**Innovative Processing Techniques Unveil the Potential of Chickpea Aquafaba**

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| **Abstract** Chickpea aquafaba, the residual liquid from cooked chickpeas, stands out as a noteworthy player in the dynamic landscape of plant-based ingredients, captivating the food science community with its distinct attributes. This study delves deeply into the physicochemical properties of chickpea aquafaba and its powder counterpart, highlighting the protein-rich profile. Chickpea aquafaba inherits these nutritional components, boasting 1-1.5% protein and 3.5% carbohydrates by weight, and distinguishing itself with exceptional foaming ability derived from a unique composition rich in soluble proteins, oligosaccharides, saponins, and starches. Aquafaba's foaming ability distinguishes it as a sought-after ingredient for vegan and egg-free recipes, excelling in achieving desirable textures. The investigation extends into cutting-edge technologies, with a focus on the transformative impact of microwave vacuum technology and custom-designed microwave equipment on aquafaba. These methods not only fine-tune critical parameters for optimized protein content and foaming ability, but they also improve protein extraction precision. The study delves deeper into spray-dried chickpea aquafaba, presenting a concentrated and shelf-stable powder that expands the benefits of this plant-based elixir. Chickpeas, aquafaba, advanced processing technologies, and custom-designed equipment have all come together to usher in a new era of plant-based innovation. The study emphasizes microwave vacuum technology which is a method that combines the advantages of microwave and vacuum drying and its superior performance, consistently yielding higher protein content and desirable properties in both liquid and powder forms of aquafaba. In the ever-changing landscape of food science, this comprehensive examination of composition, foaming ability, and advanced processing illuminates the path toward realizing the full potential of chickpea aquafaba. |
| Keywords: Chickpea, Aquafaba, Microwave-Vacuum, Spray Drying, Powder |

1. **Introduction**

In the ever-evolving landscape of plant-based ingredients, chickpea aquafaba, the liquid residue derived from cooked chickpeas, has emerged as a valuable plant-based ingredient, captivating the food science world with its unique attributes. To comprehend the full scope of its potential, it is imperative to delve into the proximate composition of both chickpeas and their liquid counterpart. Chickpeas, scientifically known as *Cicer arietinum*, boast a nutrient-rich profile, serving as an excellent source of protein, dietary fiber, vitamins, and minerals. The proximate composition of chickpeas includes approximately 19-25% protein, making them a substantial plant-based protein option [1]. Furthermore, chickpeas contain essential amino acids, particularly lysine, which is often limited in grains. Albumin and globulins are the most abundant proteins, accounting for 8%-12% and 53%-60% of total protein content, respectively[2]. The transformation of chickpeas into aquafaba involves the infusion of water with these nutritional components. Aquafaba inherits the protein content of chickpeas, typically containing around 1-1.5% protein and approximately 3.5% carbohydrates by weight, rendering it a valuable alternative for individuals seeking plant-based protein sources [3,4]. What sets aquafaba apart is this remarkable foaming ability. This might seem modest, but its real magic lies in its unique composition, rich in soluble proteins, oligosaccharides, saponins and starches, which confer remarkable foaming and emulsifying properties [5,6]. The foaming ability of aquafaba makes it a sought-after ingredient for vegan and egg-free recipes, where aeration and stabilization are crucial for achieving desirable textures. From vegan meringues and macarons to plant-based mousses and mayonnaise, aquafaba stands out as a versatile ingredient capable of creating light and airy textures traditionally associated with egg-based formulations. In the quest to enhance the production and quality of chickpea aquafaba, innovative technologies have taken center stage. Microwave vacuum technology, a marriage of precision and efficiency, has become a transformative force in the extraction and preservation of the liquid's unique properties. This advanced method allows for fine-tuning of critical parameters like temperature, power and exposure time, ensuring an optimized protein content and foaming ability in the final aquafaba product. Microwave vacuum drying is a drying method that combines the advantages of microwave and vacuum drying. The microwave energy aids in the evaporation of the aquafaba's water content, while the vacuum atmosphere accelerates the drying process by lowering the boiling point of water [7]. This combination might result in a more effective and rapid aquafaba production than conventional techniques [8]. Microwave vacuum drying has been shown in studies to retain the nutritional value of heat-sensitive components and active substances in food items [9,10]. Moreover, aside from microwave vacuum technology, custom-designed microwave equipment is also taking a next step for production of aquafaba. Crafted specifically for pasteurization of food products, these specialized units offer unparalleled precision in the application of microwave energy for production of aquafaba from chickpea. The bespoke design allows for meticulous control over the cooking process, further enhancing the extraction of proteins and other essential components. Beyond the liquid form, the exploration extends to the realm of spray-dried chickpea aquafaba, a concentrated and shelf-stable iteration of this plant-based elixir. The spray-drying process encapsulates the magic of aquafaba in a versatile powder. Whether rehydrated or employed as a convenient ingredient in food, the spray-dried form brings the goodness of chickpea aquafaba to new horizons. As embarked on these excellent properties, the convergence of chickpeas, aquafaba, microwave vacuum technology, and custom-designed microwave equipment unveils a new era in plant-based innovation. Through a detailed examination of proximate composition, foaming ability, and advanced processing methods, it is illuminated the path toward unleashing the full potential of chickpea aquafaba in the food science landscape.

