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Aquafaba, an egg substitute for food applications
Aquafaba, un substitut dels ous per a aplicacions alimentàries

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Our revolution begins with food.

Vandana Shiva

Sobretot vull agrair a la meva tutora per donar-me l'oportunitat de fer meu aquest projecte i animar-me i ajudar-me en tot moment. També agrair al meu pare i als meus germans per ajudar-me en aspectes concrets del treball. I finalment, a la meva mare i al meu xicot per donar-me ànims en tot moment.

REPORT

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1. Summary

Animal source foods cause environmental damage, animal suffering, and health problems that plant foods can reduce. Pulses are a good choice because they have a low cost and high nutritional value. The liquid that results from cooking them, aquafaba, can be used to replace the egg in sauces, patisserie and bakery products. This study explains the chemical composition, physicochemical properties (electrical and rheological), and functional properties (emulsifiers, foaming and gelling agents) of aquafaba. The variables that determine its colloidal capacity and stability are outlined in Figure 1. When these are optimized, the aquafaba achieves properties similar to those of the egg. Some variables have been studied enough and we know how to optimize them, but others, such as the pH and the method of cooking legumes, need more research to establish their optimum values.

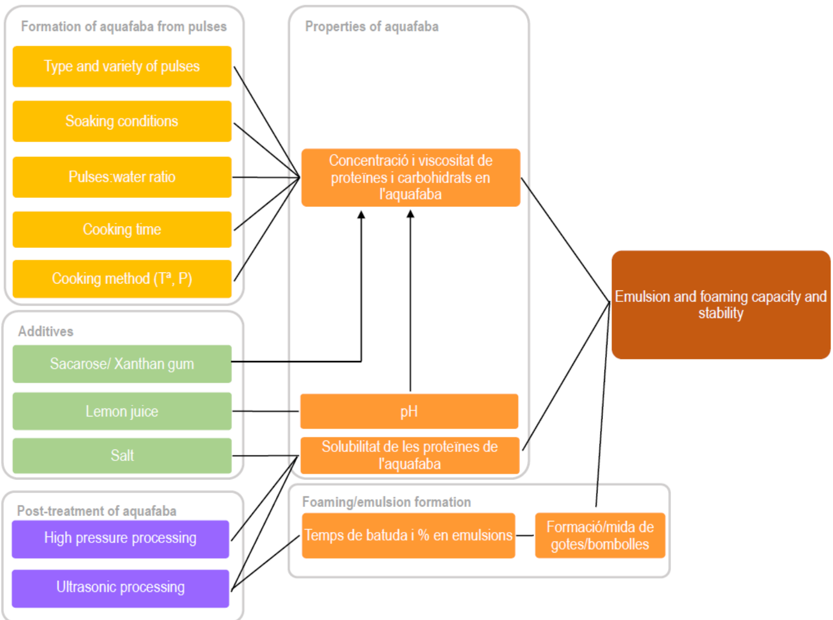

Figure 1. Aquafaba's independent and dependent variables.

Keywords: Aquafaba, egg replacers, pulses, proteins, foam, emulsion.

2. RESUM

Els aliments d'origen animal causen danys ambientals, patiment animal i problemes de salut que els aliments vegetals poden reduir. Els llegums són una bona opció pel seu baix cost i alt valor nutricional. El líquid que resulta de coure'ls, l'aquafaba, es pot aprofitar per substituir l'ou en salses, productes de pastisseria i de fleca. Aquest estudi explica la composició química, les propietats fisicoquímiques (elèctriques i reològiques) i les propietats funcionals (emulsionants, escumants i gelificants) de l'aquafaba. Les variables que determinen la seva capacitat i estabilitat col·loidal s'han esquematitzat en la figura 1. Quan aquestes s'optimitzen, l'aquafaba aconsegueix unes propietats similars a les de l'ou. Algunes variables han estat prou estudiades i sabem com optimitzar-les, però d'altres com el pH i el mètode de cocció dels llegums necessiten més investigació.

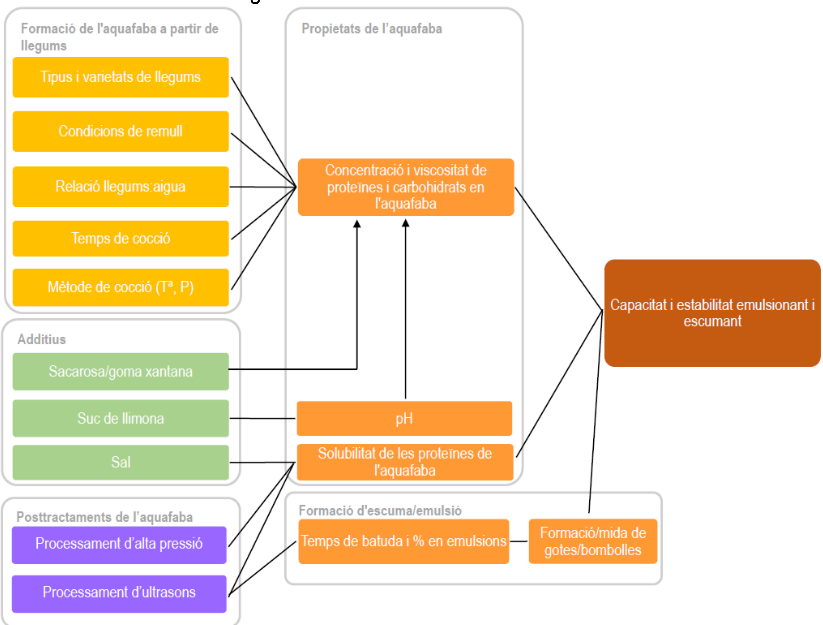

Figura 1. Variables independents i dependents de l'Aquafaba.

Paraules clau: Aquafaba, substituts d'ou, llegums, proteïnes, escuma, emulsió.

3. INTRODUCTION

The threat of climate change should force us to consider the way to feed us. Livestock accounts for 14.5% of anthropogenic greenhouse gas (GHG) emissions [1] , but it could be much higher, about 51%, if we keep in mind account indirect sources of GHG emissions from livestock [2]. Livestock farming also produces other negative effects, such as fresh water depletion, biodiversity loss, animal suffering, antibiotic resistance and zoonoses. We can combat them eliminating or reducing the meat consumption without giving up a good diet [3]–[6].

A diet based on plant products rich in protein, such as legumes, seeds and nuts, could meet these expectations [3]–[6]. Plant food is the ideal choice because of the nutritional value and low economic cost [1]. It also increase food security, sustainability and combat malnutrition [7].

Legumes, including chickpeas, beans, lentils, peas and soybeans, have a large quantity of high quality protein, so they can replace animal protein [8]. Besides, they contain lots of carbohydrates, which provide energy to the human diet. They also contain dietary fiber and a low saturated fat content. Among legumes, chickpeas have the best quality of protein and carbohydrates. Chickpeas contain 20-26% protein and 43-46% starch [9].

However, some consumers don't want to renounce the taste, appearance and functionality of some animal products. Therefore, researchers are doing efforts to find alternative products to mimic or replace the properties of meat, fish, dairy and eggs [10].

Replacing eggs is an important challenge. Apart from having a high nutritional value, they are multifunctional. They can perform at same time emulsions, foams, gels, color and flavour [11], [12]. But plant alternatives would bring many benefits. They could reduce animal suffering, environmental impact and public health risks. These benefits are specified in Figure 1.

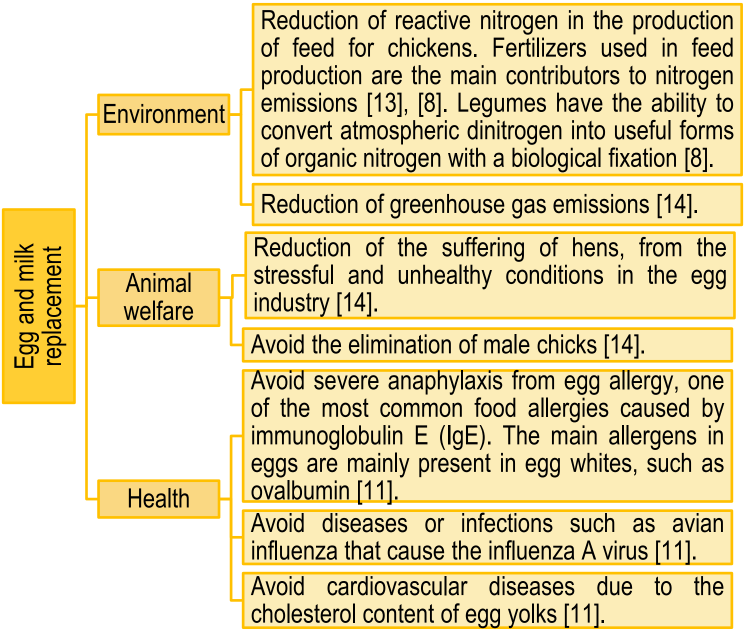

Figure 1. The reasons behind the need for egg replacement in foods.

This means that replacing eggs with plant foods is the most effective way to increase the welfare of chickens, reduce poultry farms's environmental impacts and provide healthier food for all public.

In order to find egg substitutes, the main properties of the eggs have to be understood first.

Egg yolk is known for its emulsifying properties. When it (aqueous phase) is subjected to a mechanical homogenization with an immiscible phase (lipid phase), such as oil to make a mayonnaise, a biphasic colloidal system is created. In this colloidal system the lipid phase is arranged in drops in the aqueous phase forming an emulsion. This emulsion is thermodynamically unstable, that is, the emulsion breaks down quickly separating the two immiscible phases. But this doesn't happen, because the lipids and proteins that make up the egg yolk act as emulsifiers, stabilizers of the emulsion. Lipids and proteins are adsorbed at the oil-water interface to form a condensed film around the oil droplets protecting them. This forms an oil-emulsifier-water system [12], [15].

Egg white is known for its foaming properties. It consists of an aqueous solution where more than 90% are protein, with ovalbumin being the most abundant. These proteins act as emulsifiers or stabilizers of foam, a colloidal system consisting of gases dispersed in a liquid. Therefore, the stability of the foam depends on the adsorption of the egg white proteins at the gas-liquid interface to form a continuous intermolecular network. Because egg white proteins are amphiphilic and have a relatively high surface hydrophobicity, they are efficiently adsorbed at the gas-liquid interface, especially when a certain level of denaturation is previously obtained [12], [15].

The gelling property of eggs is related to the thermal gelation capacity of egg proteins, from both yolk and egg white. This gelling activity increases the viscosity of the emulsion and therefore its stability [11], [15].

Finally, the carotenoids in the egg yolk give it its characteristic color [11].

As a result, many studies have identified partial or total vegetal egg substitutes such as those listed in Table 1. For example, protein isolated from legumes, plants, seeds, vegetable milks, and starches have been investigated. In addition, hydrocolloids (vegetable gums and jellies) and/or emulsifiers have sometimes been used as auxiliary emulsifiers.

