EVALUATION AND OPTIMIZATION OF FOAMING, EMULSION, AND RHEOLOGIC PROPERTIES OF CHICKPEA AQUAFABA AS AN EGG WHITE REPLACEMENT IN CAKE

by

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(Under the Direction of William Kerr)

ABSTRACT

Chickpea aquafaba has the potential to substitute egg white in cake, but more must be understood about what affects its functionality. The effect of cooking method (pressure-cooked versus boiled) and water:chickpea ratio on solubility, viscosity, protein content, and protein profile of aquafaba was evaluated. In addition, the influence of cooking method, ratio, and pH on functionality was determined. Foaming capacity/stability and emulsifying activity/stability index were significantly affected by pH, while the ratio was not significant. Pressure-cooked and boiled samples at a 3:1 ratio at pH 5 were selected and addition of hydrocolloid's (xanthan and hydroxypropyl methylcellulose) effects on foam overrun/texture and liquid drainage was assessed. Lastly, their influence on cake texture, color, and crumb structure was compared to egg white. While hydrocolloid addition improved pressure-cooked samples foam properties, there was virtually no significant differences in cakes made with boiled and pressure-cooked aquafaba with different types and levels of hydrocolloid.

INDEX WORDS: Aquafaba, chickpea, functional properties, cake, texture analysis, viscosity, egg white

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CHAPTER 1

INTRODUCTION

Up to 7.7% of the global protein market could be plant-based by 2030, at a valuation of 162 billion dollars (Kim & Shin, 2022). Legumes, like the highly consumed chickpea, are presumed to be a large portion of this plant-based protein sector; they are well-documented rich sources of dietary fiber, protein, and phytochemicals (Serventi, 2020). However, in the industrial processing of legumes, the cooking water is a byproduct not typically viewed as a value-added segment of the process. Today, consumer desire to reduce personal waste, and industry motives to be more sustainable while generating further value has inspired research into the novel use of legume cooking water. The resulting water is commonly referred to as aquafaba, Latin for "bean water" (Buhl et al., 2019).

Originally popularized via internet recipes, Joël Roessel used aquafaba as an egg white replacement in 2014 after observing its natural foaming properties (Mustafa & Reaney, 2020). While plant protein supplementation and substitution has been on the rise, there are currently few aquafaba products on the market, and existing research on its use in food products has been limited. The existing literature has investigated the composition and functionality of aquafaba using the liquid separated from canned chickpeas, as well as aquafaba obtained via boiling or pressure cooking (Buhl et al., 2019; Damian et al., 2018; He et al., 2019; Mustafa et al., 2018; Shim et al., 2018; Stantiall et al., 2018). Additionally, advanced treatments like ultrasound, foam mat drying, and high-pressure processing have been examined to determine their effects and possible improvements to the functionality of aquafaba (Alsalman & Ramaswamy, 2021; Aslan

& Ertaş, 2021; Meurer et al., 2020). Despite research into improving this functionality, few studies have closely examined how it functions at different pH values nor the intersection of pH modification with producing aquafaba under different methods. Currently only Lafarga et al. (2019), Alsalman et al. (2020b), and Nguyen et al. (2021) have investigated optimizing certain processing conditions. The primary food models used to demonstrate aquafaba's functionality, as well as current existing commercial products, have primarily been meringue and mayonnaise (He, Meda, et al., 2021; He, Purdy, et al., 2021; Lafarga et al., 2019; Meurer et al., 2020; Muhialdin et al., 2021, 2021; Mustafa et al., 2018; Raikos et al., 2020; Saget et al., 2021; Stantiall et al., 2018; Włodarczyk et al., 2022). Few studies have navigated the more complex system of baked goods, with only a handful of papers attempting to use aquafaba in cake and gluten-free bread (Aslan & Ertaş, 2020; Bird et al., 2017; Mustafa et al., 2018). The addition of hydrocolloids and how they interact with aquafaba protein in cake has yet to be investigated.

This study aimed to optimize the functional properties of aquafaba to use as an egg white substitute in cake using different processing methods, pH alterations, and hydrocolloid addition. Two cooking methods, boiling and pressure cooking were investigated along with three ratios (5:1, 4:1, and 3:1 water to seed) to understand the influence of varying protein quality and concentration. Aquafaba had its functional properties evaluated at a pH of 3, 5, and 7 and compared to its natural pH (a pH of ~6). Finally, xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC) were applied at three levels to examine the effect of different hydrocolloids on foam properties in addition to the texture, structure, and color of the final cake product.

CHAPTER 2

REVIEW OF LITERATURE

Aquafaba Background and Plant Protein Demand

Increased demand for vegan and vegetarian products, sustainability, and greater health awareness has driven research into novel ingredients like aquafaba and their potential use in foods as beneficial additives and animal product substitutes. In contrast to other plant proteins and their isolates, aquafaba requires little to no additional processing to provide its desired functionality (foaming, emulsifying, gelling, etc.). Additional processing can be beneficial, for example, drying of aquafaba could decreases transport costs, storage requirements, and prevents microbial growth (He et al., 2021b). Ultimately, there is an opportunity both on the industry level and the consumer level to engage in more sustainable practices. With further investigation, diverting these waste streams to use them appropriately in foods in place of egg white could prove to be beneficial on multiple fronts. As it currently stands, the value of the global egg replacement market is predicted to reach approximately 1.5 billion USD by 2026 at a compounded annual growth rate of 5.8% (Cision PR Newswire, 2016).

While the term aquafaba often refers to the cooking liquid retained after cooking chickpeas, it is technically a catch-all term for the cooking liquid from any legume. One of the initial research papers on aquafaba's functionality by Stantiall et al. (2018) compared the aquafaba from haricot beans, chickpeas, whole green lentils, and split yellow peas, while other research has explored aquafaba from lima bean, black bean, Korean soybean, kidney bean, broad

bean, small black bean, and green pea (Echeverria-Jaramillo et al., 2021; Kilicli & Toker, 2022; Nguyen et al., 2020; Shim et al., 2021). In the research of aquafaba as a direct egg white substitute, chickpea aquafaba might be preferred over other legumes due to its ability to create bright white foams (Meurer et al., 2020; Nguyen et al., 2021). Research like that of Shim et al. (2021), Kilicli and Toker (2022), and Nguyen et al. (2020) has shown improved functionality in aquafaba made from other legumes, but a lack of sensory research using these alternative aquafabas has yet to be investigated.

Chickpeas are a grain legume. They provide an excellent source of dietary protein, minerals, and dietary fiber. In addition, they are relatively inexpensive to produce, provide improved soil health and nitrogen fixation, and have less water requirements than other crops, all of which contributes to its status as a highly productive, profitable, and sustainable crop (Kumar et al., 2018). They currently rank fourth in global legume production, with 14.78 million tons being produced yearly and production only expected to further increase (Tassoni et al., 2020). While the United States provides only 1% of the worlds annual production, American consumption of legumes such as chickpea has continued to trend upward, growing by 63% over the past 47 years (USDA Economic Research Service, 2019). Increased demand for legumes leads to increased production and likewise, an increase in wastewater generation. In this context, aquafaba may serve two beneficial purposes by meeting the increasing demand for plant protein alternatives, while using a food processing by-product to meet the ever increasing need to feed a growing population.

Aquafaba Composition

Aquafaba contains what is leached out of chickpea seeds during cooking, and as such is composed of small quantities of protein, low molecular weight carbohydrates, and dry matter

fractions currently defined as "insoluble" and "soluble" fiber. The largest portion of the viscous liquid is protein, quantified at 2g/100g (Alsalman et al., 2020a; Buhl et al., 2019; Raikos et al., 2020; Shim et al., 2018; Stantiall et al., 2018). Analysis of aquafaba obtained from canned chickpea via SDS-PAGE and proteomic analysis outlined a protein profile of low molecular weight legume proteins. These studies confirmed the presence of both basic and acidic subunits of legumin, the gamma subunit of vicilin, and subunits of albumin as well as nuclear storage and membrane proteins in aquafaba (Alsalman & Ramaswamy, 2021; Buhl et al., 2019; Echeverria-Jaramillo et al., 2021; Shim et al., 2018). These small molecular weight proteins are all less than ~70 kDA and have been shown to have good emulsifying and foaming power. Vicilin fractions of chickpea have been shown to have higher solubility, foaming capacity, and emulsifying capacity, while legumin fractions had better foaming and emulsifying stability (Chang et al., 2022). These proteins' functional ability derives partially from their small size. Proteins with a lower molecular weight and a level of flexibility can migrate faster than larger proteins to the interfaces they are trying to stabilize (Narsimhan & Xiang, 2018).

Additional foaming functionality of aquafaba can be ascribed to the small sugars leached from the chickpeas. Aquafaba's sugar quantities are small, but comparable to its protein content at approximately 1.2 to 2.64 g/100g (Raikos et al., 2020; Stantiall et al., 2018). He et al. (2021) used $_1$H-NMR and identified these sugars as sucrose, stachyose, raffinose, and arabinose, confirming what was suggested by Stantiall et al. (2018) and El-Adawy (2002). Foam stability can benefit from small sugars because they can increase the viscosity of the liquid phase of the foam, but this increase in viscosity can also negatively impact the foaming capacity (Damodaran, 2005).