This paper explores the diverse attributes of chickpea aquafaba in the context of plant-based ingredients. It investigates the nutritional composition, with a focus on protein and highlights aquafaba's unique foaming ability. The study delves into innovative technologies, particularly microwave vacuum and custom-designed microwave equipment, as transformative tools in aquafaba extraction. Additionally, the paper explores spray-dried chickpea aquafaba, emphasizing advanced processing methods to enhance production and quality. The overarching goal is to contribute valuable insights to plant-based innovation, showcasing aquafaba's potential applications in food science.

1. **Materials and Methods**
	1. Production of Aquafaba

With minor modifications, aquafaba was prepared as follows [11]. The chickpeas were washed first, and any excess water was discarded. The washed chickpeas were then soaked in distilled water for 16 hours at 4°C with a chickpea to water ratio of 1:3 (w/w) [12]. Following that, two different methods were used to increase the solid content of aquafaba namely microwave vacuum (MW-V) and custom designed microwave (MW-P).

*Microwave-Vacuum*

Chickpeas were cooked in a microwave-vacuum (MW-V) oven at a chickpea to water ratio of (1:2) (IF-TECH, Ankara, Turkey) for 50 minutes at 20 kPa vacuum pressure. Chickpea to water ratio, power and time were determined with preliminary experiments.

*Custom Designed Microwave*

Chickpeas were cooked in a microwave (MW-P) oven at a chickpea to water ratio of (1:2) (IF-TECH, Ankara, Turkey) for 50 minutes at 90% power. The unique fature of this equipment is where the magnetron stands. Chickpea to water ratio, power and time were determined with preliminary experiments

After employing two microwave processing techniques, the resulting aquafaba underwent two distinct treatments before the drying process. In the initial one, the aquafaba was promptly drained and separated from the chickpeas denoted as Standard Production (“SP”). In the second method, the chickpeas and water were left undisturbed overnight at 4℃, followed by filtration after a 24-hour period denoted as Overnight Production (“OP”).

* 1. Characterization of Aquafaba
		1. *Solid Content Analysis*

The liquid samples' dry matter content was determined by placing them in an oven set at 105 °C for a duration of 3 hours. The process of measuring dry matter persisted until the variance between consecutive measurements fell below 0.5% [13].

