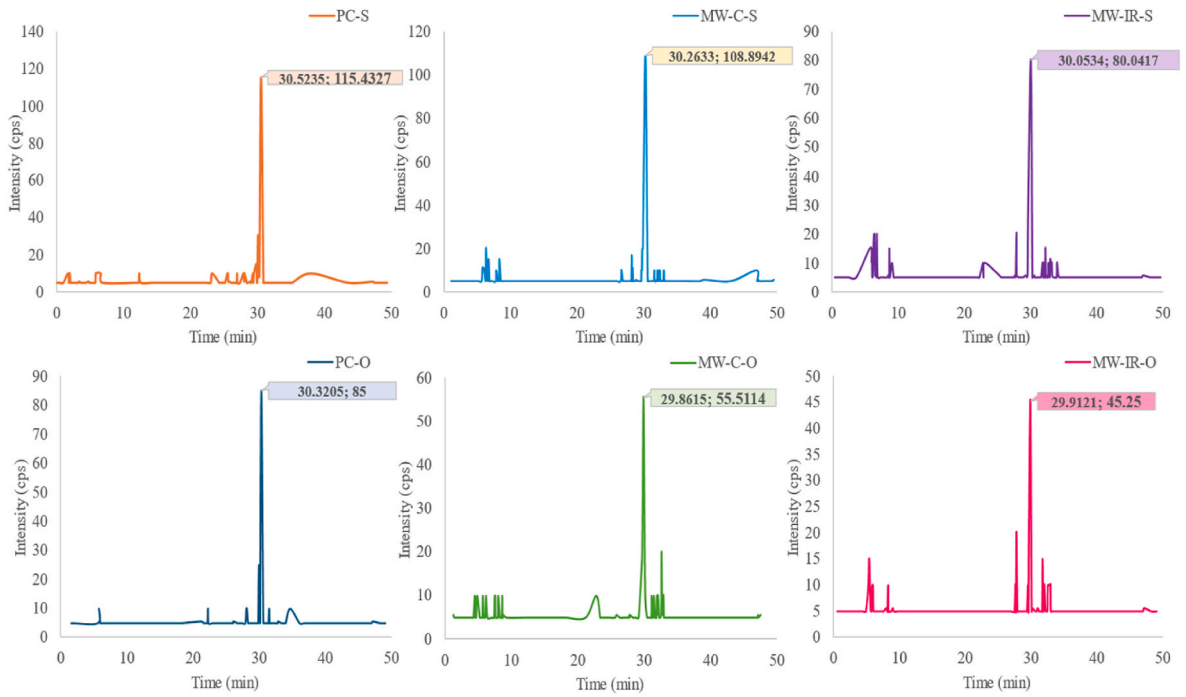


Fig. 1. LC-MS/MS Spectra of Aquafaba Saponins (Soyasaponin I).

0.05), it may indicate that saponins and proteins have more time to form complex structures, which could increase the likelihood of their combination.

As a result, it is hypothesized that the protein and saponin content does not decrease; on the contrary, the potential for complex formation increases due to the release of high amounts of protein and saponin.

3.3. Rheological analysis

Rheological analysis was performed as a complementary experiment to support visual and functional observations related to the viscosity of aquafaba obtained by different processing methods. Although the primary focus of this study was on foaming and emulsifying properties, differences in the perceived consistency among samples prompted an investigation into their flow behavior. The results in Table 3 showed that aquafaba produced using microwave-infrared (MW-IR) treatment exhibited higher viscosity values compared to samples obtained via microwave-only (MW-C) and pressure cooking (PC). This increase in viscosity is likely attributed to more extensive water evaporation and concentration during MW-IR processing, due to the combined internal (microwave) and external (infrared) heating effects. The higher viscosity may also contribute to the improved foam stability observed in MW-IR samples, as viscous continuous phases are known to slow down bubble coalescence and drainage, thereby promoting more stable foams (Datta & Rakesh, 2013). While viscosity was not a central parameter of this study, these findings complement and strengthen the overall interpretation of functional behavior.