Lipids in aquafaba could compete with proteins for adsorption at the air-water interface and negatively affect foaming (Damodaran, 2005). While chickpeas are considered to have a relatively high fat content – the "Kabuli" type averages 3.1 to 4.9% - multiple studies have observed no fat content or fat content below a detectable limit in aquafaba (Grasso et al., 2022; Mustafa et al., 2018; Stantiall et al., 2018). The exceptions to these studies measured levels of 0.14g and 0.13g in aquafaba prepared via pressure cooking, and interestingly a commercial dried form of aquafaba known as VÖR (Karatay et al., 2022).

Aquafaba Functional Properties

Soaking of chickpea seed before cooking is typically done to decreases cooking time, and has been found to also reduce the quantity of oligosaccharides/monosaccharides that can cause gastrointestinal upset, and lower anti-nutrient compounds like lectins, tannins, and trypsin inhibitors (He et al., 2021c). Chickpea and soybean aquafaba produced with presoaking had significantly higher foaming and emulsifying capacity than those without, while emulsifying stability of samples without presoaking was higher, and foaming stability was not significantly affected (He et al., 2021c). Kilicli and Toker (2022) determined that while soaking chickpeas with ultrasound shortened soaking time, it also decreased protein content and foaming capacity of all tested aquafaba (chickpea, white bean, kidney bean, green lentil, broad bean, and green pea). Different soak times have been shown to affect the functional properties of chickpea aquafaba. In chickpeas soaked for 24 hours, before boiling for 35 minutes at 120°C, there was significantly higher total soluble solids, viscosity, and peptide content in comparison to samples soaked for 12 and 18 hours (Muhialdin et al. 2021). Additionally, mayonnaise produced with the 24-hour sample had significantly higher firmness and consistency that was comparable to whole egg mayonnaise as well as a lower bacterial load than the other tested samples (Armaforte et al.,

2021; Muhialdin et al., 2021). He et al. (2021) compared short, high temperature soaking parameters to longer and lower temperatures and concluded that steeping chickpea seed at 4°C for 16 hours before subsequent pressure cooking with 75 kPa of pressure for 30 minutes at 116°C contributed to improved emulsifying performance.

Current known functional properties of chickpea aquafaba vary significantly due to differences in sample preparation, pH alteration, and technical methods used to evaluate them. However, because of this, there is more understanding of the potential factors contributing to their improved functionality. The investigation of chickpea cultivar's influence on emulsifying properties led by He et al. (2019) showed no correlation between chickpea seed composition and emulsion properties but did reveal cultivars with higher dry matter content had better emulsion properties, with the CDC leader chickpea producing the most stable emulsions of the genotypes tested. Lafarga et al. (2019) used response surface methodology to optimize foaming and emulsifying properties and determined that a cooking ratio of 1.5:1 (water:seed) for foaming properties and 1.72:1 for emulsifying properties and a pH of 3.5 would result in desirable values. Uniquely, their aquafaba samples were boiled for 190 minutes post-soaking while keeping the total water constant. When comparing different whipping times, the foaming capacity of egg white and canned aquafaba were not statistically different after selecting a canned sample with the highest foaming stability after 30 minutes (Mustafa et al. 2018). Additionally, commercial egg white liquid showed significantly lower foam volume and foaming capacity than canned aquafaba, but fresh egg white had significantly higher foam volume and capacity (Mustafa et al. 2018). A separate study using 114 pooled cans of chickpeas that were frozen, thawed, and centrifuged had lower foam overrun, foam volume, and liquid ratio in foam over time in direct comparison to egg white powder (Buhl et al., 2019). While liquid drainage over time was not

statistically different between egg white and canned chickpea, aquafaba did have a significantly higher emulsifying stability index (ESI) than egg white which increased with higher pH levels (Buhl et al., 2019).

Viscosity has not been studied much in aquafaba, nor its influence on other functional properties. Of the legumes tested by Stantiall et al. (2018) chickpea aquafaba had the highest apparent viscosity at 47 mPa·s, higher than egg white at 15 mPa·s. Ultrasound treatment of chickpea aquafaba, which did not significantly alter the protein solubility, did show a slight increase its viscosity (Meurer et al. 2020). Alsalman et al. (2020b) aimed to optimize the rheological properties of chickpea aquafaba through processing conditions and determined pressure cooking at a ratio of 3:2 (water:seed) for 60 minutes maximized the consistency coefficient and oscillatory elastic modulus.

Research into treatments or additional processing methods have shown some improvement in functional properties of aquafaba. Chen et al. (2021) discovered that spray drying yellow pea water reduced soluble fiber, did not significantly affect protein content or protein profile of samples, and did not cause significant differences in a preliminary sensory test. He et al. (2021) spray dried chickpea aquafaba that had been optimized as to soaking and cooking methods that gave best emulsifying properties. This resulted in an emulsifying activity index (EAI) not statistically different from fresh aquafaba, but with significantly lower stability. Overall, freeze drying and spray drying did not affect the functionality of chickpea aquafaba (He et al., 2021). Egg white's foaming functionality, on the other hand, is negatively affected by spray drying at standard temperatures (Phillips et al., 2011). Other alternative drying methods such as oven drying, rotovap drying, and vacuum drying produced dark and brittle, gel like, and rubbery sheets of material respectively (He, Purdy, et al., 2021). Ultrasound and high-pressure

processing at certain levels, have led to improved foaming properties when applied to chickpea aquafaba. Meurer et al (2020) noted that high intensity ultrasound did significantly improve foaming capacity and showed an increase in foam stability, color (lightness), emulsifying stability, and foam texture. High pressure processing increased the time of foaming stability from 50.5 to 67 minutes and increased the consistency coefficient of its rheological properties from 0.6 to 1.4 Pa.s.

Foam Formation and Stability

Food foams are complex systems that are the foundation of a wide variety of food products such as, whipped cream, mousse, meringues, and cakes (Foegeding et al., 2006). These foams are thermodynamically unstable colloidal systems that consist of a gas dispersed phase and a liquid, semi-liquid, or solid continuous phase (Dabestani & Yeganehzad, 2019). This instability drives the two phases to separate and the foam to break down. From a thermodynamic standpoint, the system aims to reduce the amount of interfacial contact and reduce the overall free energy. The process takes time, thus there is a kinetic barrier, and the foam may be stabilized by increasing the continuous phase viscosity as well as the disjoining pressure (Damodaran, 2005). Food foams can be stabilized by proteins, with globular proteins providing better stability because of their ability to form cohesive viscoelastic films around the air bubbles (Narsimhan & Xiang, 2018). This stability is also improved around the isoelectric point (pI) due to minimized electrostatic repulsion that allows for greater adsorption of protein at the air-water interface (Narsimhan & Xiang, 2018). Importantly, it is not beneficial to foaming when proteins are fully denatured or insoluble. This is because the protein must adsorb to the interface to form the lamella film that stabilizes it (Damodaran, 2005). A pH too close to the pI would minimize

the charges between the proteins, and lead to coagulation at the interface, a weaker lamella film, and coalescence of gas bubbles - causing liquid drainage and foam breakdown.

Egg white is most frequently used as the base in most protein foams as its foaming ability is well known and researched; it is the industry standard in food products such as meringue and angel food cake. A single egg white has enough protein to produce several liters of foam, but its water content limits the percent overrun to 500-800% (Vega & Sanghvi, 2012). The individual proteins that make up egg albumin do not all contribute to foaming, but together they make high capacity and highly stable foams. Of the approximately 40 different proteins in egg white the major portions are ovalbumin, conalbumin, lysozyme and ovomucoid (Gomes & Pelegrine, 2012). Ovomucoid contributes to higher viscosity and reducing foam drainage, while lysozyme forms protein complexes that enhance the strength of the viscoelastic film which makes the foam more stable (Sadahira et al., 2018). This stability is also determined by the air fraction in the foam. A higher air phase means more bubbles, which causes more complex paths for liquid to drain out, therefore increasing the foam stability time (Dabestani & Yeganehzad, 2019). In eggs, achieving this maximum air phase is more precarious. Excess whipping, referred to as "overbeating", can cause aggregation of ovalbumin at the interface that leads to increased drainage and foam collapse (Vega & Sanghvi, 2012). An appropriate whip time is determined by the surfactants in the solution, surface tension, and the bulk viscosity (Dabestani & Yeganehzad, 2019). Mustafa et al. (2018) observed that increasing whipping time from 2 to 15 minutes increased the foam stability of aquafaba by 10% but decreased the foam stability of egg white foams by 17% after 2 hours, while whipping times of 5 and 10 minutes were not statistically significant.

Research into plant protein foams has been driven by dietary restrictions, egg-allergies, and environmental concerns. Multiple researchers have delved into the health benefits from these plant proteins, while acknowledging the obvious drawbacks to the alternative foam source, that is, pulse protein foams generally only show stability over a few minutes to a few hours (Mohanan et al., 2020). The addition of a variety of hydrocolloids and their potential synergistic effects with plant protein has been investigated to mitigate this issue. Jarpa-Parra et al. (2016) demonstrated that the addition of guar gum, xanthan gum (XG), or pectin, did not affect the foaming capacity of lentil legumin-like protein but greatly enhanced foaming stability at pH 3 and 5. Another study investigating pea and faba bean protein concentrates and isolates discovered that without xanthan gum, only faba bean protein concentrate could be stable for more than one minute, but the addition of xanthan gum greatly improved overrun and foam stability of all protein concentrates and isolates (Mohanan et al., 2020). Soy protein isolate (SPI), which has displayed high levels of foaming capacity, has also been limited in its use in food foam applications because of its instability. Xie and Hettiarachchy (1998) determined that optimal foaming properties of SPI could be achieved with 0.1% SPI and 0.2% XG, which also achieved a significantly higher foaming capacity than egg white, and a foam stability nine times greater than SPI or egg white alone.

