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## **Aquafaba do grão de bico: revisão sistemática sobre diferentes formulações e seus aspectos tecnológicos e nutricionais**

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**Resumo:** Aquafaba é a água residual do cozimento de sementes de grão de bico em água. Possui alta capacidade gelificante, permitindo criar géis estáveis. No entanto, o desempenho dessas propriedades funcionais depende de fatores como composição da semente, genótipo, tempo de sono, tempo de cozimento, pressão e temperatura. Este estudo teve como objetivo avaliar as formas de produção da aquafaba e as características nutricionais e tecnológicas decorrentes de cada forma de produção por meio de uma revisão sistemática. Os autores realizaram estratégias de busca específicas para Scopus, Web of Science, Pubmed, Lilacs, Google Scholar e ProQuest para realizar a revisão sistemática. Um total de 16 estudos foram analisados. Destes, 18,75% (n= 3) utilizaram a água residuária de grão de bico enlatado, 18,75% (n= 3) compararam a água residuária de grão de bico enlatado e grãos secos e 62,5% (n= 10) utilizaram grãos secos de grão de bico. Os resultados da análise da matéria seca da aquafaba (carboidratos na forma de açúcares, fibras hidrossolúveis e insolúveis e proteína) foram 7,89% [2], carboidratos a 2,05 g/100ml e gordura: 0,07 g/100ml [3], tudo em base úmida. Em base seca, o valor obtido para carboidratos foi de 34,5%. Já para proteína, a quantidade mínima foi de 0,08% e a quantidade máxima foi de 2,8% em base úmida e de 6,5 a 26,8% em base seca. Em geral, os resultados mostraram as seguintes etapas: remolho por 8-10h a 4°C na proporção 1:4 (grão:água), cozimento por 30 minutos na pressão na proporção 2:3 (grão:água) e refrigerado 24h/4°C resultados em uma aquafaba caseira que teve os melhores resultados considerando a formação de espuma mais rápida e alta estabilidade nos estudos e a aquafaba de grão de bico enlatado tem maior volume de espuma e menores propriedades de emulsão em comparação com as condições de cozimento caseiro.

**Palavras - chaves:** Aquafaba; Espuma; Emulsão; Estabilidade; Grão-de-bico; Propriedades Nutricionais; Qualidade Nutricional.

## 1. Introduction

Aquafaba is the residual byproduct solution (about 90-95% water) from canning, boiling seeds or other pulses in water. The most common one is produced using chickpea. It is usually used as an egg substitute in culinary formulations due to its emulsion, foamability, stability, moisture retention, adhesion, gelation, and thickening properties. Aquafaba may improve the sensory and technological quality of egg-free food products. The properties of chickpea aquafaba are due to its proteins, carbohydrates (starch, oligosaccharide, cellulose, hemicellulose, lignin), polysaccharide-protein complexes, saponins, and phenolic compounds [1–4].

Plant-based food products as substitutes for animal sources have been considered healthy and eco-friendly in the past few years. This market growth is mainly from specific dietary choices populations like vegans and vegetarians [5]. The demand for alternative egg products has increased, especially for those that do not compromise the sensory, mainly taste and texture, and technological properties such as foaming, emulsifying, and heat coagulation that the eggs contribute to the food [6,7]. Plant-based products that replicate eggs' qualities are becoming increasingly popular among vegetarian individuals and animal-food allergic people. Among food allergies, eggs (mainly egg whites) are one of the most common, particularly among children, with prevalences ranging from 0.5 to 2% [8–10]. For those reasons, the search for products using egg substitutes increases without giving up the taste and functional properties that eggs bring to food [6].

Aquafaba's use in food products depends on its consistency, composition, and quality, and its production standardization is a difficult task necessary to ensure the products' quality. Several parameters to assure its composition and functionality should be considered in aquafaba production, such as the type of pulse, water/pulse ratio, temperature, cooking pressure, and cooking time. Some studies evaluated aquafaba production [1,2,5,7,11–18] or composition [3,5–8,11,12,14–18]. However, to our knowledge, there is no production standardization, and the nutritional and technological properties of aquafaba have not yet been well explored. Therefore, this study aimed to evaluate the different formulations of chickpea aquafaba and compare their nutritional quality and technological characteristics through a systematic review.

## **2. Materials and Methods**

This systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and its Checklist [19,20]. Also, registers of the ongoing systematic reviews were searched via PROSPERO (Centre for Reviews and Dissemination). The protocol was executed according to the following steps:

### *2.1. Inclusion and Exclusion Criteria*

The inclusion criteria were studies evaluating the properties of chickpea aquafaba (technological and nutritional) with no limitations in terms of language or time. The exclusion criteria applied were: (1) reviews, letters, conference abstracts, case reports, books, clinical studies, and review studies; (2) studies that did not evaluate the properties of aquafaba but tried to include it in the formulation of a food product; (3) studies that focused on the improvements of aquafaba through treatments; (4) studies evaluating aquafaba made from other pulses that not chickpeas (e.g.: peas, pigeon beans); The excluded studies, and their reasons were inserted as a supplementary file (Table S1).

### *2.2. Information Source*

Five electronic databases were searched in February 2022: Medline, Embase, Lilacs, PubMed, and Web of Science, complemented by gray literature research in Google Scholar and ProQuest. The reference lists of the selected papers were checked, as relevant studies may have been missed during the data search.