3.4. Foaming properties

Table 4 summarizes the foaming ability (FA) and foaming stability (FS) of aquafaba powders produced using various methods and processing techniques. FA measures the capacity of a liquid to create and sustain a foam structure, while FS assesses the ability to maintain the integrity of these foam structures over time. It was observed that both microwave methods (MW-IR and MW-C) resulted in the highest foaming ability among non-soaked methods. Despite the higher protein content of the PC-S sample, its foaming ability was significantly lower, at nearly half that of the MW-C-O sample ($p < 0.05$). Generally, the higher the protein concentration, the higher the foaming properties (Choden et al., 2023). Proteins and saponins are essential for both foam formation and stability because they interact with the air-water interface and stabilize the bubble structure (Echeverria-jaramillo et al., 2021; Stasiak et al., 2023). Additionally, a high protein concentration in the medium can cause steric repulsion and larger aggregate sizes, which can eventually lead to the degradation of the surface protein layer (Bals & Kulozik, 2003; Dissanayake & Vasiljevic, 2009). If the proteins at the interface do not form a strong network or film that effectively holds the bubbles together, the foam can easily collapse over time. Proteins can interact with each other at the interface, either forming networks that stabilize the foam or aggregating in ways that weaken the foam structure (Damodaran, 1990). A similar result was also obtained for the overnight soaking methods. Although PC and MW-IR had higher protein content than MW-C, the foaming ability of PC was lower than MW-C and MW-IR and MW-C had similar foaming ability. In addition to this, the viscosity

Table 4
Emulsification and Foaming Properties of Aquafaba Powders Produced by Different Methods.

PROCESS	EAI	ESI	FA	FS
PC-S	2067.89 ± 144.58^{b}	23.29 ± 1.16^{b}	96.67 ± 6.09^{d}	124.88 ± 1.63^{d}
Mw-C-S	1696.18 ± 140.65^{c}	43.67 ± 0.56^{a}	130.00 ± 3.65^{c}	175.50 ± 1.45^{b}
Mw-IR-S	2380.96 ± 99.81^{a}	14.76 ± 0.37^{d}	123.33 ± 2.36^{c}	181.33 ± 0.33^{a}
PC-O	2447.73 ± 168.65^{a}	19.90 ± 0.78^{c}	137.78 ± 2.72^{b}	102.40 ± 1.68^{e}
Mw-C-O	2402.44 ± 97.39^{a}	19.15 ± 1.03^{c}	228.33 ± 5.27^{a}	162.17 ± 0.73^{c}
Mw-IR-O	1786.40 ± 66.00^{c}	14.33 ± 0.29^{d}	144.44 ± 7.70^{b}	168.64 ± 1.16^{c}

*Different letters in a column means significant difference ($p < 0.05$).

of the microwave treated ones (liquid) could have increased much more than the conventional one due to the extensive evaporation that occurred in the microwave treated ones (Datta & Rakesh, 2013). While higher viscosity generally helps in foam stability, moderate viscosity is crucial for foam ability. This guarantees that the fluid is both viscous enough to generate stable and smaller bubbles when whipped and still be able to flow freely enough to capture air. Under the right conditions, proteins and saponins work together to improve this equilibrium, which makes them useful for better foam ability while providing foam stability at the same time. As a result, this high viscosity of the aquafaba may lead to higher foaming ability of the system (X. X. Hu & Meng, 2024; Rodriguez Patino & Pilosof, 2011).

Furthermore, for the foam stability, the MW-IR method consistently demonstrated superior foaming stability compared to the other methods. The conventional production method (PC) yielded relatively lower foaming stability compared to the microwave methods for both processing techniques (-S and -O), although it has a high content of saponin and protein. Protein flexibility, concentration and charge are affected by pH, and these factors can significantly affect the foaming properties of proteins (Crawford et al., 2023). Previous studies have shown that foam stability increases at pH levels close to the isoelectric point, where the net charge of proteins is zero and electrostatic forces are minimal (Crawford et al., 2023; Hammershøj et al., 2004). In this pH range, proteins coming closer increase hydrophobic interactions and hydrogen bonds. This stabilizes the foam by creating a strong interfacial film. Around the isoelectric point, the solubility of protein decreases. This is due to a thin protein layer being allowed to adsorb due to minimal electrostatic repulsion at the air-water interface, which stabilizes the foam (Amagliani et al., 2021; Narsimhan & Xiang, 2018). Since protein content measurement in this study was mostly related to soluble protein content, the solubility of protein might have been decreased for MW-C and MW-IR which leads to greater foam stability.