* + 1. *Determination of Water and Oil Holding Capacities (WHC/OHC)*

The assessment of water holding capacity (WHC) and oil holding capacity (OHC) was conducted on powder samples. For WHC, 0.1 gram of aquafaba powder was combined with 10 mL of distilled water. On the other hand, for oil holding capacity (OHC), 0.1 gram of the sample was mixed with 10 mL of sunflower oil [14]. Subsequently, these mixtures underwent centrifugation at 1960 x g for a duration of 30 minutes. Following the centrifugation process, the resulting supernatants were decanted, and the centrifuge tubes housing the sediment were weighed.



W0 represents the weight of the dry sample, W1 is the combined weight of the centrifuge tube and dry sample, and W2 signifies the weight of the centrifuge tube and the sediment.

* + 1. *Soluble Protein Content*

The soluble protein content was assessed employing the Lowry method [15,16]. 0.5 ml of the sample was combined with 2.5 ml of Lowry-ACR reagent in a tube, followed by a 10-minute incubation. Subsequently, 0.25 ml of Folin reagent (1/2 diluted) was introduced, and the mixture was thoroughly blended using a vortex. This concoction was then shielded from light and underwent a 30-minute incubation period. After completion, the absorbance at 750 nm was gauged using a spectrophotometer (Optizen Pop Nano Bio, Mecasys Co., Ltd., Daejeon, Korea). For calibration purposes, a curve was established utilizing a BSA (Bovine Serum Albumin) solution prepared at varying concentrations (0.03-10 g/L).

* + 1. *Foaming Ability and Stability*

Foaming ability was determined for both liquid and powder aquafaba [17].In this context, a powder sample weighing 1.5 grams was blended with 30 ml of distilled water at a speed of 9,000 rpm for a duration of 5 minutes in a 100 ml graduated cylinder. For the liquid aquafaba, 100 ml of liquid aquafaba was blended for 10 minutes at a speed of 14000 rpm using a 250 ml graduated cylinder.The volumes were recorded at the initial stage, after 10 minutes and after 30 minutes. The foam capacity (FC) and stability (FS) were quantified as follows:

$FC=\frac{v\_{f-}v\_{i}}{v\_{i}}\*100$ $FS=\frac{v\_{10}}{v\_{i}}\*100$

The volume values at the 30 minutes (Vf), first minute (Vi), and 10 minutes later (V10) were recorded as indicators of the foaming characteristics.

1. **Results and Discussion**
	1. *Solid Content Analysis*

The dry matter content findings show notable differences amongst the samples. The production methods have resulted in varying levels of dry matter content in aquafaba (Table 1). The distinct processing techniques employed have led to differing dry matter contents, attributed to the unique mechanisms inherent in each method. MW-P yielded lower dry matter content than MW-V. This means that special design of microwave did not work as expected.

The effect of overnight soaking was also examined. It was observed that overnight soaking significantly increased the dry matter of aquafaba for all methods (p<0.05). The change in the dry matter content was greater for MW-V produced aquafaba (2.74% 🡪 6.18%). This was attributed to the fact that higher amount of solid leached out due to the opening of the pores under the vacuum effect. Microwaves penetrate into food and cause the internal temperature to rise. High temperature generates water vapor inside the food and results in internal pressure gradient [18,19]. The internal pressure gradient is assumed to be higher in vacuum processes. Although the pores expanded during the process, time could have been insufficient for the dry matter to diffuse to the liquid phase. Hence, soaking overnight enhanced the dry matter transfer to a greater extent.