Another important factor contributing to functionality is the effect of pH on the protein source, typically dependent on its pI. Egg white's main protein portions ovalbumin and ovomucoid have a pI of 4.5 and 4.1 respectively, while conalbumin has a close to neutral pI of 6.1 and lysozyme has a significantly more basic pI at about 10.5 (Phillips et al., 2011; van den Berg et al., 2015). Lysozymes' high pI causes negatively charged residues at physiological conditions, giving it the ability to form electrostatic complexes with the other egg white proteins,

allowing for greater adsorption at the air-water interface of a foam (Phillips et al., 2011). Dropping the pH near neutral not only decreases the repulsion between proteins so they can pack closely at the air-water interface, but it also delays the formation of disulfide bonds in egg white proteins which reduces the probability of overwhipping and collapsing the formed foam (Vega & Sanghvi, 2012). The PI of canned chickpea aquafaba was determined to be similar to that of chickpea protein isolate, with a value of 4.6 based on zeta potential measurements to determine the surface charge in relation to pH (Buhl et al., 2019). The foaming expansion of egg white at pH 4 was statistically similar to that of aquafaba treated with 100% power ultrasound for 30 minutes and the foaming stability of aquafaba treated with 100% power ultrasound for 20 minutes (Meurer et al., 2020). Foam texture, foaming expansion, and foam liquid stability of egg white at physiological conditions (an average pH of 9.8) was statistically higher than egg white at pH 4, and all aquafaba treated with ultrasound (Meurer et al., 2020).

Egg Replacement in Cakes

Sponge cake is popular with consumers because it has simple ingredients, straightforward processing, and an airy texture that makes it able to absorb liquids like syrup and jam (Assad Bustillos et al., 2020). They are typically made using a combination of flour, sugar, egg, and a leavening agent. Sponge cake is a 'foam-type' cake: a type of cake with small fat quantities typically not added into the recipes as opposed to batter-type cakes that have high levels of fat (Godefroidt et al., 2019). Angel food cake is also a foam-type cake. Sponge cake has been made with whole egg and egg white, while angel food cake is only made using egg white. Both cakes need sufficient air cells to be incorporated into the batter during mixing and subsequently set the structure of the cake by the gelation of starch and proteins (Pycarelle et al., 2020).

There are multiple reasons consumers and producers may consider the substitution of egg in cake. Eggs are a costly ingredient and are high in cholesterol (Ashwini et al., 2009). Additionally, egg allergy is the second largest IgE food allergy in children and the main allergens are present in the egg white (Yazici & Ozer, 2021). Unfortunately, egg protein is challenging to replace because of its unique foaming, emulsifying and gelling properties that are important in the development of cake texture and volume (Ashwini et al., 2009). Egg replacement in cake and baked goods has primarily been with whey, soy, lentil, pea, and lupin legume proteins, which may be used in conjunction with hydrocolloids and emulsifiers (Yazici & Ozer, 2021).

The most common drawbacks to egg substitution in bakery products can be textural properties like increased crust/crumb firmness, or sensorial issues. A whey protein based commercial egg replacer was tested in sponge cake and improved the yield rate and specific volume, but in high quantities it negatively impacted the internal structure and the sensorial properties of the cake (He et al., 2015). Cake softness is dependent on the product's structure and plays a crucial role in product acceptance (Dewaest et al., 2018). Lentil protein substitution did not significantly affect cake volume but did lead to inferior hardness and chewiness texture values and lower sensory scores (Jarpa-Parra et al., 2017). Pea protein isolate addition in sponge cake decreased the fraction of air in the batter and increased the viscosity of the batter, therefore leading to denser cakes (Assad Bustillos et al., 2020). In contrast, partial substitution of wheat flour with whey protein concentrate in eggless batter decreased the apparent viscosity of the batter and led to an increase in the number and even distribution of air cells (Jyotsna et al., 2007). When pea protein was hydrophobically modified and used as an egg substitute, it did lead to improved physical properties like increased rise and firmness of the cakes (Shah et al., 2019).

Aquafaba has been used in cake and cake-like products with mixed results. Cupcakes with egg white fully substituted by lima bean aquafaba were statistically harder, more cohesive, and had higher gumminess and chewiness compared to cupcake made with 100% egg white as well as statistically lower (darker) L* color values of crust and crumb (Nguyen et al., 2020). Cake made with chickpea aquafaba had a sunken center, large inter-space voids in the crumb, and no clear dividing line between crust and crumb (Mustafa et al., 2018). The hardness and resilience of chickpea aquafaba cake crumb was not statistically different from that of egg white cake, but chewiness, springiness, and cohesiveness of aquafaba cake crumb were all statistically lower than that of egg white cake (Mustafa et al., 2018). Aslan & Ertaş (2020) determined that substituting more than 50% of egg white with chickpea aquafaba resulted in increased firmness and decreased crumb redness, while 100% aquafaba substitution resulted in statistically higher specific gravity of batter and a significantly lower volume index.

Hydrocolloid addition in cake

Hydrocolloids are water-soluble polymers, typically polysaccharides from extracts or semirefined/refined foods that can contribute to a solution's viscosity and gelation (Goff & Guo, 2019). These properties make them beneficial to add to a wide variety of foods as increasing the viscosity can help stabilize foams and emulsions, and improved gelation can in turn affect textural properties. In addition, hydrocolloids often act synergistically with other food ingredients to affect physical properties. After the hydrocolloid is dissolved in a solution, its intermolecular binding converts to molecule-water binding dependent on its particle size, the temperature of the solution, and the presence of cations (Goff & Guo, 2019).

Hydroxypropyl methylcellulose (HPMC) is a water-soluble polymer with high surface activity (Hager & Arendt, 2013). Its structure consists of a glucose backbone linked by β -1,4

glycosidic bonds that make it "rod-like" with hydrophobic methyl and hydrophilic hydroxypropyl groups that interact with each other through hydrogen bonds (Qiangzhong et al., 2009). HPMC is frequently used in bakery products particularly because it hydrates well, with only mild interpolymer entanglement during low temperature mixing, and strong gel networks formed from hydrophobic bonds between polymer chains at higher temperatures (Hager & Arendt, 2013; van den Berg et al., 2015). Xanthan Gum (XG) is a bacterial polysaccharide with high molecular weight that has high viscosity at low shear and low concentrations, is stable over a wide temperature and pH range, and has a low flow behavior index (Hager & Arendt, 2013; van den Berg et al., 2015). Its structure has repeating D-glucose units that make up the backbone with β-D mannose-(1,4)-β-D glucuronic acid – (1,2)-α-D mannose which links to alternating glucose residues (Hager & Arendt, 2013).

XG and HPMC have been tested in a wide variety of bakery products to improve volume and textural properties. HPMC's surface-active qualities, as well as its water-binding abilities, significantly influences cake texture by reducing hardness, gumminess, and chewiness of bakery products (Noorlaila et al., 2017). In gluten-free muffins chickpea flour addition decreased the springiness, cohesiveness, chewiness, and specific volume which were then improved by addition of XG (Herranz et al., 2016). When added to eggless cake, both XG and HPMC improved the grain, cell wall thickness, texture, and mouthfeel of the crumb – with HPMC addition producing the highest value`s (Ashwini et al., 2009). Noorlaila et al. (2017) investigated the effects of XG and HPMC on sponge cake and found that batter with XG had a lower flow consistency index (n) value and higher apparent viscosity than the control cake, while HPMC had a higher flow consistency index (n) value but a similar apparent viscosity to control. Cake volume is partially dependent on batter viscosity: too high viscosity can restrict the expansion of

air bubbles in the cake, while too low cannot hold the air bubbles, leading to collapsed or dense cakes (Rahmati & Mazaheri Tehrani, 2014). Sponge cake with XG produced cakes that were significantly harder than HPMC and control cakes and had a dense crumb structure, while the volume of XG and HPMC cakes was not significantly different. (Noorlaila et al., 2017).

Across the food industry there is a demand for plant-based protein as both ingredients and products. However, the ability of these proteins to function as ingredients in foods is often influenced by their extraction conditions. It's crucial that their emulsifying, foaming, gelling, and physical properties meet what is required of the food item they are a component of. As such, Chapter 3 investigates the influence of the preparation of chickpea aquafaba under different cooking conditions and pH values on its emulsifying and foaming capabilities. Additionally, the knowledge that these functional properties are often linked to protein-polysaccharide interactions or viscosity led to testing in conjunction with commonly used hydrocolloids. Chapter 4 expanded on the results of Chapter 3 to use aquafaba as an egg-white replacer in a common bakery product, hi-ratio cake. Cake physical properties and crumb structure were evaluated and directly compared to hi-ratio cake made with egg white.