Table 1. Plant-based egg replacers and their colloidal properties.

Egg replacers	Properties (Emulsifying, foaming, gelling, sensory...)	References
Soy milk	The amount of lecithin in soy milk is lower than in eggs yolks. For this reason, it has lower stability and viscosity values. However, to make mayonnaise don't affect viscosity up to 75 % egg replacement and stability up to 50 % replacement. Sensory evaluation showed no significant differences up to 50% egg replacement. To make egg-free cakes, lecithin should be included in its formulation to compensate the lower lecithin content. Regarding the high capacity of the egg proteins in foaming, it seems that soy milk is also less stable to hold air cells.	[16]–[18]
Lentil protein	It could serve as an efficient foam and emulsion stabilizer in cake formulations. Sensory evaluation showed no significant differences up to 50% of egg replacement in angel cake and a 100% of egg replacement in muffins. However, the relatively unknown relationship between its molecular structure and functionalities and the lack of knowledge of the impact of the extraction and environmental impacts has hindered the exploitation of their full potential.	[19], [20]
Chickpea protein	Its solubility is lower than lentil proteins, but its emulsifying activity and stability index, gelling property and, its water- and oil- absorption capacity are higher. Its foaming capacity and foam stability are similar.	[19]
Soy bean protein	It has lower values for solubility, foaming capacity, foam stability and oil-absorption capacity compared to lentil proteins. However, it has better results for water-absorption capacity and similar performance in emulsifying activity and stability index. It is suggested that a mixture of soybean proteins and emulsifier is a promising substitute for eggs in cakes.	[19], [21]

Pea protein	It has good emulsifying and foaming capability and stability, but are poor in gelling ability. It also has a bad sensory acceptability due to an "off – taste" and a "strange" flavor.	[22]
Lupin protein	Although lupine proteins have good emulsifying and foaming properties, they don't possess the egg coagulation capacity with heat. This problem was solved by the combined use of hydrocolloids (xanthan gum) and emulsifiers (mono- and diglycerides). Depending on the desirable level of protein, emulsifiers, hydrocolloids and oil concentration, is possible to create lupin protein emulsions with physical properties closely matching those of commercial mayonnaises.	[23], [24]
Banana	It has shown a good foaming ability.	[25]
Flaxseeds	Flaxseed proteins vary as a function of pH, so, because the emulsifying, foaming and viscosity properties depend on the proteins, they will depend directly to the pH. At pH 6 was observed a better emulsifying and foaming capacity and stability and a better viscosity. These properties are comparable to egg yolk. Products texture are aerated and light.	[26], [27]
Chia gel	It is a potential egg replacer because it presents good rheological and sensory properties and a great emulsifying capacity and stability (especially at alkaline pH). Foaming properties are not so good.	[28], [29]
Potato starch	It can serve as an emulsifier and an egg yolk replacer because it has a good emulsifying stability. However, the rheological tests elucidated that in the absence of egg yolk, potato starch may not result in a final product with consistent texture and that the best ratio of the two emulsifiers (potato starch/egg yolk) to produce stable reduced-fat, low cholesterol mayonnaise is 75/25.	[30]

Aside from the substitutes discussed, there is a recently studied ingredient that has strong potential as a substitute for eggs. This ingredient is the aquafaba, as indicated the Latin word aqua (water) and faba (bean), it is the water resulting from pulses cooking, which can also be found in pulses canned jars. It was discovered in 2014 by Joël Roessel, a vegan French musician who was trying to replace egg white with aquafaba as a foaming agent in a meringue recipe [31]. Later, in addition to the foaming property, its emulsifying, thickening and gelling properties were studied [32]. These functional properties are due to the chemical composition of aquafaba, defined during the cooking process of legumes. During cooking, proteins, carbohydrates and many other components of pulses pass into the water making it rich in composition [33].

The chemical composition and properties of aquafaba can vary due to many factors. First, it can vary according to the types, components and genotype of the different legumes. Moreover, it can vary depending on the legume soaking and cooking conditions: time, legume:water ratio, temperature, pressure and pH. And finally, it can vary if additives like NaCl or sucrose are added to it [10], [34], [35].

If it wants to guarantee the quality and standardize the aquafaba formula, it's important to extensively study the electrical properties, the rheological properties, such as flow, viscosity, and gelling capacity; and the thermal properties, because heat treatments affect the proteins and carbohydrates present in aquafaba. The heat uptake can cause the denaturation of proteins, changing the secondary structure, and the gelatinization of starch (principal carbohydrate in aquafaba) thus causing a change in its functionality [9], [34].

Some treatments such as high-pressure processing [9], [33] or the effect of high intensity ultrasound [36] have demonstrated the ability to modify protein structures and the degree of gelatinization of starch, with which its textural and functional properties can be improved.

The intention of this work is to gather all the information about aquafaba studied until now. And based on this information, make conclusions about its optimal extraction and its future use.

4. OBJECTIVES

The objective of this study is to carry out a bibliographic research on everything that has been studied about aquafaba to be able to answer two questions:

- Can aquafaba replace eggs in different food applications?
- If so, how can we get its best properties?

5. METHODS

The information to carry out this study has been found in two databases:

- Scifinder, a database of chemical and bibliographic information operated by CAS (Chemical Abstracts Service) (United States)
- Scopus, a database of sciences produced for Elsevier (Netherlands)

The search procedure was to enter the word "aquafaba" and filter the content with "articles". All the studies found on aquafaba have been used for this research work. Therefore, no date filter has been set. In the Scifinder search, 23 articles were found between 2018 and 2021. And the same search in Scopus found 31 articles until now. These databases have been reviewed throughout the work for new articles to be published, as has been the case.

In addition, in the bibliography of these found articles, other studies have been found related to legumes, legume proteins, zeta potential, etc. which have also been helpful.

6. RESULTS AND DISCUSSION

6.1. COMPOSITION OF AQUAFABA

Aquafaba composition is a complex mixture of components transferred from dried legumes to water during cooking. However, there is no exact composition for aquafaba because it depends on the type of pulse used, on the pulse structure and its cooking.

The morphology of the legumes consists of two cotyledons, separated by an embryonic axis, which occupy most of the legume seed (80-90%), and a cell wall or coat, which constitutes 8-18% of the seed [37], [38] (Figure 2). The cotyledons contain most of the nutrients in the legume, their cells are full of starch granules which all at once are embedded in a protein matrix that also contains small protein granules [38], [39]. The cell wall comprises a mixture of fibers (cellulose, hemicellulose, pectin) that provide structural support, and acts as a barrier, protecting nutrients from water, bacteria and insects [37], [39].

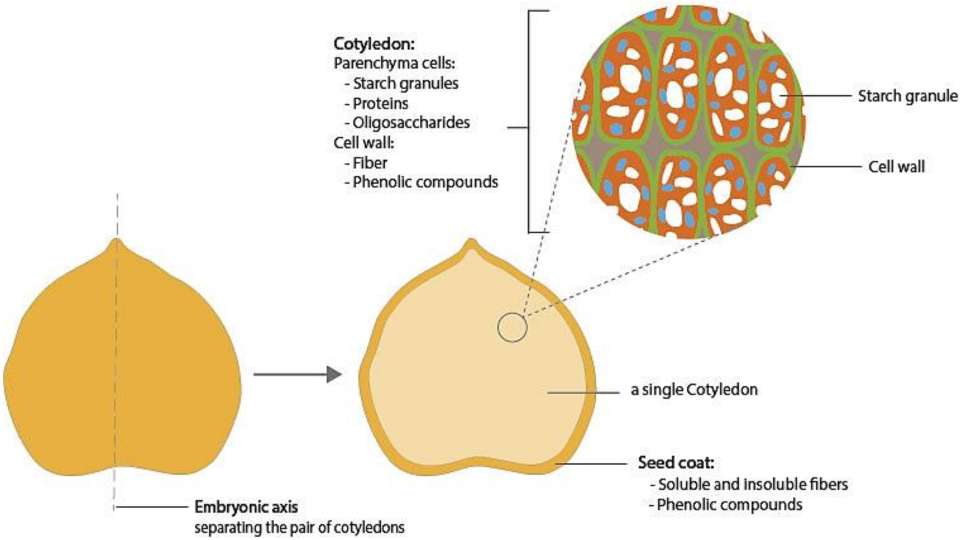

Figure 2. Structural representation of the common chickpea seed.

Soaking the pulses followed by a heat treatment in their cooking impairs the integrity and strength of the layers of the cell wall, the carbohydrates of the cell wall can be solubilized, depolymerized and/or lost, thus pass into cooking water [8], [40]. Seed coat changes depend on the pressure and temperature applied during soaking and cooking [8]. In addition, cooking causes swelling and gelatinization of starch, so cracks can form on the surface of the legume, through which valuable proteins and minerals can be lost in the wastewater [37]. This is how the aquafaba composition is determined, depending on the diffusion of seed molecules in the water during cooking.

The chemical composition of aquafaba has been studied under different conditions and analysis methods by different studies. The results are as shown in Table 2. It can be seen that the chemical composition has a large variation depending on the species of legume, but it is mainly formed of carbohydrates, proteins and water.

The moisture content of aquafaba is over 90%. Several researchers have suggested that drying or lyophilizing aquafaba would be beneficial in preventing activity of microorganisms in the water, maintaining its quality, and its shelf life. Moreover, aquafaba powder has been shown to have very good functional properties [41], [42].

The dry matter of cooking water (aquafaba) contains mainly proteins (0.5-1.72 g / 100 g), soluble (0.04-1.66 g / 100 g) and insoluble (0.93-2.4 g / 100g) carbohydrates, ash (0.4-0.78 g / 100 g), saponin (4.5-14 mg / g) and phenolic compounds (0.3-0.7 mg / g) [8], [40].

6.1.1. Proteins

As the legume hulls break during the cooking process, the release of protein into the water is facilitated [36]. These proteins give aquafaba its unique functional properties (section 6.3). Differential Scanning Calorimetry (DSC) demonstrates that heat treatment of legume cooking denatures aquafaba proteins [9]. Denaturation causes the exposure of hydrophobic groups of proteins, which improves their functional properties and digestibility. Discontinuous Electrophoretic System(SDS-PAGE electrophoresis) and peptide mass fingerprinting analyzes of aquafaba reveal that existing proteins have a low molecular weight (≤ 25 kDa) and that many of them are heat soluble hydrophilic species [10], [32]. In addition, they deduce that a large amount of aquafaba proteins belong to the group of albumin 2S, followed by the oligomeric protein vicillin 7S and the legume 11S [32], [45], [49].

Proteins vary between 18-29% in dry matter of chickpea aquafaba, 21% in haricot beans, 29% in split yellow peas, 32% in green lentils and 12% in yellow soybean. A curious case is that soybeans, the most protein-rich legume, loses a lower concentration of protein when boiled.

This is probably due to the fact that the proteins are firmly embedded in the starch matrix in the inner core of the seeds, so that they are only released in very small fractions [43].