Another reason for the high foaming and emulsifying properties despite the low amount of protein and saponins may be the complex structure formed between proteins and saponins as mentioned earlier in the saponin part. In a complex system where saponins and proteins are together, they could enhance the foaming performance of the system (Li et al., 2023; Saint-Jalmes et al., 2005; Wilde et al., 2004). Saponins are good at reducing surface tension, essential for creating and stabilizing foam. When they interact with proteins, the stability of the foam is improved. This happens because proteins are able to form strong protective layers around the foam's air bubbles. These layers can become thicker and more complex due to the interaction. Proteins adjust their structure to create a network at the surface that holds saponin molecules, reinforcing the bubble walls and making them more resistant to combining or breaking. While the improved functionality may be due to the formation of protein–saponin complexes, this remains a hypothesis based on existing literature. Future work should include structural or interfacial analyses to confirm the presence and behavior of such complexes.

3.5. Emulsification properties

The emulsifying activity index (EAI) and emulsifying stability index (ESI) were assessed for aquafaba samples subjected to different methods and processing techniques. Emulsifying activity index (EAI) is a common parameter that measures how well proteins can stabilize an emulsion (a mixture of oil and water). Generally, EAI is based on the measurement of the total surface area of droplets in a diluted emulsion stabilized by a certain amount of protein (Tang, 2017). Emulsifying Stability Index (ESI) generally measures the stability of an emulsion over a constant period of time (Cheung et al., 2014). This measurement is based on factors such as the size and arrangement of droplets in the emulsion. The ability of a protein to effectively stabilize an emulsion depends not only on its surface properties but also on the flexibility of its molecular structure, and this molecular flexibility is an important factor for emulsion stability (Sharif et al., 2018). A higher ESI may indicate that the emulsion has better stability, meaning separation between the oil and water phases is less likely. It is known that when an emulsion is formed, proteins reduce the interfacial tension between oil and water due to the hydrophobic and hydrophilic parts (Tang, 2017). Proteins in aquafaba powder gather at the interface between water and oil, creating a flexible molecular film that sufficiently stabilizes the emulsion in this region (Włodarczyk et al., 2022).

The results showed that ESI values of the samples with low saponin content were also low (Table 4). In this case, it can be concluded that generally the amount of saponin might be related to the ESI value, though it is not the only factor influencing ESI. Whereas, it has been observed that samples with lower protein content also have lower EAI values, suggesting that the EAI value is more related to the amount of protein. PC-S exhibited strong emulsifying abilities due to a higher amount of saponin and protein that can effectively lower the surface tension between the oil and water phases, leading to a more stable emulsion (Yan et al., 2022). Moreover, the overnight soaking consistently resulted in higher EAI values compared to non-soaked processing techniques (-S) suggesting that prolonging the interaction between chickpeas and water enhances emulsifying activity except for the MW-IR sample which might be most probably due to low saponin content. On the other hand, the emulsifying stability index (ESI) presented a different picture. While MW-C-S demonstrated the highest ESI, MW-IR-O and MW-C-O displayed lower ESI values despite their significant EAI levels. For MW-IR-O, although protein content is high, EAI and ESI are lower compared to other processing methods. At low protein amounts, protein adhesion at the oil/water interface is controlled by diffusion. In cases where there is less protein, it moves faster and facilitates the formation of new droplets, resulting in greater EAI. However, at high protein amounts, the movement of protein becomes difficult due to diffusion. More protein can create an activation-energy barrier that prevents the protein from sticking to the oil/water interface. In this case, the emulsion activity of the protein decreases (Guo & Mu, 2011). In addition, complex polysaccharide presence also affects the emulsion stability of proteins (Fuentes Choya et al., 2023). High molecular weight fibers and cell wall components prevented the formation of a strong protein layer which reduced the foam capacity of the samples by sterically hindering the protein–protein interaction, electrostatically interacting with proteins (Lam & Nickerson, 2013). Also, complex polysaccharides prevent emulsification both through molecular entanglement by preventing denatured proteins from adhering to the interface, and by competing with proteins at the interface (Fuentes Choya et al., 2023).