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CHAPTER 3

EVALUATING THE EFFECT OF COOKING METHOD, COOKING RATIO, PH, AND HYDROCOLLOIDS ON FUNCTIONAL PROPERTIES OF AQUAFABA[1]

[1]Crawford, K.E. and W.L. Kerr. To be submitted to *Food Hydrocolloids.*

Abstract

Chickpea aquafaba was prepared via boiling and pressure-cooking methods at cooking ratios of 5:1, 4:1, and 3:1 water to seed. The effect of cooking method on protein solubility and cooking method and cooking ratio's effect on viscosity, protein content, and protein profile of aquafaba was evaluated. Then, how foaming capacity/stability (FC/FS) and emulsifying activity/stability index (EAI/ESI) were affected by cooking method, ratio, and pH adjustment to 3, 5, and 7 alongside native pH was determined. Solubility was lowest near pH 4 and was not affected by cooking method and protein profile was not affected by method or ratio. Boiled samples had higher protein concentration and apparent viscosity. Foaming capacity was significantly affected by pH, with pH 5,6, and 7 not statistically different and pH 3 significantly lower, while foaming stability was significantly higher at pH 3 and 5. Samples with pH 3 had high emulsifying activity index but low emulsifying stability index. Overall, cooking ratio did not significantly affect FC, FS, EAI, and ESI. Pressure-cooked and boiled samples at cooking ratio 3:1 and pH 5 were selected, and then assessed for their effects on foam overrun, liquid drainage, foam texture, and viscosity with and without addition of 0.2, 0.4, and 0.6% xanthan gum and hydroxypropyl methylcellulose (HPMC). Xanthan gum had a greater effect than HPMC on aquafaba viscosity and prevented foam liquid drainage for 24 hours. Boiled samples exhibited similar overrun, with pressure cooked samples having higher overrun with hydroxypropyl methylcellulose than with xanthan gum.

Introduction

Food byproducts and waste are a concern of producers and consumers alike and delving into innovative uses of these waste streams within the food industry is both a desired and necessary development. With the current demand for plant protein only expected to increase, developing the research into proven uses of existing waste streams, like that of aquafaba, could be beneficial. Existing research has shown aquafaba's varied foaming and emulsifying properties under multiple conditions and preparatory methods (Buhl et al., 2019; Lafarga et al., 2019; Nguyen et al., 2021). Some studies have optimized cooking conditions, with varying results, but none so far have directly compared different cooking methods along with water:seed ratios and adjusted pH (Alsalman et al., 2020b; Lafarga et al., 2019; Nguyen et al., 2021). Research on adding hydrocolloids to aquafaba has been even more limited, with Nguyen et al. (2021) evaluating the effect of xanthan gum (XG) on chickpea aquafaba foams with low sugar levels as well as the additional factor of sea salt. Additionally, foaming capacity, foaming stability, and foam hardness was used to select the optimum level of each factor (cooking ratio, pH, sugar quantity, salt quantity, XG quantity) and cooking ratio was not factored into the influence on foam texture at multiple pH values and sugar levels.

This purpose of this research was to simultaneously assess the effects of cooking method, water:seed ratio, pH, and hydrocolloid gums on emulsion and foaming properties afforded by aquafaba to expand and connect factors explored in existing research alongside unexplored gum addition effects. Initial experiments focused on the effects of processing (boiling versus pressure-cooking), water:seed ratio (3:1 to 5:1) and pH (3-8) on the types of proteins extracted, the protein concentration, the viscosity, and the solubility curve of proteins in the aquafaba. From there,

some basic functional properties of the aquafaba were assessed including foaming capacity and stability as well as emulsifying activity and stability index.

To determine how the aquafaba would function in a more realistic situation, foams were formed using optimized aquafaba samples which were combined with sugar and various levels (0-0.6%) of either of two hydrocolloids (xanthan and hydroxypropyl methyl cellulose). First, the basic rheological properties of these mixes were determined as they likely determine how easily foams can be formed and how long they persist. Next, the foams were formed, and foam overrun was determined as well as the rate at which liquid drained from the foam lamella over time. In addition, basic measurements of the foam firmness were measured.

Materials and Methods

Sample Preparation

Thirty-six kilograms of Kabuli Sierra garbanzo beans were purchased from Palouse Brand chickpeas (Palouse, WA, USA). All chickpeas were stored frozen (-14°C) and used within one year. Samples were prepared using the method of Stantiall et al. (2017). Before cooking, chickpeas were soaked in tap water for 16h at 2°C in a sealed container, and at a ratio of 3.3:1 g H_2O/g seed. The soaking water was then drained and discarded. Soaked chickpeas were rinsed with tap water, patted dry, and stored in a sealed container at 2°C until cooked.

Sample Processing

After soaking, the chickpeas were cooked by either pressure cooking or boiling. Tap water and chickpea seed were weighed out to create cooking ratios of 3:1, 4:1, or 5:1 g H_2O/g seed. For samples prepared by boiling, water was added to a cooking pot and heated at 100°C

until the water began boiling. The chickpea seeds were then added into the boiling water and cooked for 1 h. For samples prepared by pressure cooking, tap water and chickpea seed was added to the 2 L pressure-cooking vessel (Hawkins, Mumbai, Maharashtra, India) and placed on a hot plate (Rosewill, Shanghai, China) at its maximum heat setting (232°C) until steam exited the open valve. To reach full pressure in the vessel a cap was then placed to seal the valve and the maximum heat setting was maintained until the cap lifted to release steam. The temperature was then lowered to 93°C and the chickpeas were cooked for 5 min. After 5 min, the pressure-cooking vessel was removed from the heat source and allowed to depressurize for 10 min. Once cooking was completed, chickpea seeds and the cooking liquid were transferred to sealed mason jars and steeped for 24 h at room temperature (20°C). After 24 h, the chickpea seeds were strained, and the cooking liquid was stored in plastic bottles at -14°C.

Adjusting Aquafaba pH

Based on existing aquafaba studies and preliminary work, three pH values of pH 3, 5, and 7 were selected to analyze foaming and emulsifying properties. In addition, these were compared to the foaming and emulsifying properties of each sample at its native pH. Aquafaba samples were defrosted overnight at 4°C and up to 2 h at room temperature if necessary. The method for changing sample pH was based on that of Cui et al. (2020). Samples were stirred using magnetic stir bars at room temperature while the pH was changed to one of the three values selected over a 1 h period, checking every 15 min with adjustment as necessary to keep the pH constant. The samples were then centrifuged at 11,000 x g for 10 min, and the supernatant separated for further analyses.

Protein Concentration and Protein Profile

The protein concentration of samples was determined using the Bradford assay (Bradford, 1976). The protein profile of each sample was determined via SDS-PAGE using a modified method by Alsalman & Ramaswamy (2021). Samples were defrosted at 2°C overnight and at room temperature for up to 2 h if necessary.

All samples were then standardized to 0.5 g/mL by diluting with phosphate buffer. Samples were prepped in both reducing and non-reducing conditions. In non-reducing conditions, 10 µl of standardized sample (0.5 g/mL) and 20 µl Laemmli buffer were added to microcentrifuge tubes. In reducing conditions, 10 µl of standardized sample (0.5 g/mL) and 19 µl Laemmli buffer were added along with 1 µl β-mercaptoethanol. The tubes were boiled for 5 min, cooled, and centrifuged at 14,000 RPM in a Sorvall RC 6 Plus Centrifuge (Thermo Fisher Scientific, Oslo, Norway) for 10 min. Next, 10µl of each supernatant was loaded onto 4-20% polyacrylamide Tris-Glycine precast gels (Bio-Rad, Berkley, CA, USA), along with 7 µl of Precision Plus Protein Standard (Bio-Rad, Berkley, CA, USA) with molecular weights from 10 – 250 kDa. The proteins were separated using 10x Tris/Glycine/SDS running buffer on a Mini-PROTEAN Tetra cell (BIO-RAD, Hercules, CA, USA) at 100V for 1.5 h. The gels were stained with Coomassie brilliant blue reagent for 45 min before destaining with ultrapure water overnight. After destaining, gels were scanned using an Epson flat-bed scanner (Los Alamitos, CA, USA). Bands were assigned using ImageJ 1.50i version software (National Institute of Health, Rockville, MD, USA) to estimate molecular weights in comparison to the protein standard bands.

Viscosity

Instrument rheological analysis of all aquafaba samples was performed using the methods of Alsalman et al. (2020) with modification. Aquafaba samples were evaluated post-steeping and before freezing. A Discovery HR-2 hybrid rheometer (TA Instruments, New Castle, DE, USA) with TRIOS software (TA Instruments, New Castle, DE, USA) was used for analyses. For each replicate, approximately 2ml of samples were dispensed onto the rheometer plate. A 60mm 2.0° cone (TA Instruments, Elstree, UK) was lowered to the trim gap at 105 µm, where the sample amount was adjusted if necessary to extend to edge of cone. The cone was then lowered to the geometry gap at 52 µm from plate. Samples were then evaluated using a linear flow rate test. First, samples went through a conditioning step of 20 s soak time and a 60 s period of pre-shear. Next, samples underwent a shear flow ramp of 0.1 s^{-1} to 100 s^{-1} over an 840 s period, while the temperature was maintained at 25°C and points sampled every 5 s. Each treatment group was analyzed in triplicate, with three replicates of each sample. A power law model was fit to the shear stress versus shear rate results using the TRIOS software.

Solubility

The protein solubility was analyzed by taking aliquots of boiling or pressure cooked aquafaba samples from one cooking ratio (1:5 g seed/g H_2O). Samples of 30 and 50mL respectively were adjusted to pH 2 with 0.1M HCl dispensed by a HI 901 potentiometric auto-titrator (Hannah Instruments, Smithfield, RI, USA). Aliquots of the same quantity were adjusted to pH 3, 4, 5, 6, 7, and 8 ± 0.1 using 0.1M HCl or 0.1M NaOH and standardized to the volume of the pH 2 sample by adding DI water. Samples were then centrifuged at 11,000 x g for 10 min, with the resulting supernatant being analyzed using the Bradford assay (Bradford, 1976).