Most studies have determined protein content by Bradford method [35], [48], [51], quantifying total nitrogen by Kjeldahl method [36], [45] or by combustion according to the Dumas principle [45], [50].

In some cases, we can see a very wide range of protein content. This wide range is due to different varieties of each legume. Depending on the size of the variety, the amount of solubilized proteins in the cooking water may change. The smaller the seeds, the higher the protein content [44], [45].

6.1.2. Carbohydrates

We can differentiate carbohydrates into two subgroups, the soluble and the insoluble. The soluble carbohydrates range from 0.8-24% of the aquafaba dry matter of chickpea, 22% of haricot beans, 23% of split yellow peas, 12% of green lentils and 30% of yellow soybeans. The insoluble ones vary in 47% of the dry matter of the cooking water of the chickpeas, 28% of the haricot beans, 37% of the split yellow peas and 44% of the green lentils and yellow soybeans. Therefore, the insoluble are more abundant in aquafaba.

Most water-soluble carbohydrates are low molecular weight (LMW) attributed to sugars, composed of 40% disaccharides, mainly sucrose, and 60% α-galactosides, such as raffinose and stachyose [38]. High molecular weight water soluble carbohydrates (HMW) are considered soluble fibers [45].

Insoluble carbohydrates are attributed to dietary fiber, an edible part of vegetables that is not digested or absorbed in the human small intestine and reaches the colon where it is fermented by the intestinal microbiota [38], [39]. These components are predominantly polysaccharides, cellulose, hemicellulose, lignin or pectin (not starches), associated with the structure of the cell walls of legumes [38], [45].

Starch, although it is the main component of legumes, is not present in aquafaba. All iodine tests made have been negative [45]. The reason is because it forms large granules of intertwined chains making them stable to heat processing [38].

6.1.3. Bioactive substances

Legumes contain many secondary plant metabolites and non-nutritive bioactive substances, such as saponins, tannins, phytic acid, phenolic compounds, oligosaccharides, among others. Some of these components are also considered anti-nutritional factors because they can have adverse effects on digestive enzyme activity, digestibility, nutrition, and health [8], [38]. For example, oligosaccharides from raffinose can cause unpleasant gastrointestinal symptoms such as flatulence, diarrhea and abdominal pain due to the lack of the enzyme α-galactosidase in the human digestive system [38]. However, soaking legumes can reduce the concentration of these components by solubilizing them. Subsequent cooking of legumes at high temperatures and pressures can also destroy or inactivate some heat-sensitive antinutritional compounds, such as saponin, phenolic compounds, and phytic acid, which are thermolabile components [40]. Therefore, the concentration and activity of these antinutritional factors in aquafaba is significantly lower than in raw legume seeds and consequently does not cause adverse effects [8].

6.1.4. Fat, tocopherols and mineral profile

Most legumes are low in lipids, that's why the fat content in aquafaba is so low that it is not detected at measurable levels [8].

Although tocopherols (mainly isomer c) were detected, they are at very low levels. To our knowledge, the vitamin and carotenoid content of aquafaba remains unknown [50].

The mineral profile of aquafaba from different legumes (chickpeas, green lentils, beans, yellow peas and soybeans) indicated that it contains large amounts of potassium (25-37 mg/100g), copper (0.06-0.17 mg/100g) and manganese (0.04-0.11 mg/100g), which is approximately 10% of the recommended daily intake (RDI) for each mineral. They are probably minerals located in the seed wall, as they could be transferred more quickly to the cooking water [46].

In addition, commercial canned chickpea NMR analysis revealed other compounds, including alcohols (isopropanol, ethanol, methanol), organic acids (lactic acid, acetic acid, citrate, succinic acid, formate, malate), amino acids (alanine), and nucleosides (inosine, adenosine) [10].

	Pulses												
Nutritional information (g/100g)	**Chickpea**									**Bean**	**Lentil**	**Pea**	**Soybean**
Moisture	-	94.97	-	-	-	-	92.98-95.12	94.90	-	-	-	-	-
Dry matter:	5.30	4.35	-	50.13	7.89	3.50-15.00	4.90-	5.10	5.13	3.28	4.69	4.41	5.59
- Protein	0.90	1.27	0.48	0.91	1.30	0.50-1.00	1.21-1.72	1.00	0.95	0.70;-; 3.19	1.51	1.27	0.68; 1.51-2.40
- Total carbohydrates	-	2.64	-	-	-	-	-	-	3.61	1.82;-; 5.97	2.70	2.74	4.12; 4.74-5.48
• LMW	-	-	-	-	-	-	-	1.20	1.20	0.73	0.54	1.02	1.66
• HMW	-	-	-	-	-	-	-	-	0.04	0.16	0.07	0.09	-
• Insoluble	-	0.69	-	-	-	-	-	2.40	2.37	0.93	2.09	1.63	2.46
- Ash	-	0.44	-	-	-	-	-	0.60	0.57	0.75	0.48	0.40	0.78
- Fat	-	<0.1	-	--	-	-	-	ND	<dl	<dl	<dl	<dl	<dl
- Saponins (mg/g)	1.01	-	-	-	--	-	-	-	4.50; 12.00	5.90; 7.90	12.00; 14.00	4.70; 9.80	6.40
- TPC (mg/g)	-	0.30	-	-	-	-	-	-	0.60	0.30;-; 0.16	0.70	0.60	-; 0.07 - 0.14
- Tannin (mgCE/g)	-	-	-	-	-	0.49-11.80	-	-	-	-	-		
- Phytic acid	-	-	-	-	-	0.00-0.06	-	-	-	-	-		
- Tocopherol (µg/ml)	-	0.11	-	-	-	-	-	-	-	-	-		
Soaking and cooking conditions	Soaking (1:4PWR, 12h, AT); cooking (pressure cooker, annealing pot, boiling water, micro-waving, 1:4PWR)	Commer-cial chickpea cans	Soaking (1:3 PWR, 24h); cooking (boiling water, 190 min)	Soaking (40ºC, 16h); cooking (pressure cooker, 1:3 PWR, 20min)	Commercial chickpea cans. For SDS-PAGE analysis: soaking (1:5 PWR,12h); cooking (boiling Milli-Q water, 1:5 PWR, 1h)	Soaking (40ºC, 2h); cooking (pressure cooker, 1:2,1:4 and 2:3 PWR, 15,30,45 and 60 min)	Commercial chickpea cans	Soaking (1:3.3 PWR,16h); cooking (boiling water, 1:1.75 PWR, 90min) [1] Without prior soaking; Cooking (pressure cooker, 2:3 PWR, 25 min at 101 kPa)					
References	[51]	[50]	[35]	[36]	[49]	[48]	[10]	[47]	[42]	[45], [46];[44][1]	[45], [46]		[43], [44][1]

LMW, Water-soluble low molecular weight carbohydrates; HMW, Water-soluble high molecular weight carbohydrates; TPC, Total phenolic compound; <dl, below detection limit; ND, no detected; PWR; pulses: water ratio

Table 2. Aquafaba composition from different pulses.

6.1.5. Factors affecting aquafaba composition

Aquafaba composition depends on two main factors, the first one is the cooking or canning conditions such as previous soaking, pulses:water ratio (PWR), temperature, pressure, and time cooking and added additives during its extraction. The second factor is the type of legume: its cultivar and genotipe, its seed and cell wall composition and structure, its crop conditions such as soil nutrition and biology, and environment conditions during plants growth, and also its the postharvest storage conditions (Figure 3) [8].

So, there are many parameters that vary the aquafaba composition, but with the studies carried out until now we can get an idea of what the composition profile is and what type of legumes are more nutritious.

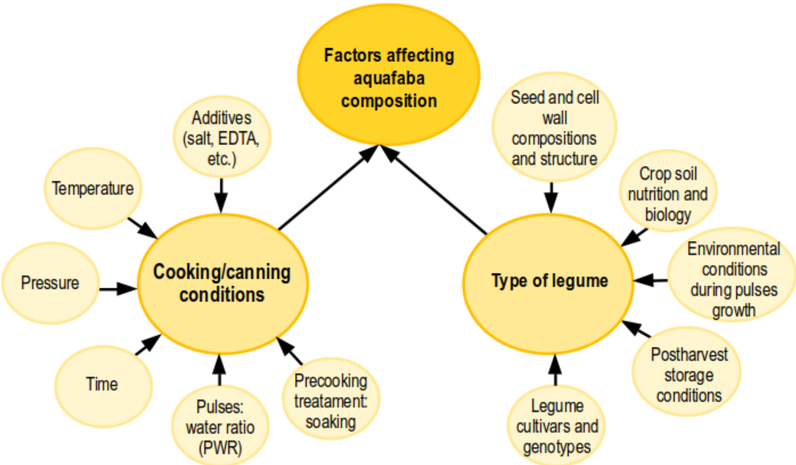

Figure 3. Factors affecting aquafaba composition.

6.2. PHYSICOCHEMICAL PROPERTIES OF AQUAFABA

The effectiveness of aquafaba to replace eggs functions depends on several physicochemical properties, apart from its the chemical composition. It is important do a detailed study in its electrical and rheological properties and its water and oil absorption capacity (WAC and OAC) [8], [34].

By studying these properties, we can have a better understanding of how to use aquafaba and the expected result of adding aquafaba as a texture.

Table 3. Aquafaba physical properties

	Chickpea	Haricot/lima bean	Green lentil	Split yellow pea	Soybean
pH	3.5-6.5; 6.26; 6.13 [35]; [45]; [49]	5.9-6.5; 6.07 [51]; [45]	6.47 [45]	6.39 [45]	6.07; 6.07-6.47 [43]; [44]
Viscosity (mPa·s)	47; 163; 5.7-114.2 [45]; [34]; [10]	4.5 [45]	25 [45]	8.7 [45]	45 [43]
Density (g/mL)	1.020; 1.01-1.18 [45]; [10]	1.017 [45]	1.025 [45]	1.021 [45]	1.25 [43]
Water absorption capacity (g/g)	1.46 [46]	0.07 [46]	0.13 [46]	2.2 [46]	1.54 [43]
Oil absorption capacity (g/g)	3.22 [46]	2.85 [46]	2.71 [46]	3.04 [46]	2.68 [43]

PWR from [51] is 1:4; from [34] is 2:3; from [35] varies from 1:1.5 to 1:5.0; from [49] 1:5; from [45] 1:75 and from [43] 1:75

6.2.1. Electrical properties

Aquafaba from chickpeas, beans, green lentils, split yellow peas and yellow soybeans have a slightly acidic pH with values between 3.5 and 6.5 (Table 3). These pH values can be affected by the legume:water ratio [35], [43], [45], [49], [51].