3.6. Amino acid composition

Amino acids are the building blocks of proteins and are necessary for the body to maintain its normal functions. Therefore, an adequate and balanced diet is very important to ensure that amino acids are taken in sufficient amounts. Essential amino acids are amino acids that cannot be synthesized by the body and therefore must be taken with food. The nine amino acids that are essential for the human body are lysine, histidine, leucine, isoleucine, valine, threonine, methionine, phenylalanine and tryptophan. Chickpeas contain many essential amino acids such as lysine, histidine, leucine, isoleucine, valine, threonine, methionine, phenylalanine and tryptophan; however, the amount of sulfur-containing amino acids such as methionine and cystine is lower. The chickpea protein isolates predominantly contain glutamic and aspartic acids, with leucine, lysine, and phenylalanine as key essential amino acids (Onder et al., 2022).

The amino acid composition influences the functional properties of proteins by determining the ratio of hydrophilic to hydrophobic groups which affects the protein's surface hydrophobicity and electrostatic interactions (Soto-Madrid et al., 2023). Globular proteins play a key role in stabilizing air/water interfaces in foams and oil/water interfaces in

emulsions, largely due to the fundamental contribution of hydrophobic amino acids (Martínez-Lapuente et al., 2015; Sagis & Yang, 2022). The amino acids with hydrophobic side chains include glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine and tryptophan (Vnučec et al., 2017). The hydrophobic properties of proteins, which are linked to foaming and emulsification, are due to the proportion of amino acids that have nonpolar side chains (Siebert, 2003).

In this study, amino acid profiles obtained for each production technique were examined (Table 5). It was observed that different cooking and processing methods had significant effects on the composition of aquafaba powders ($p < 0.05$). The amino acid contents of overnight samples were found to be higher than non-soaked samples. Also, it was found that the amino acid amount of microwave samples (MW-C and MW-IR) were higher than the PC sample. Methionine and cysteine amino acids were not detected. Approximately 30 % of the total amino acid content in the samples consisted of the five essential amino acids found in aquafaba—leucine, lysine, phenylalanine, threonine, and histidine—with the highest percentage observed in PCO sample (Aider et al., 2012). As it was mentioned earlier, aspartic and glutamic acid are the main contributors to the chickpea protein isolate amino acid content, in this study, aquafaba powders contain mainly arginine, lysine and alanine. Although glutamic acid was found in powders, it was not the one of the main amino acids. The overall quality of MWCS and MWIRS samples was somewhat considered as reduced due to their lower lysine content compared to the other. MWCS and MWIRS samples had significantly less of compared to samples especially PCO and PCS, which have higher levels of lysine ($p < 0.05$). Lysine is an important essential amino acid which is vital for protein synthesis, tissue repair, and the production of hormones, enzymes, and antibodies (Hall & Soares da Costa, 2018; Matthews, 2020). It also aids in the absorption of calcium, supporting bone health, and plays a key role in the production of collagen, which is important for healthy skin, tendons, and cartilage (Aggarwal & Bains, 2022).

In the analysis of all aquafaba samples, a correlation was observed between the foaming properties and the presence of hydrophobic amino acids, particularly alanine, leucine, and phenylalanine (Martínez-Lapuente et al., 2015; Olatunde et al., 2024). MWCO sample, in particular, shows the highest foaming capacity, which aligns with its highest levels of leucine and proline content. Similarly, the alanine and leucine contents were lower in PCS and MWIRS powders, and the foaming properties of these samples were also reduced accordingly.

Although microwave treatments improved functional properties, the observed reduction in lysine content, an essential amino acid, may be considered a nutritional drawback and should be considered in applications where protein quality is a key factor.