Foaming Capacity and Stability

Foaming capacity and stability of aquafaba samples were performed using the method of Aslan & Ertas (2020) with modification. Thirty mL of liquid (V_i) in a 250 ml beaker was foamed for 2 min at 10,000 RPM using a Polytron 2500 E homogenizer (Kinemtica Inc., Bohemia, NY, USA). Foams were transferred to 250 mL graduated cylinders and the volume of the foam was measured at 0 min (V_0) and 30 min (V_{30}). Foaming capacity and stability were calculated using Equation 1 and 2:

$$\text{Foaming Capacity (\%)} = \frac{V_0}{V_i} \times 100 \quad (1)$$

$$\text{Foaming Stability (\%)} = \frac{V_{30}}{V_i} \times 100 \quad (2)$$

Emulsifying Activity Index and Emulsifying Stability Index

Emulsifying activity and stability index of samples were analyzed following the original method of Pearce and Kinsella (1978) as modified by Buhl et al. (2019). The equation to calculate EAI was from Buhl et al. (2019) and Cameron et al. (2002) with turbidity (T) and oil volume fraction (ϕ) calculated using equations outlined by He et al. (2019). Emulsions were prepared by using a high-speed homogenizer (Kinematica Inc., Bohemia, NY, USA) to blend 10 mL of vegetable oil into 30 mL of aquafaba at 8,000 RPM for 5 min followed by 13,500 RPM for 4 min. A sample (200 µl) was pipetted from the emulsion into 50 mL of 0.1% w/v sodium dodecyl sulphate (SDS). Immediately after dilution, the absorbance was measured at 500 nm using a Genesys 150 UV-Visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Emulsifying activity index (EAI) was calculated using Equation 3:

$$EAI = \frac{2T}{c \times (1-\phi) \times 10000} \quad (3)$$

where T= turbidity in 1/m, ϕ is the oil volume fraction of the dispersed phase, and c is the concentration of aquafaba protein in the emulsion. The turbidity (T) was calculated using Equation 4:

$$T = \frac{2.303 \cdot A_0 \cdot V}{I} \quad (4)$$

where V=dilution factor, A_0= absorbance of 500 nm at 0 min, and I= path length (0.01 m). The oil volume fraction was calculated using Equation 5:

$$\phi = \frac{C - A - E(B - C)}{C - A + \frac{(B-C)\{1+E)D_0}{D_S} - E\}} \quad (5)$$

where A is the beaker mass, B is the beaker mass plus 1 mL emulsion, C is the beaker mass plus the emulsion dry matter, D_0 is the oil density, D_s is the aquafaba solution density, and E is the concentration of protein (mass per unit mass of solvent).

The emulsifying stability index (ESI) was determined by measuring absorbance after 10 minutes and calculated using Equation 6:

$$ESI = \frac{A_0}{A_0 - A_{10}} \times t \quad (6)$$

where A_0= absorbance of 500 nm at 0 min, A_{10}= absorbance of 500nm at 10 min, and t = time (10 min).

Foam Preparation

In addition to measurements on the base liquid, foams were prepared to compare overrun, liquid drainage, and foam texture of boiled and pressure cooked aquafaba at 3:1 water to seed ratio with a pH of 5, and with and without addition of xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) at 3 levels (0.2, 0.4, and 0.6 %). Samples were adjusted to pH 5 over the course of 1 h while stirring at room temperature (~18°C) and checking every 15 min to

ensure pH was constant. Samples were centrifuged at 11,000 x g for 10 min in a low-speed Eppendorf centrifuge (Eppendorf, Enfield, CT, USA), and the supernatant separated. Next, 60 mL of the supernatant was added to a KitchenAid stand mixer (KitchenAid, St.Joseph, MI, USA) with a 5-quart stationary bowl and a stainless-steel chefs whisk beater. If testing XG or HPMC levels, gum was added prior to whipping and mixed in at speed 1. Foams were then whipped at speed 10 for 7 min, then 86.4 g of sugar were slowly added while whipping for 1 min at speed 3.

Foam Overrun and Foam Liquid Drainage

Foam overrun (FO) was measured as described by Mohanan et al. (2020) with some modification. Immediately after whipping, foams were transferred from the stand mixer bowl into 4 tared 50 mL weigh boats. The weigh boats were levelled using a metal dough divider for consistent measurement. The foam weight was recorded and the average of the four measurements was used to calculate foam overrun using Equation 7:

$$Foam\ overrun\ (\%) = \frac{W_{50\ mL\ liquid} - W_{50\ mL\ foam}}{W_{50\ mL\ foam}} * 100 \qquad (7)$$

where $W_{50\ mL\ liquid}$ is the weight of an equal volume of aquafaba liquid used to make the foams.

Foam liquid drainage was measured according to Meurer et al. (2020) and Buhl et al. (2019) with some modification. The total foam formed from 60 mL aquafaba was transferred from the stand mixer bowl immediately after whipping to 150 mL plastic funnels placed on 150 mL graduated cylinders. Gauze had been placed in the funnel to help contain the foam. The drip volume in mL was recorded at 0, 1 and 24 h.

Foam Texture

Hardness, consistency, and adhesiveness of aquafaba foams were analyzed using the procedure described by Nguyet et al. (2021) and Meurer et al. (2020). After whipping, a cylindrical mold (diameter 50 mm, height 100 mm) was filled with foam and leveled with a metal dough divider. A TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) was used to assess foam texture using a compression test performed with a 35mm diameter cylindrical probe with 50% deformation. Hardness was measured as the peak force value, consistency as the positive area under the force-deformation curve, and adhesiveness as the negative area under the curve. Samples were tested in triplicate.

Egg White and Gum Solution Viscosity

The viscosity of aquafaba solutions at pH 5 with and without addition of XG and HPMC and 86.4 g sugar was analyzed using the methods of Alsalman et al. (2020) with modification. In addition, the viscosity of egg white (EW) with 86.4 g sugar was analyzed for comparison. All samples were stirred using magnetic stir bars at room temperature for 1 h to allow solubilization of sugar and gums. Prior to analysis, samples were sonicated using an ultrasonic bath (Cole-Parmer, Vernon Hills, IL, USA) for 20 min to remove bubbles formed during mixing. A Discovery HR-2 hybrid rheometer (TA Instruments, New Castle, DE, USA) with TRIOS software (TA Instruments, New Castle, DE, USA) was used for analyses. Approximately 2ml of samples were dispensed onto the rheometer plate, then a 60mm 2.0° cone (TA Instruments, UK) was lowered to the trim gap at 105 µm where sample amount was adjusted if necessary to extend to edge of the probe. The cone was lowered to the geometry gap at 52 µm and samples were then tested using a linear flow rate test at 25°C. The samples went through a conditioning step with a

20 s soak time and a 60 s period of pre-shear. Next, samples underwent a flow ramp at 25°C while shearing occurred over 840 s at a rate of 0.1 s^{-1} to 100 s^{-1}, with measurements recorded every 5 s. Samples were analyzed in triplicate. A power law model was fit with the TRIOS software.

Statistical Analysis

All samples were tested in triplicate (n=3) with aquafaba sample viscosities also measured three times per sample in triplicate (n=9). Analysis was conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Two-way analysis of variance (ANOVA) was used for analyses with two factors (cooking method and cooking ratio) while three-way ANOVA was used for analyses with three factors (cooking method, cooking ratio, and pH or cooking method, gum type, and gum level). Tukey's honest significant difference (HSD) test was also performed for significant difference at $p<0.05$.

Results and Discussion

Protein Content and SDS-PAGE

Table 3.1 shows the protein concentration of egg white and aquafaba samples for boiled and pressure cooked aquafaba samples at ratios of 5:1, 4:1, and 3:1 (water to seed). The protein concentration of boiled aquafaba at a ratio of 4:1 (1.4 mg/mL) was the highest of all samples. Overall, boiled samples had significantly greater ($P \leq 0.05$) protein concentrations than pressure cooked samples, with B4:1 (boiled at 4:1 water:seed ratio) average protein concentration being significantly greater ($P \leq 0.05$) than P4:1 and P5:1. This is likely due to boiled samples losing more water than pressure-cooked samples. Pressure cooked samples only lost water when the

pressure was released, while boiled samples continuously lost water via steam through a hole in the lid- which led to further concentration of proteins in the remaining liquid. The seed-to-water ratio was not a significant factor for protein concentration.

SDS-PAGE gels of boiled and pressure cooked aquafaba samples are shown in Figure 3.2. The bands ranged from ~10 to ~85 kDa with a similar distribution and intensity of bands across all samples, indicating different cooking methods and cooking ratios did not change the protein fractions present. Analysis of the SDS-PAGE gels revealed multiple small protein fractions in all samples; it can be estimated that these bands correspond to 2S albumin (10 and 12 kDa), the γ-subunit of 7S vicilin (~16 kDa), an unspecified 11S legumin-type protein (20 kDa), the basic and acidic subunit of 11S legumin (24 and 35 kDa respectively), and a 7S vicilin precursor (~50 kDa), with the band at ~85 kDa uncharacterized. This corresponds to the protein profiles of chickpea aquafaba determined by Echeverria-Jaramillo et al. (2021), Shimrit et al. (2013), Shim et al. (2018), Alsalman et al. (2021), and Buhl et al. (2019). These studies on chickpea aquafaba all recorded proteins with a molecular mass less than 48 kDa except for Shim et al. (2018) who identified bands at 92.1 and 92.9 as O-acyltransferase and tRNA (Cystosine-5-)-methyltransferase. Alsalman et al. (2021) was the only paper to characterize the cooking water of prepared aquafaba – all other papers that characterized the protein profiles of chickpea aquafaba used canned aquafaba. Additionally, their samples were pressure cooked for 60 min, significantly longer than the cooking time of the pressure-cooked samples used in Figure 3.2. The longer cooking time under harsh conditions could have denatured the proteins above 45 kDa observed in Figure 3.2 or caused it to split into lower MW subunits.