The pH of the solution, however, will be different from the pH at the surface of their charged particles. Most colloidal particles, such as proteins, are electrically charged. The pH of the surface of these proteins will depend on whether the pH of the solution is below or above the isoelectric point (*p*I). When the solution pH is lower than the isoelectric point of a protein, the surface is positively charged (due to the $-NH_3^+$ groups) and the protein surface pH should be higher than that of the solution. In contrast, when the pH is higher than *p*I, the surface of the protein is negatively charged (due to the $-COO^-$ groups) and the pH of the surface should be lower than that of the solution. Therefore, to a certain extent, the surface pH of a protein tends to self-adjust to the isoelectric pH [15], [49].

The existence of surface electric charge is the basis for understanding the stability of colloidal systems or the behavior of fluids. This stability is studied with the zeta potential (ζ), potential on the surface of moving colloidal particles, also defined as the potential difference between the dispersion medium and the stationary fluid layer attached to the dispersed particle (Figure 4). This is widely used to quantify the magnitude of the charge. If the potential | ζ | is high or relatively high (100 to 30 mV) means that the particles have a high surface charge. Having a high charge causes the degree of electrostatic repulsion between adjacent particles with similar charge in a dispersion to be high. A strong repulsion between particles will make the dispersion stable, not coagulate. On the other hand, if the potential | ζ | is low (below 30 mV), attractive forces can overcome this repulsion and will tend to come together coagulating, the colloidal system will not be stable [15].

The net surface charge of the aquafaba particle was evaluated, at different pH values, using a zeta potential analysis. Proteins at pH 8.5 carried a net negative charge (-2.5 mV) and towards pH 4.6 (isoelectric point) the net charge became 0 mV. As the pH decreased to 3, the potential increased to 0.3 mV and the particles carried a net positive charge [49].

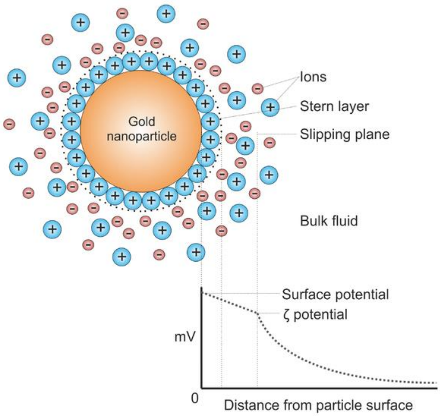

Figure 4. Interpretation of the zeta potential from Creative Commons.

6.2.2. Density

The aquafaba's density of the different legumes is similar, it only varies between 1.02-1.25 as we see in Table 3 [10], [43], [45]. A similar amount of insoluble carbohydrates (0.93–2.46 g / 100 g) (Table 2) could be responsible for these densities [43].

6.2.3. Rheological properties

Rheological properties of aquafaba provide information on its physical state (viscosity) and its gelling ability. It is important to know them to design the product that will include the aquafaba and/or the equipment that will be used for its manufacture. In other words, they are a determining factor of organoleptic characteristics (especially those related to texture) [8], [15].

The viscosity of the cooking water of the different legumes varies depending on the content of proteins and insoluble carbohydrates (especially cellulose and pectin fibers) [8], [34], [43]. In addition, it also varies significantly depending on the processing methods. Despite this, in general, the chickpeas and soybean aquafabas are quite viscous, while the aquafaba of beans, yellow peas and green lentils are very low viscous (the specific data can be seen in Table 3) [8], [43], [45].

In addition, chickpea's aquafaba has been shown to have a shear thinning effect and non-Newtonian pseudoplastic behavior (Figure 5). These concepts mean that the viscosity of this liquid decreases when shear stress is applied to it at high shear rates. The reason for this is due to the breakdown of agglomerated proteins (intrinsically disordered proteins or mis-folded proteins). These proteins align and orient, thus decreasing intermolecular frictions, and consequently the apparent viscosity decreases [33], [43]. This behavior can be described by an empirical model or a power equation (Newton's law) that establishes that:

$$\sigma = k \cdot \dot{\gamma}^{n} \quad \text{Equation 1}$$

where σ is the shear stress (Pa), $\dot{\gamma}$ the shear rate (s^{-1}), k the consistency index ($Pa \cdot s^n$) and n the flow behavior index (dimensionless) [15], [34].

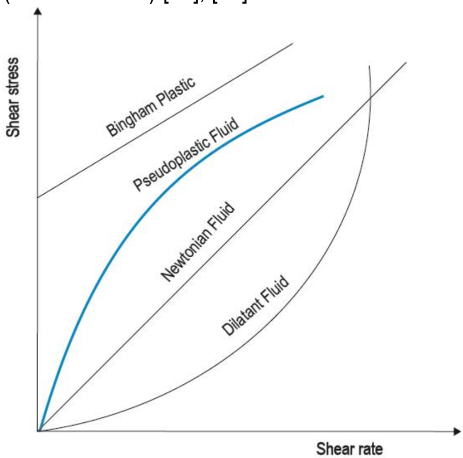

Figure 5. Types of fluid flow behavior.

In aquafaba, the consistency index or apparent viscosity coefficient (*k*) decreases as the shear rate increases, from 2.24 to 0.01 $Pa \cdot s^n$ [34]. And the flow behavior index has been found is <1, specifically to range from 0.23 to 0.69 [34]. These figures prove pseudoplastic behavior.

Higher concentrations of pulses make a higher apparent viscosity because the higher concentration makes it difficult for particles to move and increases molecular friction [33]. Finally, a longer treatment time decreases the viscosity, although this effect is often relatively small [33]. This explains, for example, that the highest apparent viscosity of aquafaba of chickpea (163 mPa·s) was at the highest concentration (2:3 PWR) and cooked in a pressure cooker and short cooking time (60 minutes) [34].

The gelling activity of aquafaba depends mainly on the protein content. During heating, proteins are denatured and aggregated by thickening the water and resulting in the formation of gels [33], [34]. Gels are semi-solid systems made up of small amounts of solids retained in relatively large amounts of liquid that impart more solid than liquid characteristics. The stiffness of the gel can be assessed by the dissipation factor (tan δ) which is the quotient between the elastic modulus and the viscous modulus. When tan δ < 1, the sample is a gel, and when tan δ > 1 the sample is a viscous elastic network [33], [34]. As a result, the aquafaba is not a gel, as low values of tan δ came out. However, the desired gel function can be obtained by optimizing the concentration and cooking pressure [33].

6.2.4. Water and oil absorption capacities (WAC and OAC)

The water and oil absorption capacities of aquafaba are influenced by the soluble/insoluble carbohydrate content, protein content and cooking time.

As we can see in Table 3, the WAC of cooking water for chickpeas, peas and soybeans is higher than that of lentils and beans. These values are related to the concentration of water-soluble carbohydrates contained in the aquafaba of the different legumes. Soluble carbohydrates, or oligosaccharides, are responsible for the absorption of water because they have a high affinity for it. As we saw in Section 6.1, these carbohydrates were more abundant in chickpeas, peas, and soybeans [8], [43], [46].

The OAC of aquafaba is high and similar among the different legumes. In this case, the absorption capacity of the oil is marked by the content of insoluble carbohydrates and proteins. All aquafabas of legumes contain similar significant amounts of these (Table 1). Proteins and fibers (cellulose, hemicellulose or lignin) are able to absorb oil as they contain lipophilic groups. In addition, the fact that proteins have been denatured when boiled can increase the affinity of the oil because it more easily exposes its hydrophobic sites [8], [43], [46].

Finally, increasing cooking time can reduce WAC, as it can cause more protein denaturation and decrease its ability to bind to water by exposing hydrophobic zones. Consequently, the OAC increases [33].

6.3. FUNCTIONAL PROPERTIES OF AQUAFABA

Aquafaba attracts attention mainly for its functional properties (emulsifying, foaming and gelling) that make it comparable to the properties of the egg. Unlike eggs, aquafaba works in a wide range of pH and temperatures, and it can be heated and cooled repeatedly without altering its functionality.

6.3.1. Emulsibility

Emulsions called oil in water (O/W), such as mayonnaise, can be created from aquafaba. In these emulsions, the oil is arranged in drops in the bosom of the aquafaba, separated from each other by a film of emulsifier. Being two immiscible liquids, they tend to separate in two phases due to the large surface energy they have. That is, they are thermodynamically unstable ($\Delta G > 0$). Therefore, the emulsions must be stabilized with the addition of an emulsifier. These protect the oil droplets formed, thus reducing the free energy of the system.

What determines the formation of an O/W emulsion, and not a W/O emulsion, is the nature of the emulsifier. According to Bancroft's rule, the phase in which the emulsifier is most soluble constitutes the continuous or dispersing phase. In this case, the main emulsifier is the proteins dissolved in the aqueous phase (aquafaba). Therefore, aquafaba acts as a dispersing phase [9], [15].

The aquafaba emulsion is stabilized by solid particles (Pickering stabilization), as we have said, mostly with proteins [9]. To achieve stabilization, the solid particles must coat the oil droplets. This is achieved with a strong mechanical energy that forces the incorporation of the oil in the bosom of the aquafaba. During these homogenizing forces, the proteins are adsorbed on the surfaces of the droplets, and they are deployed exposing their hydrophilic part to the aquafaba, and their hydrophobic part to the oil. This reduces the water-oil surface tension and develops the emulsion formation process [8], [9], [15]. At the same time, strong interactions develop between neighboring proteins, forming an interfacial cohesive film with mechanical properties similar to those of a three-dimensional gel. This viscoelastic film can extend and compress in order to absorb the deformations of the interface, which prevents a breakdown of the colloid [15].

Apart from proteins, saponins are also thought to play an important role in stabilizing emulsions. Saponins can also be adsorbed at the water-oil interface by mitigating unfavorable interactions between the two phases. They are considered surfactants, compounds that reduce the interfacial tension between the two liquids [45], [46], [52].

Aquafaba polysaccharides can also provide emulsifying stability. Non-adsorbed polysaccharides generate an increase in the viscosity of the aqueous phase thus delaying the movement of the droplets and preventing breakdown. Whereas, the adsorbed act at the interface forming a physical barrier between the drops, thus delaying or preventing the collision of the drops [53]. In addition, they can also interact with proteins to form protein-carbohydrate complexes. These complexes are predominantly negatively charged and stabilize the repulsive forces on the surface of droplets, which increases emulsion stability and decreases flocculation and creaming [44]. Also, due to the intense pressure and temperature applied during the legumes cooking, a more disordered protein-polysaccharide complex could form due to the Millard reaction. These complexes can form a thick film that is adsorbed at the oil-water interface, thus improving emulsion capacity and stability [44].

There are several instability mechanisms that cause the emulsion to break down. One of them is flocculation, a process that the droplets are joined by van der Waals forces to form aggregates, the repulsive forces are overcome. Another is the maturation of Ostwald, a process in which large drops grow at the expense of the integration of smaller drops, a problem due to the greater solubility of small drops in the continuous phase. These two mechanisms facilitate coalescence, an irreversible union of droplets to form large droplets that destabilize the system and subsequently cause phase separation. It can also suffer sedimentation or creaming, produced by the different densities between the phases that move with the influence of gravity. Sedimentation represents the displacement of the dispersed phase downwards, and in creaming the dispersed phase goes upwards. These mechanisms also lead to a separation of the phases. Finally, a phase inversion can occur, moving from O/W to W/O, due to a change in temperature, component concentration, or the addition of a new component to the system (Figure 6) [15], [54].