3.7. Fourier transform infrared spectroscopy

FTIR spectra of aquafaba powders produced with different methods can be seen in Fig. 2. The first peak that is seen is in the 1000–1100 cm^{-1} region, which is a characteristic peak for polysaccharides and shows that the C=O or C–C stretching modes have been coupled (Tas et al., 2022). PC-S and MW-C-O showed the highest intensity means higher content of polysaccharides. Moreover, Amide I and Amide II bands observed in the FTIR spectrum of proteins are important indicators used to analyse changes in the secondary structures of proteins. The amide I band is located between approximately 1600-1700 cm^{-1} and represents the C=O stretching vibration in the amide group of proteins (Parker, 1971). This band may reflect changes in the secondary structure of proteins. In particular, changes in C=O stretching vibration and band peak heights are used in the analysis of secondary structures of proteins (α-helix, β-sheet) which could be an indicator of protein quantity by reflecting the density of the amide group within the proteins. MW-IR samples for both processing techniques (-S and -O) showed lowest intensity which could mean that density of amide groups is the lowest. Although soluble protein content of MW-IR-S and MW-IR-O is high, total protein content might be limited. The amide II band is located between approximately 1500 cm^{-1} and contains the N-H and C-N stretching vibrations in the amide group of proteins (Parker, 1971). The changes in the amide II band can be used to observe structural changes in protein samples (Tas et al., 2021). Amide A and Amide B bands observed in the spectrum of proteins express certain vibrational modes resulting from hydrogen bonds of water molecules. Changes in the FTIR spectra of Amide A provide valuable information about the water absorption and hydration state of protein samples, which is important for understanding how proteins respond under various environmental conditions. Shifts in the Amide A band indicate changes in the interactions between hydrogen bonds inside a molecule (Ertugrul et al., 2021). Amide A is located at a wave number of approximately 3300 cm^{-1} and reflects the interaction of N-H (nitrogen-hydrogen) groups, usually found within proteins, with water molecules. This mode of vibration is highly responsive to the strength of a hydrogen bond but is not dependent on the backbone conformation (Krimm & Bandekar, 1986). This band shows a higher intensity in PC samples, which may indicate stronger hydrogen bond formation and increased water hydration rate of proteins obtained by this production method. On the other hand, the Amide B band is located at a wave number of approximately 3100 cm^{-1} and again indicates the interaction of N-H groups in proteins with water molecules.

3.7.1. Limitations of the study

This study has several limitations that should be acknowledged. First, due to equipment constraints, the microwave power levels could not be standardized between MW-C and MW-IR treatments, which may affect comparability. Second, while improved functional properties were observed, the proposed mechanism of protein–saponin complex formation remains hypothetical and was not directly confirmed. The observed reduction in lysine content also raises potential concerns regarding nutritional quality. Finally, while the findings are promising for industrial applications, scalability and cost-effectiveness of these

Table 5
Amino Acid Profile of Aquafaba Powders Produced by Different Methods.