Viscosity

The apparent viscosity and flow behavior index for egg white and each cooking method and ratio of aquafaba samples is listed in Table 3.1. The apparent viscosity (mPa·s) is the calculated viscosity at a low value of shear, that is the ratio of shear stress to shear rate as the shear rate approaches zero. The flow behavior index (n) describes the relationship between the shear rate and shear stress of the fluid, with $n = 1$ indicating a Newtonian fluid and $n < 1$ indicative of pseudoplastic fluids (Ansari et al., 2020).

The apparent viscosity of aquafaba samples ranged from 9.6 to 81 mPa·s. The sample with the lowest apparent viscosity was the pressure cooked aquafaba sample at the 5:1 water to seed ratio, however this was found to also not be significantly different ($P \geq 0.05$) from the mean apparent viscosity of all ratios of pressure-cooked samples, as well as the most dilute boiled sample (cooked at a 5:1 ratio). The sample with the highest apparent viscosity was the boiled sample with a 3:1 ratio, and the boiled sample at a 4:1 ratio was significantly lower ($P \leq 0.05$) than the 3:1 ratio but significantly higher ($P \leq 0.05$) than the 5:1 ratio. Viscosity has been shown to have a strong correlation to total soluble solids and protein content in aquafaba (Muhialdin et al., 2021; Stantiall et al., 2018). Both cooking method and cooking ratio were significant factors. This could be related to total soluble solids, although this was difficult to prove as the protein concentration of different cooking ratios was not significantly different.

The flow behavior index ranged from 0.66 to 0.95, with pressure cooked samples at a 5:1 ratio having the highest flow behavior index and samples boiled at a 3:1 ratio having the lowest. The pseudoplastic (shear thinning) behavior and its relation to the cooking method and cooking ratios can be observed in Figure 3.1 which displays the shear stress versus the shear rate of all aquafaba samples. Samples with lower ratios had lower flow behavior index values which

translated to more observable shear-thinning behavior, as well as curves with higher overall shear stress values. This is consistent with the results of Alsalman et al. (2020) that demonstrated decreased flow behavior index, and likewise increased pseudoplasticity, when the cooking ratio of pressure cooked aquafaba increased.

Solubility

Figure 3.3 shows the solubility curve of pressure cooked and boiled aquafaba samples from pH 2 to pH 8. Pressure cooked samples had a protein concentration of ~0.5 mg/mL at pH 2 which decreased to around 0.3 mg/mL at pH 4, then increased to 0.65 mg/mL at pH 6 and maintained that level at pH 7 and 8. Boiled samples had a similar shaped curve but with overall lower protein concentrations. Thus, both boiled and pressure-cooked samples had their lowest solubility at ~pH 4. This agrees with results by Buhl et al. (2019), who detected an isoelectric point (pI) of 4.6 for centrifuged canned aquafaba and which was similar to the isoelectric point of chickpea protein isolate (pH 4.5). Protein solubility is dependent on the hydrophilic and hydrophobic interactions that balance protein-solvent and protein-protein interactions (Ladjal Ettoumi et al., 2015). Solubility is lowest near the pI because the proteins lack a significant net charge (positive or negative) that provide electrostatic repulsion, thus allowing stronger protein-protein interactions and greater aggregation (Chan et al., 2013).

Foaming Capacity and Stability

Figure 3.4 shows the foaming capacity of boiled and pressure-cooked aquafaba at ratios of 5:1, 4:1, and 3:1 (water to seed) prepared at pH values of 3, 5, and 7 along with the foaming capacity at the native pH (~6). The highest foaming capacity (200%) was observed for the boiled

3:1 aquafaba at pH 5, while the lowest foaming capacity (133%) occurred for the pressure-cooked 5:1 aquafaba at pH 3. Cooking ratio was not a significant factor, which aligns with the statistical analysis of the protein concentration. Furthermore, while pH did significantly affect foaming capacity, samples at pH 5- 7 were not significantly different ($P \geq 0.05$) and only those at pH 3 had a significantly lower foaming capacity. Additional details are shown in Figure 3.5, where the foam volume was multiplied by protein concentration to standardize the results. It was found that the foam volume per mg of protein across seed-water ratios remained constant. As shown in Table 3.2, all aquafaba samples had significantly lower ($P \leq 0.05$) protein concentrations at pH 3 which likely led to the decreased foaming capacity. As shown in Figure 3.5, however, samples at pH 3 had significantly greater ($P \leq 0.05$) foam produced per mg of protein than all other pH values tested. This suggests that while additional protein did allow for more foam creation it came with some diminishing returns. A study evaluating the functional properties of 0.1% chickpea protein concentrates showed that samples had highest foaming capacity at pH 2, followed by pH 10 and 8 respectively, with pH values near the pI having the lowest foaming capacity (Tontul et al., 2018). Other research showed that chickpea aquafaba foaming capacity was not affected by pH, however a different foaming method was used (shaking in an enclosed tube) and it is unclear if samples were centrifuged after the pH was changed (Buhl et al., 2019). It is possible high variability or overall low foaming capacity could have influenced results.

Figure 3.6 shows the foaming stability after 30 minutes of boiled and pressure-cooked aquafaba at ratios of 5:1, 4:1, and 3:1 (water to seed) with pH values of 3, 5, and 7, along with the foaming stability at the native pH (~6). The pressure-cooked 3:1 samples at pH 3 had the highest foaming stability (86.5%), while pressure-cooked 4:1 samples at pH 7 had the lowest

(59.8%). Foaming stability was affected by pH and cooking method, with samples at pH 3 and 5 having significantly greater ($P \leq 0.05$) values than those at pH 6 and 7, and with pressure-cooked samples having greater values than boiled samples. Protein flexibility, concentration, and charge are all affected by pH and can significantly affect the foaming properties of proteins. Stability is improved around the pI due to minimized electrostatic repulsion between proteins allowing for greater adsorption at the air-water interface, creating a thicker layer of protein that can act as a mechanical barrier to prevent foam instability (Amagliani et al., 2021; Narsimhan & Xiang, 2018). Buhl et al. (2019) also demonstrated that pH significantly affected the foam stability of canned chickpea aquafaba, with the foams produced at pH 4.5 remaining stable for up to 1 h.

Emulsifying Activity Index and Emulsifying Stability Index

Figure 3.7 shows the emulsifying activity index (EAI) of boiled and pressure-cooked aquafaba at ratios of 5:1, 4:1, and 3:1 (water to seed) with pH values of 3,5, and 7 alongside the EAI at the native pH (~6). The EAI is a calculated value that measures the interfacial area stabilized by the protein in m^2/g. In this experiment, the EAI of aquafaba samples ranged from 17.5 m^2/g to 92 m^2/g, with the pressure-cooked 5:1 sample at pH 3 producing the highest EAI and the boiled 3:1 sample at pH 7 producing the lowest. Additionally, both pH and cooking method were found to significantly affect the EAI. This appear to be directly related to the protein content of the aquafaba solutions. As displayed in Table 3.2, samples at pH 3 had significantly low levels of protein, which translated to high EAI values in Figure 3.7. Overall, this experiment displayed that even extremely low quantities of protein can stabilize emulsions. EAI is not easily compared between studies, as differences in method, oil type, oil volume fraction, protein concentration, and equipment can all cause significant differences in EAI values

and outcome (Pearce & Kinsella, 1978). The EAI of samples at pH 5, 6, and 7 were comparable to those obtained by Buhl et al. (2019) who had EAI values around 15 m^2/g which was also significantly higher than that of egg white powder with the same protein concentration.

Figure 3.8 shows the corresponding emulsifying stability index (ESI) of boiled and pressure-cooked aquafaba. The ESI uses the change in turbidity of the emulsion after ten minutes to calculate the stability of the emulsion over time. Of the samples and pH values tested, the boiled 4:1 sample at pH 6 had the highest ESI at 22 min, and the boiled 4:1 ratio sample at pH 3 had the lowest ESI at 11.6 min. It was determined that pH significantly affected the ESI time, with samples at pH 6 and 7 having significantly higher values ($P \leq 0.05$) than those at pH 3 and 5. These results are comparable and consistent with the results obtained by Buhl et al. (2019) where canned chickpea aquafaba showed an increase of ESI from ~15min up to ~25 min with an increase in pH, and with pH 7 and 8.5 having a significantly higher ($P \leq 0.05$) ESI than pH 3 and 4.5. The ESI of multiple legume isolates have been shown to be pH dependent, typically attributed to their higher surface charge and solubility (Ge et al., 2021). Additionally, protein composition can impact emulsion stabilization. Due to their full and partial solubility in water, globulins and albumins are more suited for interface stabilization than other legume storage proteins such as prolamins and glutelins. In a study on rapeseed, their albumins and globulins were found to interact synergistically, with albumins adsorbed to the oil-water interface and globulins weakly bound around the albumins (Yang & Sagis, 2021). As was demonstrated by the SDS-PAGE in Figure 3.2, all the identified protein bands in chickpea aquafaba were albumins or globulins.