### *2.3. Search Strategy*

The appropriate combinations of truncation and keywords were selected and adapted for searching each database. The software Rayyan® (Qatar Computing Research Institute-QCRI) was used to aid in the selection and deletion of duplicate articles. The Mendeley desktop software was used to manage all the references (Table S2 - Indexers used to select publications that jointly or separately address words related to aquafaba and its properties).

### *2.4. Studies Selection*

There were two phases to the study selection process. In phase one, all identified references in the databases had their titles and abstracts reviewed separately by two reviewers (B.B.M, G.S.H). The items that did not match the eligibility criteria were discarded. In phase two, the entire texts of the selected articles were subjected to the eligibility criteria by the same reviewers (B.B.M, G.S.H). In cases of conflict, regardless of the phase, the topic was debated until the two reviewers agreed. In circumstances where there was no agreement, the final judgment was made by a third reviewer (D.C.M). The final decision was always performed after reading the full papers.

### *2.5. Data Collection Process*

The following items were collected in the data collection process: authors and year of publication, country of research, the study's objective, the proportion of water and chickpeas , methods, and main results. The report was based on the PRISMA flowchart (Figure 1).

### *2.6. Risk of Bias (RB)*

A particular instrument was constructed for this study to evaluate the Risk of Bias using well-established classical and literature criteria and expert guidance, based on instructions provided by the Joanna Briggs Institute. Six questions were included in the assessment instrument for the bias risk of the chosen 17 studies: (1) Was the Study design appropriate?; (2) Was the statistical analysis adequate to the objective of the study?; (3) Were objective, standard criteria used for measuring the condition?; (4) Did the results answer the main question?; (5) Were strategies to deal with confounding factors stated?; (6) Were the outcomes measured in a valid and reliable way?. When the study received a score of up to 49 percent "yes," the risk of bias was classified as High, and when the study received a score of 50 percent to 69 percent "yes," the risk of bias was classified as Moderate and Low when the study reached more than 70% yes (Table S3).

## **3. Results**

A total of 1243 articles were initially found in the electronic databases. After removing duplicates, 250 articles titles and abstracts were selected and read. After reading the abstracts, 46 studies were selected for full-text reading. No study records were chosen from the references list of full-text articles. After reviewing the papers, 26 articles were excluded (Table S1—

Supplementary materials). By the end of the process, 17 studies met inclusion criteria and were considered for this systematic review. The flowchart of the study identification, screening, and inclusion process is in Figure 1.