mg/gr-powder	PCS	MWCS	MWIRS	PCO	MWCO	MWIRO
Alanine	127.75 ± 0.53^{f}	184.64 ± 0.45^{d}	328.58 ± 0.41^{b}	173.93 ± 0.66^{e}	205.92 ± 0.65^{c}	369.20 ± 0.14^{a}
Arginine	203.50 ± 0.35^{e}	190.90 ± 0.64^{f}	210.99 ± 0.70^{d}	244.72 ± 0.51^{a}	226.78 ± 0.55^{c}	229.77 ± 0.54^{b}
Glutamic Acid	9.45 ± 0.32^{f}	68.54 ± 0.38^{d}	87.02 ± 0.01^{b}	61.04 ± 0.03^{e}	109.69 ± 0.49^{a}	79.76 ± 0.54^{c}
Histidine	14.68 ± 0.23^{b}	11.18 ± 0.13^{d}	18.61 ± 0.28^{a}	14.13 ± 0.09^{c}	7.47 ± 0.16^{e}	15.24 ± 0.17^{b}
Leucine	8.14 ± 0.10^{d}	8.64 ± 0.45^{d}	10.36 ± 0.25^{c}	9.32 ± 0.09^{c}	12.93 ± 0.05^{a}	11.33 ± 0.23^{b}
Lysine	166.21 ± 0.15^{b}	115.35 ± 0.25^{f}	140.66 ± 0.46^{e}	172.66 ± 0.24^{a}	149.21 ± 0.15^{d}	157.86 ± 0.10^{c}
Phenylalanine	21.62 ± 0.27^{a}	15.77 ± 0.55^{c}	20.57 ± 0.40^{b}	22.69 ± 0.22^{a}	19.22 ± 0.15^{b}	23.09 ± 0.07^{a}
Proline	7.85 ± 0.11^{b}	5.69 ± 0.49^{d}	5.95 ± 0.04^{cd}	3.85 ± 0.11^{e}	13.95 ± 0.03^{a}	6.56 ± 0.31^{c}
Serine	12.45 ± 0.32^{c}	11.34 ± 0.24^{d}	14.03 ± 0.02^{b}	10.36 ± 0.25^{e}	12.90 ± 0.07^{c}	16.11 ± 0.08^{a}
Threonine	37.41 ± 0.29^{e}	41.90 ± 0.64^{c}	44.92 ± 0.06^{b}	39.79 ± 0.56^{d}	45.35 ± 0.25^{b}	51.30 ± 0.21^{a}
Tyrosine	15.70 ± 0.21^{a}	10.81 ± 0.57^{c}	13.50 ± 0.36^{b}	15.45 ± 0.32^{a}	12.21 ± 0.15^{b}	14.43 ± 0.41^{a}

*Different letters in a column means significant difference ($p < 0.05$).

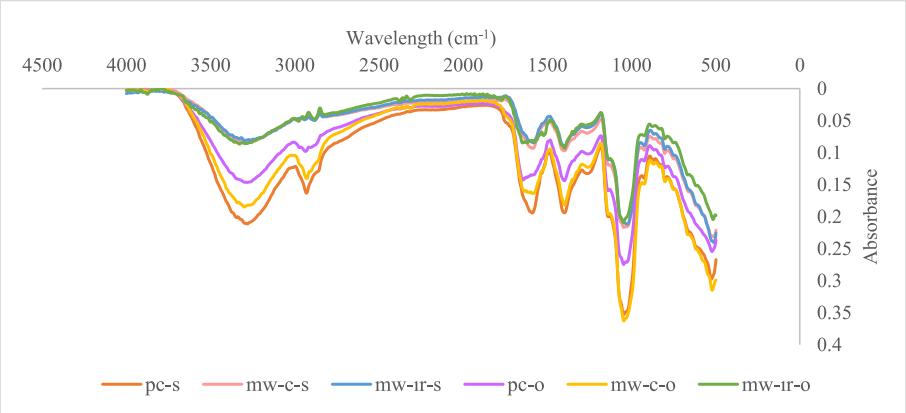


Fig. 2. FTIR Spectra of Aquafaba Powders.

microwave-assisted methods were not assessed.

4. Conclusion

Microwave-assisted methods were found to significantly enhance the functional properties of aquafaba powders, with superior foaming capacity and stability compared to conventional pressure cooking. Despite lower protein and saponin concentrations, the microwave-treated samples showed improved functionality, attributed to enhanced protein-saponin interactions that optimized foaming and emulsifying performance. The overnight soaking step further contributed to these improvements by increasing the extraction of soluble proteins and improving overall functionality. The results demonstrated the potential of microwave-assisted methods as innovative and efficient alternatives for producing aquafaba with enhanced properties, offering promising applications in plant-based and egg-free food formulations. Although the treatment time was fixed at 50 min in this study, microwave methods are considered more energy-efficient due to their rapid volumetric heating and lower overall energy input. In industrial applications, microwave-based processes also offer potential for faster heating and reduced total processing time under optimized conditions. Future work should aim to confirm the role of protein–saponin complex formation in functional performance, nutritional quality of aquafaba powders and evaluate the scalability of these methods for industrial application.

CRediT authorship contribution statement

Elif Gokcen Ates: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gokcem Tonyali Karsli:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Ozcan Dilara Ozcan:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Emin Burcin Ozvural:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition. **Mecit H. Oztop:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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