Sample Reduction

The foaming ability of model systems containing aquafaba, hydrocolloid gums and sugar was also analyzed. These mixtures would be more reminiscent of a meringue, whipped topping or material used in a foam-based cake. This allowed us to test whether these additional ingredients would affect the performance of the foam. As previous results showed that cooking ratio was not a significant factor for foam capacity or stability, only the most concentrated solutions (that is from the 3:1 water to seed ratio) were used. As the chickpea cooking method was significant, aquafaba from both boiling and pressure-cooking was used. Finally, only one pH was selected to move forward (pH 5) as aquafaba at this pH was significantly higher ($P \leq 0.05$) than pH 3 in foaming capacity and had significantly higher stability than pH 6 and 7.

Foam Overrun and Foam Liquid Drainage

Figure 3.9 (a) shows the foam overrun of boiled and pressure-cooked aquafaba with XG and HPMC added at 0%, 0.2%, 0.4%, and 0.6% by mass. The overrun ranged from 66.2% to 421%, with pressure-cooked samples with no gum added exhibiting the lowest overrun and boiled samples with 0.4% as well as pressure cooked samples with 0.6% HPMC having the highest overrun. All factors including cooking method, gum type, and gum level were shown to significantly affect foam overrun. Interestingly, in pressure-cooked samples the effect of gum type and gum level was more evident. The boiled aquafaba samples all exhibited similar percent overrun between 351 – 421%. Pressure-cooked samples, on the other hand, showed significantly lower ($P \leq 0.05$) overrun even when gum was added, with HPMC producing significantly higher ($P \leq 0.05$) overrun than XG.

The pressure-cooked aquafaba showed a loss of functionality in comparison to boiled aquafaba, with it either unable to properly stabilize the air-water interface or perhaps unable to support the increase in bulk viscosity of the continuous aqueous layer. While XG did significantly improve the overrun of pressure-cooked samples, those samples still had lower overrun than those with added HPMC. XG is not considered to be surface active (Patino & Pilosof, 2011). In solutions with limited thermodynamic compatibility, conditions that inhibit proteins and polysaccharides from forming a complex or associating, polysaccharides can cause the protein to perform as a more concentrated film as well as increase surface pressure via exclusion volume effects thereby giving a similar effect to that of increasing protein concentration (Grinberg & Tolstoguzov, 1997; Patino & Pilosof, 2011). XG also increases the viscosity of the continuous aqueous phase through water binding. While beneficial to foam stability by reducing drainage this quality can also inhibit air incorporation and restrict the mobility of surface active molecules (Dabestani & Yeganehzad, 2019). This could be why boiled aquafaba showed a slight decrease in overrun with an increase in XG levels, and why increased levels of XG with pressure-cooked samples did not improve the % overrun. On the other hand, HPMC is a surface-active polysaccharide (Patino & Pilosof, 2011). For surface active polysaccharides, one of two processes may occur. Polysaccharides can adsorb at the air-water interface and be in competition with protein or the surface-active polysaccharide can form a complex with the adsorbed protein through electrostatic interactions or hydrogen bonding (Patino & Pilosof, 2011). As this experiment was done at pH 5, the charges of HPMC were likely minimal, which could limit its surfactant behavior. Polysaccharides with and without surface activity are able to complex with adsorbed protein however, and neutral complexes have been

shown to form denser viscoelastic interfacial films, therefore contributing to improved foaming properties (Patino & Pilosof, 2011).

As seen in Figure 3.9 (b), egg white had an overrun of 225%. In comparing the average egg white foam overrun % to the boiled and pressure cooked aquafaba foam with no added gum, the egg white foams were found to be significantly higher ($P \leq 0.05$) than pressure cooked samples, while significantly lower ($P \leq 0.05$) than boiled aquafaba samples.

Figure 3.10 (a) shows the liquid drainage from boiled and pressure-cooked aquafaba foams with added XG and HPMC measured at 0 h, 1 h, and 24 h. Foams at 0 and 1 h had very little liquid drainage, with pressure-cooked samples with no gum added having an average liquid drainage of 18.3 mL. After 24 h, both boiled and pressure-cooked samples had high levels of liquid drainage at 61.3 to 75 mL. All levels of XG reduced liquid drainage to 0 mL regardless of cooking method. As previously mentioned, XG's ability to bind water increases the bulk viscosity of the aqueous phase, and it is extremely beneficial for foam stability. HPMC levels did lead to observable differences after 24 h, with higher levels of HPMC exhibiting lower amounts of drainage. Liquid drainage is reduced by increasing the viscosity of the solution or the disjoining pressure- that is the force per area needed to separate two bubbles separated by the liquid lamella. As HPMC does not significantly increase the viscosity of aquafaba at the experimental temperatures, then the greater HPMC levels most likely increased the disjoining pressure. The disjoining pressure is associated with the development of osmotic pressure differences between the bulk phase and the lamella fluid, and as such protruding surfactant chains, counter ion clouds around surfactant layers, and hydration repulsion forces all contribute to these pressure differences (Damodaran, 2005).

Figure 3.10 (b) shows the foam liquid drainage of egg white to that of boiled and pressure cooked aquafaba foam with no added gum. Egg white had no observable drainage until 24 h, where drainage averaged 66.3 mL. As with foam overrun, the average drainage of egg white was greater than for pressure-cooked aquafaba samples and lesser than that of boiled samples.

Foam Texture

The hardness, consistency, and adhesiveness of foam produced by egg white and by boiled and pressure-cooked aquafaba with xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC) are listed in Table 3.3. The hardness value (N) is the measurement of the maximum force exerted during compression. Consistency (N·s) is the positive area from the compression while adhesiveness (N·s) is the negative area.

Hardness of aquafaba foams ranged from 0.74 N to 2.02 N. Pressure-cooked aquafaba foams with no gum added produced the softest foams, while both boiled and pressure-cooked aquafaba foams with 0.6% HPMC produced the hardest foams. Interestingly, for boiled aquafaba samples, the hardness was lower at 0.2% XG and HPMC than with no added gums, but higher at 0.4 or 0.6% levels. At the maximum XG level (0.6%), the hardness of boiled aquafaba foam was no different than that without XG, while foams with 0.6% HPMC were harder than boiled samples without HPMC. Pressure-cooked aquafaba with no gums produced foams that were less hard (0.74 N) than those made from boiled aquafaba with no gums (1.47 N). Pressure-cooked samples with XG at all levels were harder (1.28-1.35 N) than those with no gum (0.74 N) but were not significantly different from each other and were not different than boiled aquafaba foams with XG at all levels (1.24-1.48 N). Pressure-cooked aquafaba foams with HPMC were also harder than those with no gum, and samples with 0.6% HPMC were harder (2.02 N) than all

others except boiled aquafaba foams with 0.4-0.6% HPMC. Egg white had a hardness value (1.48 N) similar to that of boiled aquafaba with no added gum as well as boiled aquafaba with 0.6 % XG.

Similar trends were observed with the consistency of aquafaba foams. The consistency ranged from 27.2 N·s to 78.4 N·s, with the least cohesive being the pressure-cooked foam with no added gum, and the most cohesive the pressure-cooked sample with 0.6% HPMC. Additionally, boiled aquafaba samples with 0.4% and 0.6% HPMC (69.8-74.5 N·s) had an average consistency that was no different from that of pressure-cooked sample with 0.6% HPMC. As with foam hardness, the consistence of boiled aquafaba samples decreased at 0.2% XG and HPMC addition and increased with increased levels. At 0.4 and 0.6% XG, boiled aquafaba had a consistency similar to that of a boiled sample without added gum, while 0.6% HPMC produced foams that were more consistent than boiled samples without added gums. Pressure-cooked aquafaba samples with no gum had relatively low consistency (27.2 N·s) while added XG increased consistency (48.0-52.5 N·s). All pressure-cooked samples with XG had consistency values similar to boiled samples with the same levels of XG. HPMC addition also improved the consistency of pressure-cooked samples, with addition of 0.6% HPMC producing similar consistency to boiled samples with 0.4 and 0.6% HPMC.

The adhesiveness of aquafaba foams exhibited similar trends to those found for hardness and consistency. The range of adhesiveness was from 24.3 N·s to 57.2 N·s, with the boiled aquafaba foam with 0.2% HPMC resulting in the lowest adhesion, and both the boiled and pressure-cooked samples with 0.6% HPMC having the greatest adhesion. The addition of 0.2% XG or HPMC had the same effects on boiled aquafaba samples as it did on foam hardness and consistency, that is adhesiveness values were significantly lower than that of boiled samples with

no gum. As HPMC levels increased, so did the adhesiveness of boiled samples, with 0.6% addition resulting in higher adhesiveness than boiled samples with no gum. Maximum levels of XG in boiled samples resulted in similar adhesiveness to boiled samples with no gum. Lastly, pressure cooked samples had increasing adhesiveness with higher levels of HPMC and XG, with 0.6% HPMC resulting in a value similar to boiled samples with 0.6% HPMC. All levels of XG in pressure-cooked samples were statistically similar to boiled samples with 0.4 and 0.6% XG. Egg white foam hardness and consistency had values most similar to the boiled aquafaba with 0.6 % XG, although they were more adhesive with values most similar to boiled or pressured-cooked samples with 0.6% HPMC.