* 1. *Determination of Water and Oil Holding Capacities (WHC/OHC)*

WHC and OHC results of two different techniques were given in Table 2. Overnight storage was found to decrease WHC of MW-V. The decrease observed in microwave vacuum produced aquafaba is explained by the fact that the vacuum process expanded the pores more. With the opening of the pores, while dry matter transfer increased, the transfer of higher molecular weight constituents such as starch and insoluble fiber which can decrease WHC could also have taken place more. It is known that chickpea contains higher insoluble fiber content than soluble fiber [2]. Because insoluble fibers and starch are weak water binders, they might have decreased WHC [20,21]. The most known chickpea proteins are albumin and globulin which are water soluble and salt soluble fractions, respectively. With the opening of the pores, globulin transfer which is the larger and insoluble protein fraction may have enhanced [22]. It has been also reported that as temperature increased, less amount of starch leaches out to the solution where boiling occurs. This is explained by the rupturing of granules due to the local temperature rise around each granule. Increase in temperature results in increase in local viscosity which restricts the mobility and prevents more leaching [23]. In addition to expanding of the pores, considering that the vacuum microwave operates at lower temperatures (~70℃), more starch transfer is likely to occur. Overnight soaking had a significant increase in MW-V sample (p<0.05). It was observed that overnight soaking had the opposite effect on WHC and OHC. It is possible to state that WHC and OHC work in opposite directions for aquafaba powders. This is expected since a decrease in WHC might mean a decrease in polar groups, while an increase in OHC might mean a decrease in these polar groups [24,25].

* 1. *Soluble Protein Content*

This study assessed the protein content of liquid (Table 1) and powder (Table 2) aquafaba derived from various production methods. For both, MW-V sample showed higher protein content than MW-P sample for both standard and overnight production. The application of vacuum treatment in conjunction with microwave heating contributes to enhancing the protein extraction process. Vacuum conditions could play a role in the enlargement of pores within the cellular structure of plant materials. This phenomenon is often referred to as "vacuum impregnation" or "vacuum infusion," and it can contribute to enhanced mass transfer of solutes, including proteins, from the interior of the cells to the surrounding liquid [26]. When vacuum is applied, the pressure is reduced, causing air and gases within the cellular structure to expand and escape. This can lead to the enlargement of cell pores and intercellular spaces. As a result, when the plant material is immersed in a liquid medium, such as water or an aqueous solution, the enlarged pores can facilitate the movement of solutes, including proteins, from the plant cells into the liquid. Vacuum conditions could promote the solubility of proteins in the aquafaba. It is known that vacuum conditions can alter the physical properties of substances by reducing the pressure and altering the intermolecular forces which eventually leading to increased solubility of proteins. This might be caused by the dissociation of the quaternary structure and depolymerization of the protein aggregates at a moderate power and vacuum level [27]. Also, while equilibrium between protein release and reabsorption occurs in all production methods, the microwave vacuum technique, characterized by its application of microwave heating and reduced pressure, offers distinct advantages. The vacuum environment in MW-V leads to lower boiling points and enhanced moisture evaporation, contributing to controlled and relatively lower temperatures within the sample. This environment, coupled with reduced oxygen exposure, potentially minimizes protein denaturation and degradation, resulting in stable protein content between immediate and overnight separations. In the MW-V-S samples, where immediate separation takes place, the protein content remains relatively stable. This stability can be attributed to the combined effects of microwave heating under reduced pressure. The vacuum conditions in MW-V-S likely contribute to the preservation of protein integrity by lowering the boiling point of liquids, leading to controlled moisture evaporation at lower temperatures. This mitigates the risk of excessive denaturation or degradation of proteins during the heating process. Intriguingly, even with overnight processing (MW-V-O), the protein content maintains its stability. The vacuum environment continues to play a crucial role in maintaining protein structure, preventing oxidation, and overall enhancing protein content stability. This consistency between immediate and extended separation underlines the potential of MW-V as a method capable of efficiently preserving and extracting proteins from chickpea aquafaba, making it a valuable option for applications demanding stable protein content.