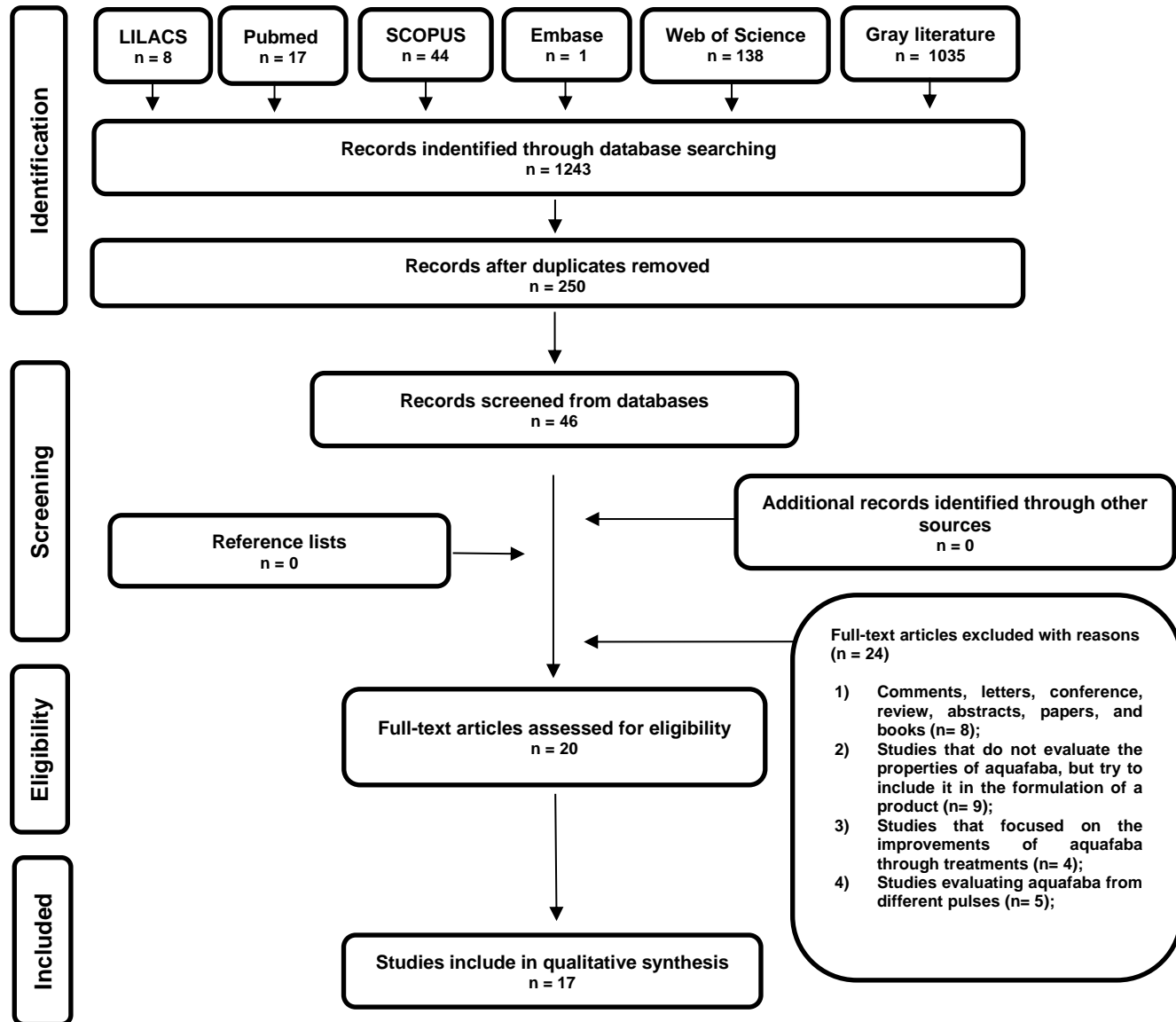


Figure 1- Systematic review flowchart adapted from PRISMA.

### *3.1. Studies characteristics*

The studies were carried out in the following countries: Canada (n = 7; 41.17%) [1,2,5,8,9,15,16], Vietnam (n = 3; 17.64%) [15; 18; 19], Brazil (n = 3; 17.64%) [7,11, 42], China (n = 2; 11.76%) [5,9], USA (n = 1; 5.88%) [17], Spain (n = 1; 5.88%) [12], France (n = 1; 5.88%) [21], Lebanon (n = 1; 5.88%) [8], Turkey (n = 1; 5.88%) [13], Denmark (n = 1; 5.88%) [6]. The date range for the included studies was between 2009 and 2018 (Table 1).

**Table 1. Main descriptive characteristics and results from the included studies.**

Reference, Year, and Country	Objectives	Proportion of chickpea/water	Methods	Results of aquafaba nutritional composition	Results of technological properties
He et al. (2019) [5] Canada	Prepare aquafaba from a variety of chickpea cultivars and use it to make food oil emulsions, then compare the properties of those emulsions	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes               <ul style="list-style-type: none"> <li>○ Proportion (dry grain:water): 1:4</li> <li>○ Was the water discarded: Yes</li> </ul> </li> <li>● Cooking:               <ul style="list-style-type: none"> <li>○ Pressure cooker for 30 minutes</li> <li>○ Proportion (hydrated grain:water): 1:1</li> </ul> </li> </ul>	Emulsifying stability Method used to determine protein content: Association of Official Analytical Chemists (AOAC) methods [22]	No information.	Aquafaba emulsion capacity and stability ranged from 1.10 to 1.30 m <sup>2</sup> /g and 71.9 to 77.1%, respectively. Significant correlation between the proximate composition of chickpea (carbohydrate, protein) and emulsion capacity and stability. The lower the chickpeas' carbohydrate content, the lower the emulsion properties.



Buhl & Christensen & Hammershøj (2019) [6] Denmark	Determine the protein content of aquafaba made from canned chickpeas and test for functional properties in foams and emulsions, as well as the effect of pH and NaCl on these properties.	<ul style="list-style-type: none"> <li>● Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ The proportion of grain/water was not mentioned.</li> </ul> </li> </ul>	Foaming properties, emulsifying activity, and stability  Method used to determine protein content: BCA, Thermo Scientific™, USA	Protein: 1.3 % The dry matter content of centrifuged aquafaba (carbohydrates in the form of sugars, water-soluble and insoluble, fiber, and protein): $7.89 \pm 0.09\%$ w/v of the herbal liquid of aquafaba.	Foam capacity: Centrifuged aquafaba had a significantly lower foam capacity when compared to egg white. However, it was not affected by changes in the pH level or the NaCl within the studied range. The foam produced by the centrifuged aquafaba was more moisture than the foam produced by the egg white, as it had a higher proportion of liquid. In addition, the protein surface charge affects foam stability.  Emulsion properties: the centrifugated aquafaba-based emulsions showed a significantly higher emulsifying activity index and stability index than emulsions prepared by egg white. The emulsifying activity and stability index were not affected by changes in NaCl. However, the change in pH affected the diameter of the mean particles
Aslan & Ertas (2020) [13] Turkey.	Optimize the variables of the aquafaba foam in the drying process.	<ul style="list-style-type: none"> <li>● Hydrated grain: No</li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Boiling water for 30 min</li> <li>○ Proportion (dry grain:water): 1:5</li> </ul> </li> </ul>	Foaming properties	No information.	The most optimum temperature for the foam-mat drying of aquafaba liquid was 70°C. The optimum formulation was determined as 0.716% carboxymethylcellulose, 0.165% Na-alginate, and 0.119% polydextrose for foaming properties.