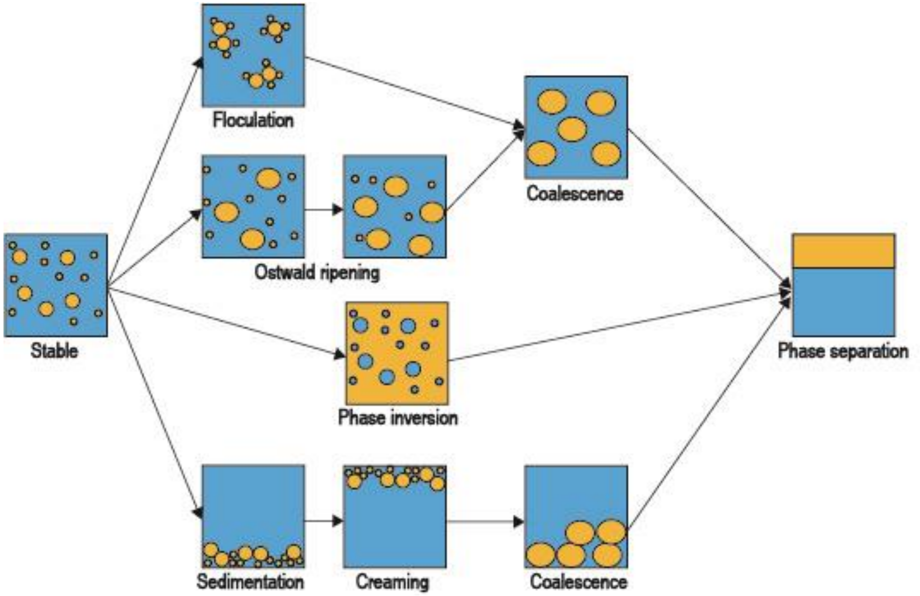

Figure 6. Breakdown of emulsions.

Emulsifying properties can be quantified by emulsion capacity (EC), emulsion stability (ES), and emulsion activity index (EAI). EC is defined as the percentage of emulsion generated [46] and it can be calculated using equation 2.

$$\%EC = \frac{h_f}{h_i} \times 100 \qquad \text{Equation 2}$$

where h_f is the height of emulsified layer after homogenization and h_i is the total height of mixture before homogenization.

ES is expressed as the percentage of emulsion remaining after homogenization [32], [35]. It is calculated with equation 3, measuring the aqueous phase generated after a phase inversion or collapse of the emulsion. The higher the volume of the aqueous phase, the less stable the emulsion.

$$\%ES = \frac{v_{t=0} - v_{t=x}}{v_{t=0}} \times 100 \qquad \text{Equation 3}$$

where, $v_{t=0}$ is the dispersing phase before homogenization and $v_{t=x}$ is the aqueous phase volume after x min.

And finally, using Equation 4, we can calculate the EAI. This index tells us the ability of the emulsifier to cover the oil-water interface area [43], [46], [49], [55]. A high EAI is more functionally positive for foam.

$$EAI(cm^2/g) = \frac{4.606 \, x \, A_0 x DF}{\emptyset \, x \, C} \qquad \text{Equation 4}$$

in which A_0 is the emulsion absorbance at 0 min, *DF* is the dilution factor, Ø is the oil volume fraction of dispersed phase, C is the emulsifier concentration and 4.606 is the interfacial area of the emulsion.

In order to compare these parameters of aquafaba with those of eggs, we know that EC and ES are 115% [75] and 92% [76,] respectively.

6.3.1.1. Factors affecting the emulsifying property

The EC, ES and EAI properties of aquafaba are affected by many factors that need to be taken into account when producing it. These factors are:

1. Chemical composition of aquafaba:

As we have seen in section 6.1.5., the composition of the aquafaba also depends on many parameters. Controlling these parameters during the preparation of aquafaba is important to improve the emulsifying properties.

a) Type of legume:

EC and ES varies depending on the type of legume. In general, the ES does not have many differences between the different legumes [44], but EC does have them, black soybean and small black beans have a higher capacity, followed by chickpeas (Table 4).

Table 4. EC, ES and EAI from aquafaba of different types of legumes.

Type of legume	Chickpeas	Haricot beans	Whole green lentils	Split yellow peas	Yellow soybeans	Black soybeans	Small black beans
EC (%)	54.20; 68.5	46.30	52.70	49.00	49.30; 75.48	80.76	78.89
ES (%)	-; 74.50	-	-	-	-; 72.58	76.85	75.11
EAI (cm^2/g)	38.60	22.80	47.10	16.00	20.30	-	-
References	[46]; [44]		[46]		[43], [44]	[44]	

b) Legume variety:

The stability of the emulsion also changes depending on the variety of legumes. In a study of 5 different varieties of chickpeas, it was found that Kabuli Leader and Amit chickpeas have better stability than Orion, Consul and Luna chickpeas (Table 5) [55]. And in another study, which compared the emulsifying capacity of aquafaba of three types of Korean soybeans, Backtae soybean (yellow soybeans) had better properties than Seoritae and Jwinunikong soybeans (Table 6) [56].

Table 5. ES from aquafaba of different Kabuli chickpea variety [55].

Kabuli chickpea variety	Leader	Amit	Orion	Consul	Luna
ES (%)	77.1	76.4	75.6	74.7	71.9

Table 6. EC from aquafaba of different Korean soybeans variety [56].

Korean soybean variety	Backtae	Seoritae	Jwinunikong
EC (m^2/g)	1.18±0.04	1.03±0.01	1.04±0.05

c) Cooking conditions (pH, pulses:water ratio, soaking, pressure):

The effect on pH change requires further research as contradictory results are shown between different articles (Table 7). Some have found that EC and ES increase at a lower pH. This is because at low pH proteins increase their net charges, thus weakening hydrophobic interactions but increasing their flexibility. Flexible proteins can reduce surface tension and thus improve the formation and stability of emulsions [35]. In contrast, others have observed higher EAIs at pH> 6. They conclude that at a pH near or below the isoelectric point (4.6) the forces of attraction between the proteins adsorbed in the interracial zone are weak due to the zero net charge. In addition, as the pH decreases, a decrease in the average diameter of the droplets forming the emulsion was observed [49]. A smaller droplet diameter will prevent the emulsion from sedimentating or creaming [15].

Table 7. EC, ES and EAI from chickpea's aquafaba at different pHs.

pH	3.5	5	6.5	3	4.5	6	7	8.5
EC (%)	15.7±0.0 -72.3±1.0	6.3±1.2 -62.8±1.5	3.9±0.0 -58.1±0.5					
ES (%)	0.0±0.0 -76.3±2.3	0.0±0.0 -66.0±2.7	0.0±0.0 -32.7±2.3					
EAI (m^2/g)				~13	~13.5	~17.5	~16	~16
References		[35]				[49]		

The proportion of legume:water (PWR) also affects. A low proportion (e.g., 1:1.5), which means a higher concentration of protein in aquafaba, increases the emulsifying properties (Table 8).

Table 8. EC and ES from chickpea's aquafaba at different PWR [35].

Chickpea:water ratio	1:1.50	1:3.25	1:5.00
EC (%)	58.1±0.5 -72.3±1.0	40.1±1.4-61.5±0.9	3.9±0.0-15.7±0.0
ES (%)	32.7±2.3-76.3±2.3	12.5±1.8-44.8±3.4	0.0±0.0-0.0±0.0

Presoaking has an important role in the effect of the functional properties of Aquafaba. The EC of the Aquafaba samples produced with a previous soaking of the legumes, is significantly higher than that obtained without presoaking. However, the ES of the aquafabes without a previous soaking is higher than those produced with a soaking (Table 9). This is because ES is inversely correlated with aquafaba moisture content. Therefore, presoaked aquafaba samples, having a higher moisture content, have a lower emulsion stability than non-soaked ones [56].

Table 9. EC and ES from aquafaba of Canadian chickpeas and Korean soybeans with/without presoaking [56].

	Canadian Chickpea		Korean Soybeans	
Conditions	With presoaking	Without presoaking	With presoaking	Without presoaking
EC (m^2/g)	1.39±0.05	0.78±0.03	1.03±0.01-1.18±0.004	0.78±0.03 – 0.84±0.02
ES (%)	74.91±0.94	78.05±1.66	80.38±4.39 - 89.49±3.72	94.66±0.46 – 96.80±1.06

In addition, the properties of the aquafaba also vary depending on the soaking time and temperature. One study standardized chickpeas soaked in water for 16 h at 4 ºC, and with a small percentage of added baking soda, followed by cooking for 30 min at 116 ºC, give better emulsifying properties to aquafaba (EAI of 1.30 ± 0.05 m2/g) [42]. Baking soda is used to soften the seed coat and cotyledons and increase the concentration of seed compounds in the cooking water [42].

Finally, high pressure and cooking time also improve properties [35]. A very short cooking time do not soften the walls of the chickpea seed enough to speed up protein leaching, limiting the concentration of aquafaba solid material and emulsion properties. And, it should be noted that, a very long cooking time could also reduce the emulsion properties, as heat and water would damage the cell walls by allowing larger molecular compounds to be transferred to the aquafaba [42].

d) Concentration of proteins:

Protein concentration has a positive effect on emulsion stability. A high concentration of protein is important to accomplish its function as an emulsifying agent [9].

e) Concentration of saponins:

As already mentioned, saponins improve the emulsifying capacity of legumes' cooking water. Thus, the higher the saponin content, the higher the emulsion capacity of the aquafaba. The lower concentration of saponins in split yellow peas and beans could explain the greater fragility of their emulsions [46].

2. Post-treatment of aquafaba

a) High pressure processing (HPP):

On top of being a food preservation technique that inactivates harmful pathogens and microorganisms through the use of pressure, it is also used to alter the functional properties of proteins. Proteins can be denatured with HPP in the absence of high temperature [57]. Denaturation is beneficial because this way proteins can interact with both phases of the interface. For this reason, a high-pressure level and high pressurization time had a positive effect on the capacity and stability of the emulsion. In addition, high pressure decreases the size of the particles and prevents coalescence [9].

b) Ultrasonic processing:

Ultrasound can also be used to improve the functional properties of food proteins and emulsification [54]. One study applied ultrasound to aquafaba and concluded that it increased the emulsion capacity, especially at higher power intensities and processing times. This is due to the fact that ultrasound can increase the denaturation of proteins, which facilitates their adsorption at the water-oil interface. In none of the sonicated samples in the study was there phase separation, either after 1, 12, 24 h or 4 days. The EAI had no significant difference compared to the egg yolk [36].