* 1. *Foaming Ability and Stability*

The performance of four different samples (Mw-V-S, Mw-V-O, MW-P-S, and MW-P-O) reveals interesting differences in their foaming ability and foaming stability. Out of all the powder samples, Mw-V-S has the highest foaming ability, suggesting a tendency to create foam easily. Despite having a reduced foaming stability, the combination points to a dynamic interaction between adequate stability and rapid foam formation. Mw-V-O, on the other hand, has a lesser foaming ability but a greater foaming stability, indicating a more durable foam structure once created. The samples with moderate foaming abilities, MW-P-S and MW-P-O, suggest that there may have been a compromise in the initial creation of foam. However, their foaming stability values are on par with or even greater than the samples with higher foaming abilities. As mentioned above, the microwave-vacuum (MW-V) method resulted in relatively good foaming stability, implying that the vacuum treatment might contribute to maintaining the structural integrity of the foam. Pulse proteins could be a major factor contributing to the discrepancy between high foaming ability and low foaming stability. Proteins play a crucial role in both foam formation and stability due to their ability to interact with the air-water interface and stabilize the bubble structure. 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. Also, 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.

1. **Conclusion**

The solid content analysis in this extensive investigation showed notable variations between aquafaba samples made using various techniques. The dry matter content of the microwave-vacuum (MW-V) methodology was found to be higher than that of the microwave-only (MW-P) method, indicating that the intended outcome of the microwave's particular design may not have been achieved. All techniques of aquafaba showed a considerable increase in dry matter after overnight soaking; however, MW-V showed the largest increase, which was attributed to pore opening under vacuum. Production techniques and overnight soaking have an impact on the water and oil holding capacities (WHC/OHC). Because of its larger pores and the transfer of components with higher molecular weights, MW-V showed a lower WHC. The results of the soluble protein content study demonstrated that MW-V consistently had a greater protein content than MW-P, highlighting the contribution of vacuum conditions. Additionally, even after processing for an entire night, the protein content of the microwave vacuum approach remained consistent, suggesting that it has the ability to effectively preserve protein. Evaluations of the samples' foaming ability and stability revealed subtle variations, with MW-V-S exhibiting a strong foaming ability and respectable stability. The integrity of the foam structure in MW-V was probably preserved in part by the vacuum treatment. Overall, our results highlight the complex interactions between manufacturing processes and aquafaba composition and qualities, providing important information for maximizing its use in a variety of sectors, including the food industry. The complex molecular and structural dynamics underlying these reported effects could be the subject of future investigation.

Table 1. Characterization of liquid aquafaba

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample ID | Dry Matter (%) | Protein Content (mgBSA/L) | Foaming Ability | Foaming Stability |
| Mw-V-S | 2.71±0.11c | 5.25±0.21a | 222.00±1.51b | 97.03±1.20b |
| Mw-V-O | 6.18±0.04a | 5.18±0.16a | 262.00±6.71a | 106.73±0.83b |
| MW-P-S | 1.21±0.07d | 2.85±0.22c | 120.83±5.83d | 104.54±6.13b |
| MW-P-O | 4.23±0.07b | 4.30±0.45b | 180.00±3.41c | 129.36±0.1.18a |

Table 2. Characterization of powder aquafaba

|  |  |  |  |
| --- | --- | --- | --- |
| Sample ID | WHC | OHC | Protein Content (mgBSA/g-powder) |
| Mw-V-S | 2.14±0.11a | 3.12±0.12d | 1.69±0.07b |
| Mw-V-O | 1.63±0.15b | 4.84±0.31a | 1.75±0.07a |
| MW-P-S | 0.63±0.06d | 3.31±0.34c | 0.57±0.06b |
| MW-P-O | 1.02±0.08c | 3.73±0.34b | 0.85±0.11a |

Table 3. Foaming ability and stability of powder aquafaba

|  |  |  |
| --- | --- | --- |
| Sample ID | Foaming Ability | Foaming Stability |
| Mw-V-S | 223.33±5.77a | 99.58±3.69b |
| Mw-V-O | 122.50±4.19b | 106.51±4.03a |
| MW-P-S | 111.67±1.75c | 107.22±1.24a |
| MW-P-O | 115.00±4.08c | 104.56±05.78a |

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