Mustafa et al. (2018) [9] Canada and China	1) Investigate foaming and emulsifying capabilities for aquafaba made from commercially available chickpeas. 2) Choose aquafaba with the best functional properties to replace egg white in a sponge cake recipe. 3) Compare physiochemical and textural properties of aquafaba and egg white in cake.	<ul style="list-style-type: none"> <li>• Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ Brand (10 different companies in Canada): AGT Foods and Ingredients Inc. (Regina, SK), Arz Group Inc. (Scarborough, ON), Sobeys Inc. (Calgary, AB), Grace Foods Canada Inc. (Richmond Hill, ON), Loblaws Inc. (Cambridge, ON), Primo Foods Inc. (Toronto, ON) and Unico Inc. (Concord, ON).</li> </ul> </li> </ul>	Foaming capacity and stability, emulsifying capacity and stability	No information.	The tested commercial brands have different foaming and emulsifying capabilities. Foaming capacity and stability ranged from 182 to 476% and 77 to 92%, respectively, with emulsion stability varying from 60 to 80%.
Alsaman et al. (2020) [2] Canada	Use a statistical sound experimental design to evaluate different ways of cooking chickpeas to obtain aquafaba by varying the chickpeas, the proportion of water (CPCWR), and the cooking time	Wastewater from chickpeas and dried grain <ul style="list-style-type: none"> <li>• Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ Brand: CLIC</li> </ul> </li> <li>• Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the hydration water discarded? no information</li> </ul> </li> <li>• Cooking: <ul style="list-style-type: none"> <li>○ Pressure cooker</li> <li>○ Proportion (hydrated grain:water): 1:2, 1:4, and 2:3</li> </ul> </li> </ul>	Foaming capacity  Method used to determine protein content: Bradford, 1976 [23]	Protein content: 0.5 -1% (wasterwater)	The optimal conditions were 2:3 chickpea to water ratio cooked for 60 min.
Landert & Zaminelli & Capitani [11] (2021) Brazil	Standardize the process of obtaining homemade aquafaba for application in vegan cooking	Wastewater from chickpeas and dried grain <ul style="list-style-type: none"> <li>• Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ Brand: Tetrapak</li> </ul> </li> <li>• Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the hydration water discarded? Yes</li> </ul> </li> <li>• Cooking: <ul style="list-style-type: none"> <li>○ Pressure cooker for 20 minutes</li> <li>○ Proportion (hydrated grain:water): 2:2; 2:3 and 2:4;</li> </ul> </li> </ul>	Foaming capacity and stability  Method used to determine protein content: Association of Official Analytical Chemists (AOAC)	Protein content: 1.7g/100ml (wastewater) and 3% (homemade)	Aquafaba from canned chickpeas has a higher foam volume. As for homemade aquafaba, the best result was achieved with a ratio of 2:3

Alsaman & Ramaswamy (2020) [1] Canada	Investigate the enhancement of gel strength, crystallinity, and starch digestibility of aqueous aquafaba slurry and compare them to those from untreated samples	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the water discarded? -</li> </ul> </li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Pressure cooker for 60 minutes</li> <li>○ Proportion (hydrated grain:water): 1.5:3.5</li> </ul> </li> </ul>	Foaming capacity and stability, emulsion capacity, stability, gel strength, crystallinity, and starch digestibility	No information	Foaming capacity and stability: An increase in CWR (chickpea: boiling water ratio) and pH will cause a decrease in foam capacity.  Emulsion capacity and stability are higher with lower ph and CWR values
Lafarga et al. (2019) [12] Spain	Optimize the pH and domestic boiling conditions (chickpea: water ratio) required to increase the foaming and emulsifying capabilities of CCW (RSM) using response surface methodology.	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion (dry grain:water): 1:3</li> <li>○ Was the hydration water discarded? Yes</li> </ul> </li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Boiling water for 190 minutes</li> <li>○ Proportion (hydrated grain:water): 1:3.25; 1:5 and 1:1.5</li> </ul> </li> </ul>	Foaming capacity and stability, emulsion capacity, and stability	The protein concentration of the aquafaba obtained at CWR of 1:5, 1:3.25, and 1:1.5 was measured as $0.48 \pm 0.01$ , $0.23 \pm 0.04$ , and $0.08 \pm 0.00$ %, respectively	Both the boiling conditions and the pH adjustment were critical when preparing chickpea aquafaba at home to maximize the foaming and emulsifying abilities of the chickpea aquafaba and the stability of the generated foams and emulsions.
Shim et al. (2018) [8] Canada and Líbano	Determine the components of aquafaba that contribute to foaming properties.	<ul style="list-style-type: none"> <li>● Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ Brands: 10 brands with no information on the names</li> </ul> </li> </ul>	Protein and carbon content, functional properties of aquafaba, foaming capacity, chickpea seed color parameters  Method used to determine protein content: Association of Official Analytical Chemists (AOAC)	Protein content (22.65 - 26.8% - dry basis)	The aquafaba from 10 commercial products varied significantly in foam volume and stability. Brands D and H showed greater foam stability after 14h at storage, and no additives were included other than water and chickpeas