3. Additives

a) NaCl:

EAI decreases with the addition of NaCl. With the addition of added salt, there is a decrease in the ζ potential, attributed to the accumulation of Na^+ counterions around the surfaces of the negatively charged droplets. A low ζ potential indicates system instability [58].

But as we will see in the addition of salt to foam (Chapter 6.3.2.1. 3. c), a small amount of salt could slightly increase the emulsifying capacity [15].

b) EDTA:

It can increase the viscosity, which is beneficial for the stability of the emulsions [34].

6.3.1.2. Rheology of aquafaba emulsions

The rheological character of aquafaba emulsions is non-Newtonian pseudoplastic, but in highly concentrated emulsions it can become plastic and have viscoelasticity [15]. A high concentration may be due to the association of proteins and polysaccharides, which increases the viscosity of the system to a viscoelastic behavior. These protein-polysaccharide complexes can form networks at the air / water interface that promote the stability of the foam [8].

The viscoelastic behavior of the emulsions is analyzed by the yield stress value (Pa). This stress is defined as the force per area required to deform the system constantly. A food system exhibits different behavior depending on whether the stress applied to it is above or below its level of performance stress. The yield stress (Pa) of aquafaba emulsions was affected by the change in pH. At acidic pH values, the yield voltage was higher (> 50 Pa) than at basic pH (<47 Pa). That is, at neutral and basic pH, emulsions require less force to exceed their yield stress than at acidic pH values. Therefore, the more basic the pH indicates less stability. It was also observed that aquafaba-based emulsions have a significantly higher yield stress value than egg white-based emulsions [49].

6.3.2. Foamability

Foams are colloidal systems made up of air bubbles dispersed in a liquid, in this case, aquafaba. The liquid separates the adjacent gas bubbles in the form of thin layers of liquid, also called lamella. Due to the large extent of the surface of the aqueous phase, the foam has little stability, has a tendency to break due to sedimentation of the liquid, gravitational forces cause the liquid to drain. In addition, because the air is essentially apolar, there is an ordering of the aquafaba molecules that leads to high surface tension and energy of the system [15]. These two facts mean that the foam is also thermodynamically unstable. Its breakup causes a decrease in the free energy of the system [35]. Therefore, the role of the foaming or stabilizing agent is also essential in this case to increase the stability of the foam.

The formation of a foam is similar to the formation of an emulsion. The forces involved in incorporating air into the aqueous phase are similar to those needed to mix oil with water. The formation of the foam is done by a beating of the solution, a mechanical action. The whipping causes the air to be retained in the viscous liquid in the form of large bubbles. With a long whipping time, the bubbles break into smaller bubbles and increase the volume of the foam. The diameter of the foam bubbles usually ranges from 1µm to a few centimeters. The size of the bubbles marks the density of the foam [15].

Air bubbles are at ~10^3 times larger than the drops in the emulsion, as a gas is more soluble in water than oil. But a foam with very large bubbles has a tendency to drain or form a cream because, apart from the size of the bubbles, the difference in density between the phases of the foam is 10 times greater than that of the emulsion [15].

For the same reasons as in emulsions, foaming agents are the solid particles of aquafaba (especially proteins, polysaccharides and saponins) [9], [15], [40]. In the case of proteins, the whipping helps them to deployment by facilitating their adsorption at the water-gas interface [15].

The main cause of the breakage of a foam is the reduction in the thickness of the lamellas (critical point: 5-15nm thickness) which causes coalescence. This loss of thickness may be due to the force of gravity, to the forces of deformation exerted by the movements of the gas (diffusion) or to a capillary drainage [15]. Coalescence due to thinning of the lamellas can be prevented in three ways:

i. **Increasing the elasticity of the lamella.** An increase in elasticity results in the lamella being able to counteract external forces that deform it in order to reduce surface tension. The elasticity increases with the increase of foaming agent molecules adsorbed on the surface of the lamella.

ii. **Increasing the viscosity of the aqueous phase**. The association of polysaccharides and proteins on the surface to form larger entities increases the viscosity of the liquid phase. Higher viscosity reduces the rate of liquid drainage and retains more air bubbles. Sometimes sugar is added to the mixture to increase the viscosity.

iii. **Increasing of protein concentration.** The more protein there is, when the lamella thins and the molecules get closer to each other, electrostatic repulsion can be experienced avoiding coalescence. However, overcharging should be avoided, as this would lead to charge repulsion during the foaming process, with incomplete coating of the air bubbles.

As in emulsions, the capacity (FC) and stability (FS) parameters of the foam can be defined. First, we will define as FC the volume of foam generated as a percentage of the initial volume of solution [36], [49] and it is calculated using the following equation 5:

$$\%FC = \frac{v_{foam}}{v_{liq}} \times 100 \qquad \text{Equation 5}$$

where $v_{foam,\,t}$ is the volume of foam after whipping and v_{liq} is the initial volume of dispersing phase.

And we can define FS as the percentage of the volume of foam that remains over time [32], [35], [59], or with the percentage of liquid that has drained over time after beating [36], [49].

$$\%FS = \frac{v_{foam,t}}{v_{liq,t=0}} \times 100 \qquad \text{Equation 6}$$

where $v_{foam,\,t=0}$ is the volume of foam just after whipping and $v_{foam,\,t=x}$ is the volume of foam remaining after t=x min.

$$Liquid\ drainage = \frac{v_{liq} - Dv_{liq,t=x}}{v_{liq} - Dv_{liq,t=0}} \qquad \text{Equation 7}$$

where v_{liq} is the initial volume of the dispersing phase samples before whipping, $Dv_{liq,\,t=0}$ is the volume of liquid drainage at time t=0 min after whipping and $D_{vliq,\,t=x}$ is the volume of liquid drainage after t=x min.

In order to compare these parameters of aquafaba with those of eggs, we know that FC and FS are 684%– 311% [18],[59], and 98% [59], respectively.

6.3.2.1. Factors affecting the foaming property

The following describes the factors that affect the newly defined FC and FS parameters:

1. Chemical composition of aquafaba:

a) Types and varieties of legumes:

It is clear that there will be significant differences between different types of legumes, because the chemical composition changes in each. The foaming capacity of green lentils, yellow peas and soybeans seems to be greater than that of chickpeas, and beans (Table 10). But none can be compared to the great foam capacity of the egg white [43], [45]. However, other factors that affect this ability need to be considered. According to one study, some brands of canned chickpeas have a foaming capacity of 297%, which is comparable to egg white (311%) [32].

Table 10. FC from aquafaba of different types of legumes.

Type of legumes	Chickpeas	Haricot beans	Whole green lentils	Split yellow peas	Yellow soybeans	Egg white
FC (%)	58±7; 443±14	39±2	97±6	93±12	65±2; 544±6	400±49
References	[45], [56]		[45]		[43], [56]	[45]

b) Cooking conditions (pH, pulses:water ratio, soaking, pressure)

As emulsifying properties, soaking legume before cooking has significant effects on foaming. Presoaked aquafaba samples have a significantly higher FC than those that have not been presoaked (Table 11). And there are no significant differences in FS with cooking conditions with or without presoaking. The FS is generally above 80% (82.35±1.1 - 91.34±1.23 %) [56].

Table 11. FC from aquafaba of Canadian chickpeas and Korean soybeans with/without presoaking [56].

	Canadian Chickpea		Korean Soybeans	
Conditions	With presoaking	Without presoaking	With presoaking	Without presoaking
FC (%)	443±14	311±10	544±19 - 567±6	422±14 – 437±23

The pulses:water ratio (PWR) also has a significant effect on FC and FS. A higher PWR (e.g., 1: 5) means that it contains a lower protein content. Due to the importance of proteins as foaming agents, it will have worse foaming capabilities and stabilities. However, a very low PWR (e.g., 1: 3), also does not ensure good foaming properties. Having a low water level causes the pulses to break, releasing starch granules that break the foam membranes. Therefore, the best FC and FS are obtained at PWR averages (e.g., 1: 4) (Table 12) [35], [59].

Table 12. FC and FS from chickpea's aquafaba at different PWR [59].

Chickpeas:water ratio	**1:3**	**1:4**	**1:5**
FC (%)	510.00±17.30	610.67±10.10	504.71±10.0
FS (%)	81.17±1.15	85.55±1.18	82.10±0.99

Cooking time also affects the properties of the foam. A longer cooking time results in higher FC and FS. This is because a longer cooking time allows a greater migration of soluble protein from the seed into the cooking water [32], [44], [45]. In addition, heat treatment also aids in the deployment of proteins, causing protein bonds to adhere to bubble interfaces [51].

Apart from PWR and cooking time, the method used to cook legumes also varies FC and FS. According to a study, high pressure (pressure cooker) does not promote foaming [51]. It appears that complete denaturation of proteins, caused by increased pressure, exposes hydrophobic groups to the outside too soon and there is a loss of their solubility [60]. It concluded that the method of cooking with a normal pot and the microwave are the most effective methods for producing foams (Table 13) [51].

Table 13. FC and FS from chickpea's aquafaba at different cooking methods [51] .

Cooking methods	**Using pressure cooker**	**Using annealing pot**	**Cooking for 30 min**	**Cooking for 15 min + microwaving for 15 min**	**Cooking for 60 min**	**Cooking for 45 min + microwaving for 15 min**
FC (%)	348.33± 22.48	410.00± 17.80	450.00± 25.00	438.33± 16.07	626.67± 15.28	660.00± 15.00
FS (%)	23.00± 1.72	46.53± 6.01	40.98± 2.41	48.89± 2.95	82.08± 2.83	79.85± 6.43

c) Protein concentration:

There is a strong correlation between protein content and foaming capacity. As we know, proteins are the main foaming agents that stabilize foams. Therefore, the higher the protein concentration, the better the foaming properties [45].

2. Post-treatment of aquafaba

a) Ultrasonic processing:

Ultrasonic treatment to aquafaba improves its foaming capacity (from 259%, for the untreated sample, to 548% for the treated sample with greater power and longer pressurization time) and stability (from 45%, for the untreated sample, 93%, for the treated sample with greater potency and longer time). In addition, the foaming capacity of the ultrasound-treated sample of higher power intensity (67 W/cm^2) can be compared with that of the egg white (610% at natural pH, 510% at pH 4) [36]. During sonication, proteins are partially denatured by exposing their hydrophobic parts. This increases the adsorption of proteins at the air-water interface and reduces surface tension [61]. It also decreases the size of protein-protein aggregates, so faster protein particles will be adsorbed at the interface [36].

3. Foam processing conditions:

a) Whipping time:

Increasing the whipping time improves the stability and capacity of the aquafaba foam, while for the egg white, an increase in the beating time negatively affects the properties of the foam. As we can see in Figure 7, it is necessary to whip the aquafaba for at least 5 minutes to obtain properties similar to egg white [32].

However, after reaching the maximum capacity of the foam, an excessive whipping would be counterproductive. The stability of the foam would decrease due to excessive protein deployment leading to coagulation of the proteins at the air-water interface. This coagulation would lead to a loss of water retention capacity [15], [32].