Nguyet & Quoc & Buu (2021) [14] Vietnam	Use high-pressure processing to improve chickpeas and their byproduct “aquafaba” qualities and functional properties	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion (dry grain:water): 1:4</li> <li>○ Was the water discarded: no information</li> </ul> </li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Boiling water for 45 minutes</li> <li>○ Proportion (hydrated grain:water): 1:3, 1:4, and 1:5</li> </ul> </li> </ul>	Foaming capacity and stability	No information.	The ratio of 1:4 is considered to be the most suitable ratio to obtain the highest values of foaming capacity and stability.
He (2019) [15] Canada	<p>1) Determine which chickpea cultivar produces aquafaba with the best emulsion properties;</p> <p>2) Determine grain composition and physicochemical properties of the different chickpea cultivars used in this study;</p> <p>3) Determine correlations among AQ emulsion properties, chickpea composition, and chickpea physicochemical properties; and</p> <p>4) Standardize the conditions for aquafaba preparation and compare the influences of different commercial drying methods on aquafaba emulsion properties.</p>	<ul style="list-style-type: none"> <li>○ Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion (dry grain): 1:4</li> <li>○ Was the hydration water discarded? Yes</li> </ul> </li> <li>○ Cooking: <ul style="list-style-type: none"> <li>○ Pressure cooker for 30 minutes</li> <li>○ Proportion (hydrated grain:water): 1:1</li> </ul> </li> </ul>	Emulsion turbidity and capacity	No information	Aquafaba prepared by soaking chickpea seed in 4 °C water for 16 h and cooking for 30 min displayed the highest emulsion capacity (1.30 m <sup>2</sup> g <sup>-1</sup> ) and stability (77.1%).
Alsaman (2020) [16] Canada	Enhance chickpea and its byproduct “aquafaba” qualities and functional properties by high-pressure processing, especially for reducing antinutritional factors,	<p>Wastewater from chickpeas and Dried grain</p> <ul style="list-style-type: none"> <li>● Wastewater from chickpea canning <ul style="list-style-type: none"> <li>○ Brand: CLIC</li> </ul> </li> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the water discarded: no information</li> </ul> </li> <li>● Cooking:</li> </ul>	Aquafaba yield and protein content, color, turbidity, functional properties, tannin, phytic acid, hydrophobicity,	Protein content: 0.5 – 1%	Emulsion properties were the maximum at 2:3 and cooking time of 60 min. Foaming capacity was the highest (120%) at 2:3 cooked for 30 min. The most stable foam was at 1:2 with 45 min cooking.

	soaking/hydration time, and improving functional properties of the associated proteins and carbohydrates.	<ul style="list-style-type: none"> <li>○ Pressure cooker for different times (15, 30, 45, and 60 minutes)</li> <li>○ Proportion (hydrated grain:water): 1:2; 1:4 and 2:3</li> </ul>	emulsion particle size  Method used to determine protein content: Bradford technique (Bradford, 1976)		
Meurer (2019) [7] Brazil	Evaluate the effects caused by the use of ultrasound in the foaming and emulsifying functional properties of water cooking of chickpeas (aquafaba), to make it more efficient in replacing of egg in a food	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the hydration water discarded? Yes</li> </ul> </li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Pressure cooker for 20 minutes</li> <li>○ Proportion (hydrated grain:water): 1:3</li> </ul> </li> </ul>	Foam capacity and stability  Method used to determine protein content: Method Kjeldhal, 036/IV (Instituto Adolfo Lutz, 2008)	Carbohydrates: 2.05 % Fat: 0.07 % Protein: 0.52 %	The results prove that the application of ultrasonic waves in aquafaba, at different times and intensities, favors its foaming capacity.
Nguyen et al (2021) [17] Vietnam and USA	Determine: 1) Effects of the different treatments on foaming capacity, foaming stability, hardness, and bubble size of foaming aquafaba and (2) Properties of cakes with the different treatments of chickpea cooking water as an application for eggless baking processes.	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information-</li> <li>○ Was the hydration water discarded: Yes</li> </ul> </li> <li>● Cooking: <ul style="list-style-type: none"> <li>○ Electric stove for 40 minutes</li> <li>○ Proportion (hydrated grain:water): 1:4</li> </ul> </li> </ul>	Foaming capacity and stability	Protein: 2.8 %	The highest foaming ability was of the aquafaba solution with pH adjustment and table salt. with citric acid (pH of 4), table salt (3,000 µg.mL-1)
Nguyêt (2019) [18] Vietnam	Investigate the factors that affect the foam structure of chickpea cooking water for application in the	<ul style="list-style-type: none"> <li>● Hydrated grain: Yes <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the hydration water discarded? no information</li> </ul> </li> </ul>	Foaming capacity and stability	No information.	The result shows that the ratio of dry grains: water = 1:4 demonstrated high values of foam stability (%) and capacity (%).

processing of egg/milk/fat-free cold dessert products.

- Cooking:
  - Boiling water for 45 minutes
  - Proportion (hydrated grain): 1:3; 1:4 and 1:5

Escadellas et al. (2019) [21] France	Highlight the characteristics of aquafaba as a foaming food matrix and characterize its properties (rheology, foamability) with a view to the implementation of transformation processes	<ul style="list-style-type: none"> <li>● Hydrated grain: -           <ul style="list-style-type: none"> <li>○ Proportion: no information</li> <li>○ Was the water discarded? no information</li> </ul> </li> <li>● Cooking:           <ul style="list-style-type: none"> <li>○ Proportion: no information</li> </ul> </li> </ul>	Foaming properties	Composition on dry basis: Protein: 6.5% Carbohydrates: 34,5% Hydrogen: 5.6% Nitrogen: 3.7% Sulfur: < 0.5%	Aquafaba has foaming properties as its foam has small, stable bubbles.
Ricci (2018) [42] Brazil	Physicochemical and rheological characterization of aquafaba analyzing the stability of the systems formed.	<ul style="list-style-type: none"> <li>● Hydrated grain: -           <ul style="list-style-type: none"> <li>○ Proportion: 2:3</li> <li>○ Was the water discarded? Yes</li> </ul> </li> <li>● Cooking:           <ul style="list-style-type: none"> <li>○ Proportion: no information</li> </ul> </li> </ul>	Emulsions, foams, stability, and rheology  Method used to determine protein content: Association of Official Analytical Chemists (AOAC) methods [22]	Composition on dry basis: Protein: 8.4% Carbohydrates: 10.3% Fat: 0.5 %	All samples (emulsions and foams) from aquafaba and albumin showed phase separation; however, the separation remained stable after 5 hours of observation.

Abbreviations: CCW: chickpea cooking water;RSM: response surface methodology;CWR: chickpea:boiling water ratio

The evaluation of the nutritional composition of the aquafaba was performed in 58.8% (n = 10) of the included studies [1,2,6–8,12,17,18,21,42]. Of them, two [21,42] analyzed (11.76%) composition on dry basis, and eight (47%) [1,2,6–8,12,17,18] on wet basis. Only 3 (17.64%) studies [6,7,21] assessed the proximate composition of the aquafaba. On a wet basis, the dry matter of the aquafaba (carbohydrates in the form of sugars, water-soluble and insoluble fiber, and protein) was 7.89% [6], carbohydrates at 2.05%, and fat at 0.07 % [7]. On a dry basis, the value obtained for carbohydrates varied from 10.3% to 34.5% [42,21], and for fat, it was 0.5% [42]. Considering protein, 47.05% of the studies performed the analysis, the minimum amount was 0.08%, and the maximum amount was 2.8% on a wet basis [1,2,6–8,12,17,18] and 6.5 to 26.8% on a dry basis [21,42]. Table 1 also presents the methods used to analyze the protein content in aquafaba, since it is the main nutrient involved in foam production.

52.9% of the studies performed protein content analysis. The most frequently (n = 5; 29,38 %) used method was the Association of Official Analytical Chemists (AOAC 981.10) which is based on the Kjeldahl Method [5,8,11,15,42]. The second most used method was the Brad Ford technique (n = 2; 11.76%) [2,16]. One study used the Kjeldahl Method without mentioning the correspondent AOAC method [7]. Another study used the BCA method (Thermo Scientific™, USA) [6].

The studies had different ways of obtaining the aquafaba, 17.64% [6,8,9] used the wastewater from canned chickpeas, 17.64% [2,11,16] compared the wastewater of canned chickpeas, and home cooking of dry grains of chickpea and 58.82% [5,7,12–15,17,18,21,42] used home cooking of dry chickpea grains. Furthermore, the most used aquafaba formulation was with the grain previously hydrated with a proportion of 1:4 (dry chickpea/water) that was used in four studies [5,12–14], as well as the wastewater from chickpea canning [2,6,8,9,16]. Most studies did not mention whether water was discarded or not [1,2,16–18,21] and the majority used pressure-cooking for 30 minutes [1,2,5,7,11,15,16].

Most of studies (n =12; 70,58%) used soaking in aquafaba production [1,2,5,7,11,12,14–18, 42]. However, only five [5,12,14,15,42] reported the proportion, three of which used a proportion of 1:4 (chickpea:water), one study used the proportion of 1:3, and another employed the proportion of 2:3. Only one study did not use the soaking technique [13], and the others used wastewater from chickpea canning.

As for the methods used for cooking, 32.29% of the studies (n = 6) [1,2,5,7,15,16] used the pressure cooker. Nearly 20% of the studies (n = 3) used boiling water [12,14,18]. The

cooking method employed in the other 8 studies (47.05%) [6,8,9,11,13,17,21,42] was not mentioned.

### 3.2. Risk of Bias (RB)

The studies are heterogeneous, but the majority, 88.23%, had a low risk of bias, 5.88% had a moderate risk of bias, and 5.88% presented a high risk of bias (Table S3—Supplementary File). All studies answered the main question.

**Table S3.** Summarized risk of bias assessment.

Author (year)	Risk of bias	Risk (%)
He et al. (2019) [5]	Low	100
Buhl, Christensen & Hammershøj (2019) [6]	Low	100
Aslan & Ertas (2020) [13]	Low	100
Mustafa et al. (2018) [9]	Low	100
Als Salman et al. (2020) [2]	Low	100
Landert & Zaminelli & Capitani [11]	Low	100
Als Salman & Ramaswamy (2020) [1]	Low	100
Lafarga et al. (2019) [12]	Low	100
Shim et al. (2018) [8]	Moderate	50
Nguyet & Quoc & Buu (2021) [14]	Low	100
He (2019) [15]	Low	100
Als Salman (2020) [16]	Low	100
Meurer (2019) [7]	Low	100
Nguyen et al. (2021) [17]	Low	100
Nguyêt (2019) [18]	Low	100
Escadellas et al. (2019)[21]	High	16.6
Ricci (2018) [42]	Low	100

## 4. Discussion

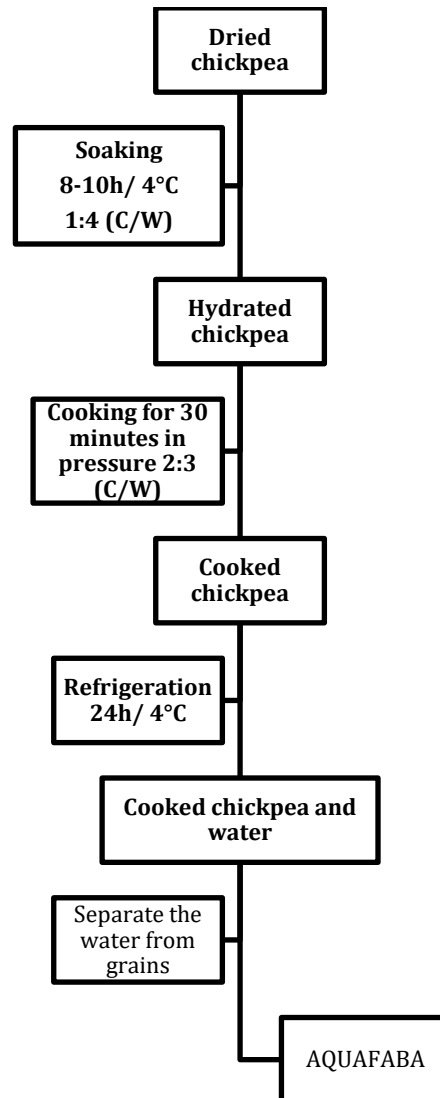
The number of vegetarianism and veganism followers has grown and, consequently, the search for products that can replace food and ingredients of animal origin [22]. This tendency of growth is mainly in high-income countries [23,24]. About 10% of the total population