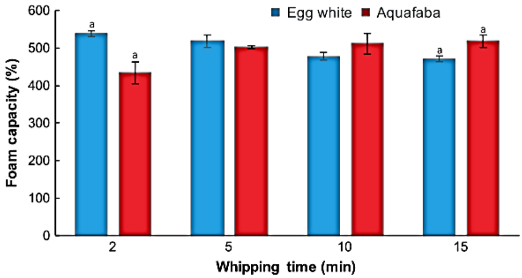

Figure 7. Influence of whipping time on foaming capacity of egg white and aquafaba. [32]

b) pH (lemon juice or Potassium bitartrate addition)

There are also conflicting opinions about the change in pH in the formation of foam. According to some studies, FC and FS are maximum at pH values close to the isoelectric point of the soluble proteins involved [15], [49], [59], and according to others they are maximum at pH more acidic or much more basic than the *p*I [35], [51] (Table 14).

At pH near *p*I (e.g., 4 or 4.5), as the net protein charge is zero, the repulsive forces between protein molecules are not sufficient to inhibit the formation of protein aggregates. In this way, the viscosity of the lamellas increases and helps to retain air bubbles, slowing down its the movement to avoid them crash into. Foam stability increases [15], [49], [59].

On the other hand, at lower or higher pHs of the *p*I (e.g., 3.5 and 5) the net charge of the proteins increases. At this pH hydrophobic interactions weaken, there is less protein clustering, but flexibility increases. This allows the proteins to spread more quickly at the air-water interface to retain the bubbles and improve the stability of the foam [9], [32].

In both cases, if you want to lower the pH of the aquafaba, it is advisable to add a small amount of lemon juice or cream of tartar [15], [51].

Table 14. FC and FS from chickpea's aquafaba at different pHs.

pH	**3**	**4**	**5**	**3.5**	**5**	**6.5**
FC (%)	636.06±7.21	650.33±6.51	610.67±7.57	294±1 - 324±15	162±1 - 270±3	175±7 - 199±3
FS (%)	86.61±0.35	91.55±0.51	87.82±0.15	78.3±1.7 - 93.4±1.9	36.9±0.6 - 70.2±3.4	3.4±3.1 - 44.8±4.0
References		[59]			[35]	

3	4.5	6	7	8.5	4	5
~109	~106	~92	~94	~90	521.67±22.55 - 550.00±43.59	621.67±22.55 - 638.33±16.07
					72.66±4.82 - 78.23±2.8	79.63±0.82 - 84.77±1.01
		[49]			[51]	

c) NaCl addition

The FC and FS depend a lot on the salt concentration that is added to the foam. If a low concentration is added, the salt ions interact with the ionic charges of the proteins, creating a contraion screen that solvates the proteins (a phenomenon known as "salting-in"). In this way, the solubility of the proteins increases and they move more easily towards the air-water interface [62]. Good adsorption of proteins at the interface decreases surface tension and stabilizes the colloid.

In contrast, at a higher salt concentration (C>1M), the high ionic strength decreases the solubility of proteins, they coagulate and precipitate (a phenomenon known as "saling-out") [15]. This phenomenon is a consequence of the union of water molecules with salt ions to such an extent that there are not enough water molecules left to hydrate the protein molecules [49]. There is a competition for solvation water. Due to the low degree of solubility, proteins do not move easily at the air-water interface to reduce the surface tension [59]. The foam is destabilized.

We can see the two phenomena (salting-in and salting-out) represented in Figure 8:

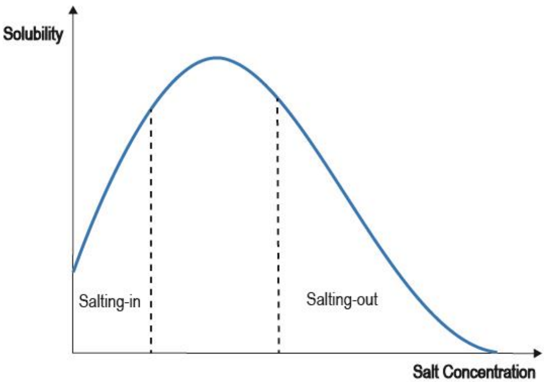

Figure 8. Salting-in and salting-out.

d) Sugar and Xanthan gum addition

Both sugar and Xanthan gum are polysaccharides that increase the viscosity of the aqueous phase due to the association of the polysaccharide with proteins. Higher viscosity increases FC and FS as it reduces the rate of liquid drainage and retains more air bubbles. Thus, these two additives are perfectly suitable for improving the properties of foams. [15], [59]

6.3.3. Gelling

Gelling and thickening properties are responsible for the rheological properties of food. These properties are attributed to increased viscosity, increased adhesion and improved water retention. This is due to the formation of a three-dimensional network of proteins and polysaccharides formed during the cooking of legumes [8]. With heat treatment, proteins are completely or partially denatured and interact with polysaccharides to form this solid ice network, but with many characteristics of a fluid, as it retains water molecules [63]. Heat also induces the Maillard reaction (chemical reaction between amino acids and reducing sugars), under specific conditions, to complex structures and improved ice characteristics [40]. Only water-soluble polysaccharides, such as pectin, and simple sugars contribute to the gelling of aquafaba [40].

In uncooked foods such as mousse, aquafaba demonstrates gelling properties comparable to those of egg whites. However, in cooked foods such as meringue, aquafaba has a low gelling capacity due to its low dry matter content, which is mostly insoluble fiber [45].

6.3.3.1. Factors affecting gelling property

The gelling properties also play an important role in the stabilization of emulsions and foams. Although little is known about the gelling properties of aquafaba, several articles have noted some factors that affect gels. We see them below:

1. **Chemical composition of aquafaba**

a) Types and varieties of legumes

The gelling capacity differs according to the type of legume and the variety. For example, the gelling capacity of chickpeas and soybeans has been found to be higher than other legumes [43]. Also, Kabuli chickpeas have a greater ability to form ice than Desi chickpeas [40].

b) Cooking conditions

The gelling properties also depend on pH and temperature. High heat treatment improves the gelling properties, although overheating can cause weak gel [40]. These altering factors are supposed to depend on the composition of the legumes being cooked and the interactions that take place during the heat treatment.

c) Soluble/insoluble fiber content

As we have already mentioned, only soluble fibers contribute to the gelling of aquafaba, and the insoluble fibers are inversely correlated [43].

6.4. AQUAFABA USES

Many food recipes have been created using aquafaba to replace or minimize the use of eggs. There are even explicit books from a collection of recipes that use aquafaba [64]–[68]. With aquafaba, it is possible to make pastries, baked goods, ice cream, sauces, and even cheeses. However, commercial production of aquafaba-based foods is still limited due to little scientific research.

Below, we will look at aquafaba-based food products that have been scientifically studied (Figure 9) and see some related commercial products.

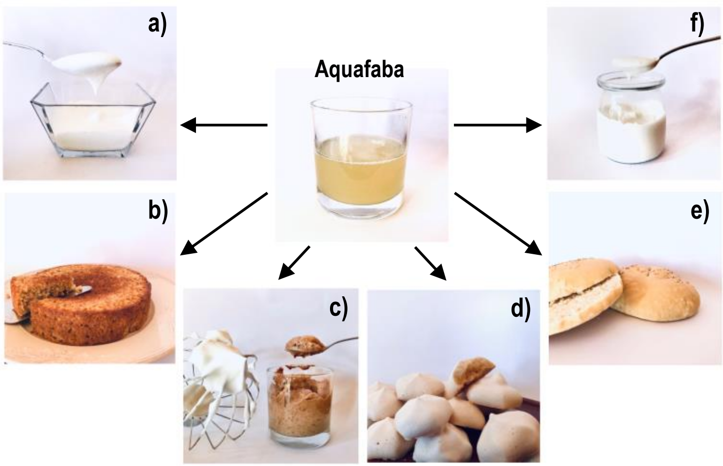

Figure 9. Aquafaba-based foods own images.

a) **Mayonnaise**

Due to the emulsifying and stabilizing properties of aquafaba, it can be used as a substitute for egg yolk in mayonnaise.

The aquafaba was able to form a stable emulsion comparable to that of the egg. The best stability of the emulsion was obtained with an aquafaba:oil ratio of 15:80% [50]. However, the stability over time of aquafaba mayonnaise (21-28 days at 4 °C) [32], [50] is less than that of egg yolk mayonnaise (6 months refrigerated) [50]. This is due to the polydispersity (different sizes) of the oil droplets dispersed in the aqueous medium, which over time can cause instability due to a maturation of Ostwald [32], [50]. In contrast, the stability of egg mayonnaise is higher due to the presence of a highly efficient emulsifier, egg lecithin, which is able to reduce the inter-phase tension between the dispersed and continuous phases [50].

Hardness (strength needed to compress food between molar teeth), adhesive strength (strength needed to overcome the forces of attraction between mayonnaise) and adhesiveness (energy needed to separate mayonnaise from spoon) decrease with the increase of the aquafaba:oil ratio [50], [69]. However, the cohesion or consistency (strength of the internal bonds of the mayonnaise and the degree to which it can be deformed before breakout) was not affected by the aquafaba:oil ratio [50].

Finally, the sensory assessment of mayonnaise, assessed by consumer acceptability, showed no significant differences in aroma, texture, or taste [35], [50], [69]. It only presented a different color; aquafaba mayonnaises have a higher color intensity than egg mayonnaises [35].

There are many commercial mayonnaises made with aquafaba such as Vegan Mayonnaise by Naked Byron Foods, Classic Fabanaise by Sir Kensington's, Vegan Mayo Classic by Veggie-Naise, Aquafaba Mayo by Rubies in the Rubble and Classic Vegan Mayo by Chosen Foods.

b) **Cake/cupcake**

Aquafaba is a potential substitute for egg whites or whole eggs in cake and cupcake recipes. In the formulation of these, aquafaba is added for its foaming and texturizing properties [8].

The nutritional composition, appearance, texture and color are very similar to those of egg-containing cakes. However, elasticity and cohesion are lower than for egg whites [32]. Because of these drawbacks, there are articles that have suggested only replacing a maximum of 50% of eggs with aquafaba, as it gives a better uniformity [51], [70].

There are other aspects that have been improved with the addition of aquafaba, such as loss of density (a more porous result) and weight loss [32], [51].

In terms of color analysis, the crust of the cake or cupcake made with aquafaba may be a darker color due to Millard's reactions (caramelization) between simple sugars and aquafaba proteins [32], [51].

The English trademark OGGS® makes various types of cakes and cupcakes made with aquafaba.

c) Mousse/whipped cream

Due to the foamy and emulsifying properties of the aquafaba, it has been possible to find a texture that very well simulates the whipped egg white and a mousse. In other words, no significant differences in appearance (texture and color), aroma, or palatability were found in the studies [46], [59].

In terms of taste, although there are no significant differences with mousse made with egg white, it may have a slight bitterness and salty taste that could be correlated with the concentration of calcium, sodium and saponins in legumes [46].