declares themselves vegetarians in countries like Australia, New Zealand, Israel, and Sweden. In India, given the prominent religion, one-third of the population is vegetarian [25]. Therefore, following the trend of the recent growth of the vegetarian movement, the studies on this systematic review topic were conducted recently.

In general, most of the studies were performed in Canada (n = 7; 41.17%) [1,2,5,8,9,15,16], followed by Vietnam (n = 3; 17.64%) [15; 18; 19], Brazil (n = 3; 17.64%) and China (n = 2; 11,76%) [5,9]. Among the countries included in the studies evaluated in this review, data suggest that vegetarianism is most prevalent in Brazil (14% of the population) [26], followed by Canada (12.2%) [27], Vietnam and Denmark (10%) [28,29], France (5.2%) [30], China and USA (5%) [31,32] and Spain (1.4%) [33]. Therefore, among the countries included in this review, the countries that have most of the studies on this topic were the ones with the highest prevalence of vegetarianism, except for Denmark.

Aquafaba can be obtained using two main sources: from the wastewater resultant of homemade cooking of chickpeas or from separating the viscous liquid from canned chickpeas. These different processes interfere with the foam capacity and stability properties because of the individual characteristics of the grain, the type of implemented storage (if refrigerated or not) and time of storage, cooking time and temperature, and the use of pressure [5,9]. The homemade process of aquafaba production (Figure 2) in most of the studies was: the dried chickpeas were soaked for 8-10h at 4°C on a proportion of 1:4 (chickpea:water) [5,14,15]. After that, the water was discarded, and the hydrated grains went into pressure cooking for 30 minutes on a proportion of 2:3 (hydrated chickpea:water) [2,11,16]. Subsequently, the cooked chickpeas with the cooking water were stored on refrigeration for 24 hours at 4°C. By the end, the wastewater (aquafaba) was separated from the grains. None of the studies described exactly the protocol displayed in Figure 2; however, this protocol was constructed based on the most frequent processes in the evaluated studies.



**Figure 2. Homemade chickpea aquafaba production flowchart.**

Regarding the soaking step, 94.11% of the studies [1,2,5–9,11,12,14–18,21,42] previously hydrated the chickpea grains, but only 29.41% of them [5,12,14,15,42] mentioned the proportion of water and chickpeas. The hydration capacity of the grain during soaking is generally related to the physical properties of the grain, thus, different effects on aquafaba may be noted. A study showed that it was impossible to obtain foam when chickpeas were not soaked [11]. Soaking is a common process among pulses since the mechanical process of adding water before the cooking process and letting the grains rest underwater for a minimum of eight hours might improve digestion by reducing antinutritional phytates and oxalates while also softening the soaked grain[34]. The hydration promoted by soaking results in swelling of the seed's cotyledons, making the seed coat crack more permeable [34,35]. At the same time, the

introduced water partially hydrates starch molecules inside the matrix, in a rheological phenomenon called gelation [36]. The combination of these processes reduces the cooking time and regulates the chemical diffusion into the cooking water (aquafaba). Some studies mentioned that after soaking and cooking, the total amounts of sugar, oligosaccharides (raffinose, stachyose, verbascose), and protein in chickpeas decreased, given that a part of these compounds was diffused into the cooking water [2,4,5,16]. Among the compounds diffused into the cooking water, proteins related to aquafaba formation might be more prominent than seeds that skipped the soaking process. Also, it is important to note that most studies discarded the water residual from soaking because of antinutritional compounds [2,4,5,16].

The ratio 1:4 (hydrated grains:water) was the most used proportion (n = 5; 29.41%) in the cooking step [2,14,16–18]. However, regarding the technological characteristics of aquafaba, it was not the one with the best results. Therefore, we used the proportion with the best results mentioned in the studies to construct the flowchart (Figure 2). Although the 2:3 ratio was only tested in three studies, this ratio showed better characteristics, emulsion properties, and foaming capacity in studies that compared it with the 1:4 proportion [2,16]. Only one study showed that on the proportion of 1:4 with the pH adjustment with the addition of table salt and citric acid (pH of 4), table salt (3.000  $\mu\text{g}\cdot\text{mL}^{-1}$ ) performed the highest foaming ability [17]; however, this study did not compare with the aquafaba using the proportion of 2:3 on cooking.

According to a study [37], the total sugar content of chickpeas was significantly reduced after boiling in water (32% of reduction). Non-galactoside sugars (fructose and sucrose) decreased slightly more than galactoside sugars (38% and 50% decrease, respectively) [38]. These carbohydrate losses can partly be explained by the solubilization of carbohydrates in water during the soaking and cooking process. However, because other water-soluble nutrients were also eliminated, the soluble sugar losses are significantly higher than the percentages shown on a dry basis [37]. In that case, the aquafaba produced through boiling water could have good foaming stability, given the solubilization of carbohydrates in the cooking water exerts a positive influence on aquafaba's foam stability [37].

Protein denaturation causes structural changes, which cause protein modifications. These modifications either increase molecule size through aggregation (lower solubility) or decrease it through breakdown into smaller compounds (increased solubility) [1]. As for high-pressure cooking, rupturing non-covalent connections between protein molecules or forming new intermolecular links (such as hydrogen bonds and hydrophobic interactions) promotes protein aggregation]. Due to changes in solvation volume, where non-covalent bonds are

ruptured and reorganized with solvent molecules, pressure cooking can also increase protein volume [1].

The different proportions of water seem to affect the technological properties of the aquafaba. Lower quantities of water make the chickpeas soft and crumbly, allowing the starch granules to easily disperse in the cooking water and break the foam membranes, thus lowering the foaming capacity and stability [14]. Excess water also impairs foam formation by excessive solubilization of starch and protein, thus reducing this compound's concentration [11]. In regard to refrigeration, according to Landert et al. [11], this practice, if carried out for 24 hours after cooking the beans, significantly improves foam formation and stability. Probably, the cooling time favors chemical reactions such as the starch gelation and extravasation of proteins from the cooked grain to the wastewater, thus resulting in a greater amount of gelated starch and solubilized proteins, favoring a more technologically stable aquafaba [11].

The studies that investigated aquafaba from the wastewater of canned chickpeas [6,8,9] showed that the manufacturers that produced the commercial brands have different genetic chickpea cultivars causing changes in the nutrition composition of the aquafaba, as well as foaming and emulsifying properties [5,9]. In general, the proximate composition of the utilized chickpeas did not influence the stability of aquafaba; nevertheless, it seems that grains with higher amounts of dry matter displayed better emulsion properties, resulting in better results for aquafaba [5,9]. Nevertheless, the dry matter content relies mainly on the chickpeas' genotype, given that the aquafaba produced by the "*CDC Leader*" genotype presented the highest amount of dry matter and, subsequently, the most adequate aquafaba [5].

In addition, some commercial brands include food additives, such as salt, and preservatives like disodium ethylene diamine tetra acetic acid (EDTA), which might suppress viscosity and foam stability by increasing the molecular weight of the formulation [8,9]. In this manner, the aquafaba from brands that had no addition of salt or additives produced more viscous foam with greater capacity, and stability [8,9,39].

Some other studies compared the aquafaba with dried beans (homemade) and canned chickpeas. They concluded that the proportion of 2:3 of homemade aquafaba had the best results, forming foam more quickly and with high stability. The aquafaba from canned chickpeas has a higher foam volume and lower emulsion properties than the homemade cooking conditions, possibly because of the utilized chickpea cultivars; however, these studies did not describe the used chickpea cultivar [2,11,16].

Aquafaba is mainly composed of carbohydrates and protein, but the protein is the most evaluated compound in the studies due to its foaming properties [2,6,8,11,21,42]. Proteins may

present hydrophilic amino acids interacting with water, whereas the hydrophobic amino acids stabilize interactions with the gaseous phase. In this sense, aquafaba foaming capacity strongly correlates with protein content [15]. A lack of a standard for protein measurements was observed among studies. Also, some studies showed the content on a dry basis and others on a wet basis. Studies [8,11,42] used the same technique for analyzing proteins (AOAC). The only difference in the method is that one of the articles multiplied the nitrogen content by 6.25 and the other by 5.75, probably because of the difference in the type of chickpeas. Despite this, the studies showed different results regarding protein, 1.7% protein for canned chickpeas, and 3% protein of protein for homemade chickpeas. The differences in protein content also occur because of the different cooking methods. Boiling can change nutrients' concentrations. There may be solubilization of proteins or even a higher concentration [11,12]. The protein fraction in aquafaba of commercially canned chickpeas ranged from 92.9 to 8.8 kDa; these included some globulins and heat-soluble proteins [8].

Only two studies evaluated fat content in chickpea aquafaba, 0.07% [7] on a wet basis and 0.5% on a dry basis [42]. This data is important, since fat can influence the foaming capacity of aquafaba. The presence of unsaturated fatty acids reduces the volume and stability of the foam, and chickpea cultivars may contain 2.70–6.50% of fat. It is an important source of unsaturated fatty acids [40,41]. E esses dois que avaliaram gordura apresentaram menor ou maior foaming capacity. Acho que essa informação fecharia bem a discussão

## 5. Conclusion

This study aimed to evaluate different formulations of aquafaba and compare their nutritional and technological characteristics. The results showed the following steps to prepare aquafaba: soaking for 8-10h at 4°C at the proportion of 1:4 (chickpea: water), pressure cooking for 30 minutes in the proportion of 2:3 (chickpea: water), and refrigerating 24h/4°C. Most of the studies used soaking in water as a strategy to home cook chickpeas, improving the diffusion of compounds to the water in the cooking process. The proximate composition of chickpeas did not alter the quality of produced aquafaba; however, species with higher concentrations of dry matter produced better foam. According to the studies, there was also an indication that aquafaba from wastewater canned chickpeas produced by the *CDC Leader* chickpeas genotype presented better results regarding foam formation, emulsion capacity, and stability compared

to homemade aquafaba. Also, canned chickpeas without added salt or EDTA produced aquafaba with better technological characteristics.

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