However, egg white foam is more stable because the foam particles have a smaller size and a thicker membrane [59].

d) Meringue

Aquafaba of chickpeas and yellow peas can replace egg whites in meringues due to their frothy and gelling properties [45]. The meringues show palatability and color comparable to the egg white meringue. However, it has significant differences in hardness and consistency, which are lower [35], [45].

One study affirms that ultrasonic treatment of aquafaba improves the sensory properties (taste, texture and color), stability and emulsifying activity rate of meringue compared to meringue made with untreated aquafaba. It also significantly increased hardness, consistency, and adhesiveness [36].

Aquafaba-based meringue powders have been commercially developed to facilitate their production as Vegan Meringue Powder by Vör® and Meringue Powder by Meringueshop.

e) Bread/crackers

Aquafaba can also be used to improve the texture of gluten-free bakery products due to its gelling properties and high water retention capacity.

Making gluten-free bread with aquafaba can improve its properties. A softer texture can be achieved by reducing the hardness of the spring and improving the homogeneity of the spring and the retention of gases, possibly due to the emulsifying activity of saponins and insoluble fiber. Insoluble fiber can act as a stabilizer of the protein network, and saponins act as surfactants by delaying the recrystallization of starch [47].

In a study of gluten-free crackers made with aquafaba, many benefits were obtained with respect to the same recipe without aquafaba. Soy aquafaba improved the texture, increasing the softness and lightness, and also increased the moisture content of the product, probably due to the high water adsorption capacity. Having more moisture prevents them from hardening when stored for 2 days at room temperature. Also, the addition of aquafaba to the recipe improved the color of the crackers. Gluten-free crackers tend to be pale in color due to their low protein content and therefore lower Maillard reaction. As it has more protein, it reacts with polysaccharides, giving it a more yellowish color. The only disadvantage was that they had a higher breaking rate due to their weak protein-starch network resulting from the foaming and emulsifying capabilities of soy protein [43].

f) Oat yogurt with aquafaba

The use of aquafaba in the production of yogurt with oat milk was used to improve its consistency and texture due to its gelling character. The results of the recipe study showed a reduction in syneresis (spontaneous separation of a colloidal system) and an increase in water retention capacity during storage. These two aspects are attributed to the significant increase in added solids, proteins and fibers. The increase in solids and fibers strengthens the yogurt by increasing the uptake of water within the ice network, reducing the syneresis [71].

Apart from the commercial products we have named, there are also several brands that sell natural aquafaba, such as Aquafaba by Vegadénia and Egg alternative Aquafaba by OGGS®; and aqufaba powder, Aquafaba Powder by Vör®.

Finally, thanks to the good properties of aquafaba, it can also be used for many other products without the need to replace eggs. The gelling properties of aquafaba can be used to create a substitute for meat [72]. And its emulsifying properties could be used to make natural cosmetics [40].

6.5. ENVIRONMENTAL IMPACT

An environmental impact study should be carried out before conducting a market study to replace the eggs with aquafaba. It is true that the mere fact of not wasting this byproduct is already a sustainable practice, and post-treatment of wastewater is avoided. However, a study evaluating the environmental impact of mayonnaise production concludes that egg-based is more sustainable than aquafaba-based [73]. Aquafaba mayonnaise would only be environmentally sustainable if renewable energy sources were used to cook the legumes [73]. What this study does not take into account, however, is the animal suffering of the hens and the large amount of wasted aquafaba produced as a result of the production of other products. Aquafaba could be recovered from the cooking water of legumes cooked for cans or for the production of hummus. If we used this byproduct in liquid form without any additional treatment, we would save energy and costs. In addition, legumes that are squandered in the screening process, because they do not meet the classification standards (although they generally have a normal biochemical composition), could be used to produce aquafaba. The rest of the cooked seed could be used for animal feed or dried and used as chickpea flour for other applications [40].

It is unknown what is the amount of leftover aquafaba in the cooking processes of legumes, but it is known that there is a growing trend in the consumption of legumes and hummus due to the increase of vegan and vegetarian persons [74]. And it is expected that there will be an increase in its consumption to fight hunger, as it is a very cheap product. In this way, it would also lead to an increase in the amount of aquafaba produced [40].

Another alternative that can be sustainable is to dry the aquafaba. Although the drying process increases the costs, the service life is extended. Because aquafaba has a water content of over 90%, it can be harmed over time by undesirable microbial growth. In addition, the efficiency of transport is also improved, as the required storage space is reduced, which would reduce shipping costs [42].

7. Conclusions

Aquafaba can replace the egg as it has similar functional properties. Aquafaba has been experimentally shown to achieve similar levels of colloidal capacity and stability as eggs. The highest values of emulsifying capacity and stability obtained from the aquafaba (EC=139% and ES=97%) even exceed the values from eggs (EC=115% and ES=92%), and the foaming capacity and stability of aquafaba (FC=660±15% and FS=92%) are very similar to those of eggs (FC=684-311% and FS=98%). The higher the emulsion and foaming capacity and stability, the better the colloidal systems will form. But these properties depend on several interrelated variables that are outlined in Figure 10.

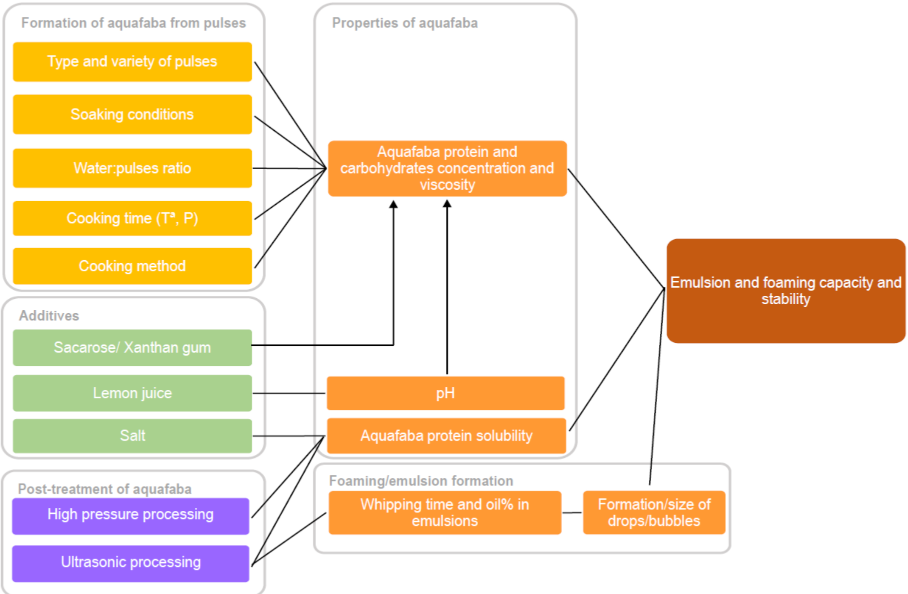

Figure 10. Aquafaba's independent and dependent variables.

In order to obtain the most optimal aquafaba, each of the variables has to be analyzed separately. **Protein and carbohydrate concentrations** correlate with viscosity. The higher the concentration/viscosity, the greater the stability of the colloidal systems. To ensure that they are maximum, the seven parameters on which they depend must be keep in mind:

a) **Types and varieties of legumes**: There are many varieties and types of legumes around the world, and each has a different composition. According to studies, the different types of soybeans, chickpeas, and small black beans are the ones with the highest protein content and carbohydrate content. However, each country should investigate which of the local varieties contains the highest concentration of these components. This can improve the sustainability of consumption.

b) **Soaking conditions**: Two studies reveal that presoaking increases the viscosity of aquafaba because it softens the wall of legumes and allows a greater transfer of protein and carbohydrates to the water. In addition, the longer the soaking time (16–24 hours), the higher the viscosity.

c) **Pulse-water ratio**: The more quantity of legumes per water, the higher the protein and carbohydrate concentration in the aquafaba (1: 1.5 > 1:2 > 1: 4).

d) **Cooking time**: A study shows that the longer the cooking time, the higher the protein and carbohydrate content (60 > 45 > 30 > 15 min). Also, in other studies that directly measure the capacity and stability of the foam/emulsion of aquafaba, they agree that a longer cooking time improves these properties.

e) **Cooking method**: A study of various cooking methods of legumes (with different temperatures and pressures) concludes that the use of a combination of cooking and microwaving shows better properties. However, we cannot draw firm conclusions from this variable as only one study has been conducted, and only one type of legume has been used.

f) **pH**: A study concludes that protein concentration is not influenced by pH. Other studies, which take into account the pH of proteins denaturalization and how proteins charge changes, differ in what is the ideal pH (between 3 and 6). More thorough research is needed to reach a conclusion on pH.

g) **Sugar/Xanthan gum**: A study explains that the addition of sugar or xanthan gum to aquafaba increases the carbohydrate content.

High **solubility of proteins** facilitates the adsorption of proteins at the air/oil-water interface, thus reducing surface tension and stabilizing the colloid. This solubility can be increased by adding a small amount of salt. Studies show that the ideal amount would be 0.3%.

The **formation of drops or bubbles** in a colloidal system and their size are determined by the time of whipping in foams and the amount of oil in emulsions. According to one study, whipping the aquafaba for a longer period of time (10–15 minutes) than whipping the egg (2–5 minutes) contributes more to the formation of drops/bubbles and protein absorption at the gas-liquid interface. A longer whipping time also decreases the size of the drops/bubbles and prevents sedimentation of the system. Another study found that an 80% oil content in emulsion is more stable. However, this fact should be corroborated by some other study.

High pressure and ultrasound processing have also been found to improve emulsifying and foaming capacity. Both techniques do this by denaturing the proteins, thus facilitating their adsorption to the water-oil/air interface. In addition, the ultrasonic processing increases the viscosity and reduces the average size of the bubbles/drops.

To conclude, it has been found that more thorough research is needed on some of the variables that affect the properties of aquafaba. Especially, more emphasis needs to be placed on how pH affects proteins and, consequently, their emulsifying and foaming properties. It is also necessary to analyze the chemical composition of national pulses and further examine the effect of the cooking method. Finally, study the minimum amount of oil in an emulsion. With this complete research, the conditions for the extraction of aquafaba could be standardized in the most efficient way. And then we will be able to experiment the effects of using the corresponding aquafaba for the production of commercial foods. These end products would have a lower residual and environmental impact, as well as reduce the burden on the poultry industry and improve the health of the population.

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9. ACRONYMS

DSC: Differential Scanning Calorimetry

EC: Emulsion Capacity

ES: Emulsion Stability

EAI: Emulsion Activity Index

FC: Foam Capacity

FS: Foam Stability

GEH: Greenhouse Gas

HPP: Hight pressure processing

HMW: Hight molecular weight

LMW: Low molecular weight

OAC: Oil Adsorption Capacity

PWR: Pulses:Water ratio

TPC: Total Phenolic Compounds

WAC: Water Adsorption Capacity