Regardless of cook type, 0.6% HPMC resulted in the highest values for hardness, consistency, and adhesiveness. This is likely due to its complex concentration dependent behavior, its ability to alter and/or overtake the interfacial characteristics of the adsorbed protein layer, and its water binding capabilities (Arboleya & Wilde, 2005; Patino & Pilosof, 2011). The initial decrease in hardness, consistency and adhesiveness observed at 0.2% HPMC and XG could be due to the adsorption of neutral protein-polysaccharide complexes at the interface. These complexes can form dense second layers that prevent proteins from moving through them to form a protein layer that stabilizes the air-water interface (Patino & Pilosof, 2011). Alternatively, it could be that the proteins and polysaccharides are competitively adsorbing at the interface, making it so the surface pressure is being primarily affected by the component that is able to adsorb more rapidly (i.e. is more surface active) therefore affecting foam structure (Patino & Pilosof, 2011).

Viscosity of Egg White and Aquafaba/Gum Blends

Table 3.4 lists the apparent viscosity and flow behavior index (n) for egg white along with boiled and pressure-cooked aquafaba with 86.4g sugar and XG and HPMC at levels of 0%, 0.2%, 0.4%, and 0.6%. The apparent viscosity ranged from 137 mPa·s to 3339 mPa·s. As expected, all samples were much more viscous than samples with no added sugar or gums (Table 3.1). For example, boiled aquafaba with no sugar had an apparent viscosity of 81 mPa·s, while that with sugar had a viscosity of 137 mPa·s, that with sugar and 0.6% HPMC had a viscosity of 198 mPa·s, and that with sugar and 0.6% XG a viscosity of 2820 mPa·s. Thus, it is apparent that XG provided the most pronounced effect on the aquafaba viscosity.

Boiled aquafaba with no gum had the lowest viscosity, but it was not significantly different ($P \geq 0.05$) than boiled samples with 0.2-0.6% HPMC, boiled samples with 0.2% XG, or pressure-cooked samples with 0-0.6% HPMC. HPMC forms weak thermo-reversible gels upon heating around 63-80°C while XG displays non-Newtonian behavior above a critical concentration referred to as the "overlap concentration" (Saha & Bhattacharya, 2010; van den Berg et al., 2015). As all viscosity tests were performed at 25°C, samples with HPMC did not show any increased viscosity or changes in pseudoplasticity and were similar to samples with no added gum. The sample with the highest apparent viscosity was the pressure-cooked sample with 0.6% XG, which was not different from the boiled aquafaba with 0.6% XG. Egg white with added sugar had a measured apparent viscosity of 561 mPa·s, which was between the apparent viscosity of boiled aquafaba samples with all levels of HPMC and boiled samples with 0.2% XG.

The fitted value for flow behavior index (n) ranged from 0.65 to 0.93, with the pressure-cooked aquafaba sample with 0.6% XG having the lowest flow behavior index and therefore greater pseudoplastic (shear-thinning) behavior, while the pressure-cooked sample with no added

gum having the highest flow behavior index and thus more Newtonian (linear) behavior. All samples with any level of HPMC had flow behavior index values that were statistically similar ($P \geq 0.05$), with values all greater than 0.88 indicating more Newtonian behavior. These results can also be observed in Figure 3.11 (a), which displays the shear stress versus the shear rate of boiled aquafaba with sugar and gum added and compared with egg white with added sugar, and in Figure 3.11 (b), which displays the same data for pressure-cooked aquafaba. As shown in Table 3.4, egg white had a flow behavior index of 0.95 indicating more Newtonian behavior. This is also visible in Figure 3.11 (a) and Figure 3.11 (b) where the shear stress versus shear rate of the egg white is mostly linear, especially when visually compared to boiled and pressure-cooked aquafaba with 0.4 and 0.6% XG.

Conclusion

The cooking ratio did not significantly affect the functional properties of aquafaba, while cooking method did significantly affect functional properties. This was attributed to the difference in water loss via evaporation leading to a difference in aquafaba concentration. As cooking method and ratio did not influence the protein profile, protein concentration is likely what affects functionality regardless of the method of preparation. However, cooking ratio and method were significant factors in the apparent viscosity of aquafaba solutions, implying either an unknown factor such as fiber content or a greater sensitivity to small protein concentration changes. Furthermore, aligning with the results of the protein profile analysis, the cooking method did not affect the protein's solubility which was lowest around pH 4. While the altering of aquafaba pH significantly affected foaming capacity/stability and the emulsifying activity/stability index, this also was likely primarily influenced by the concentration of protein

in the solution. A cooking ratio of 3:1 water to seed of both cooking methods at pH 5 displayed balanced improvement in both foaming capacity and stability and was further investigated for use in food foams and cake.

Depending on the needs of the formed foams, like that of meringue or whipped topping, hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG) can provide a wide range of textural properties, overrun, and stability up to 24 hours. HPMC-based foams displayed higher levels of overrun overall, significantly improving the low overrun values of pressure-cooked aquafaba. However, HPMC-based foams had greater liquid drainage at 24 h in contrast to xanthan gum (XG) that had virtually no drainage. Although, increasing the level of HPMC decreased the overall liquid drainage at 24 h and increased the hardness, consistency, and adhesiveness of the foam texture. Essentially, HPMC displays the capability to increase foam texture qualities and the air fraction in chickpea aquafaba (and possible other plant-based foams) with some long-term stability limitations that can be mitigated with higher percent addition. In direct comparison, low levels of XG decreased textural values, with foams with 0.6% XG having similar texture to those with no added gum. The addition of any level of XG can produce highly stable plant-based foams at the expense of its textural qualities. However, different foods or food foams may desire vastly different foam textures. Boiled aquafaba foams, with no gum addition, had higher stability and a greater overrun than that of egg white foams, and addition of 0.6% XG led to a similar foam texture. These results indicate that chickpea aquafaba can produce foams with similar likeness to egg white foams, and possibly improved qualities of those foams with different levels of either XG or HPMC addition.

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Tables

Table 3.1 Protein concentration, apparent viscosity, and flow behavior index (n) of the power law model ($R^2 > 0.99$) of aquafaba samples.

Sample[1]	Protein Concentration[2] (mg/mL)	Apparent viscosity[2] (mPa·s) at 25°C[2]	Flow Behavior Index[3] (n)
B5:1	1.1 ± 0.1^{abc}	20 ± 7^{c}	0.78 ± 0.07^{b}
B4:1	1.4 ± 0.07^{a}	52 ± 4^{b}	0.70 ± 0.07^{c}
B3:1	1.3 ± 0.09^{abc}	81 ± 3^{a}	0.66 ± 0.03^{c}
P5:1	0.94 ± 0.1^{c}	9.6 ± 2^{c}	0.95 ± 0.04^{a}
P4:1	0.97 ± 0.2^{bc}	12 ± 6^{c}	0.89 ± 0.05^{a}
P3:1	1.3 ± 0.07^{ab}	21 ± 9^{c}	0.81 ± 0.04^{b}

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 5:1, 4:1, and 3:1.
[2]Mean values (mean value ± standard derivation, n = 3) in columns not followed by the same superscripts are significantly different ($p<0.05$).
[3]Mean values (mean value ± standard derivation, n = 9) in columns not followed by the same superscripts are significantly different ($p<0.05$).

Table 3.2. Protein concentration of aquafaba samples at pH 3, 5, and 7.

Sample[1]	pH	Protein Concentration[2] (mg/mL)
B5:1	3	0.47 ± 0.05^{hi}
B5:1	5	1.1 ± 0.1^{cdefg}
B5:1	7	1.5 ± 0.06^{ab}
B4:1	3	0.32 ± 0.1^{i}
B4:1	5	0.98 ± 0.15^{defg}
B4:1	7	1.4 ± 0.1^{abc}
B3:1	3	0.35 ± 0.1^{i}
B3:1	5	1.3 ± 0.1^{abcde}
B3:1	7	1.6 ± 0.2^{a}
P5:1	3	0.26 ± 0.05^{i}
P5:1	5	0.76 ± 0.08^{gh}
P5:1	7	1.2 ± 0.04^{bcdef}
P4:1	3	0.40 ± 0.08^{hi}
P4:1	5	0.84 ± 0.1^{fg}
P4:1	7	1.2 ± 0.2^{abcde}
P3:1	3	0.36 ± 0.07^{i}
P3:1	5	0.92 ± 0.1^{efg}
P3:1	7	1.3 ± 0.2^{abcd}

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 5:1, 4:1, and 3:1.
[2]Mean values (mean value ± standard derivation, n = 3) in columns not followed by the same superscripts are significantly different ($p<0.05$).

Table 3.3. Foam texture of aquafaba samples with added xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) and egg white.

Sample[1]	pH	Gum	Level (%)	Hardness[2] (N)	Consistency[2] (N·s)	Adhesiveness[2] (N·s)
B3:1	5	----	----	1.47 ± 0.07^{bc}	57.0 ± 3.1^{cde}	40.8 ± 3.3^{bcde}
B3:1	5	HPMC	0.2	0.94 ± 0.1^{ef}	34.1 ± 1.2^{fg}	24.3 ± 3.2^{g}
B3:1	5	HPMC	0.4	1.76 ± 0.1^{ab}	69.8 ± 6.1^{abc}	47.0 ± 2.9^{abc}
B3:1	5	HPMC	0.6	1.89 ± 0.2^{a}	74.5 ± 12^{ab}	56.6 ± 6.4^{a}
B3:1	5	XG	0.2	1.24 ± 0.3^{cde}	46.6 ± 11^{ef}	33.4 ± 8.3^{defg}
B3:1	5	XG	0.4	1.31 ± 0.2^{cd}	49.9 ± 9.4^{de}	35.4 ± 7.7^{cdefg}
B3:1	5	XG	0.6	1.48 ± 0.08^{bc}	55.0 ± 3.2^{de}	41.5 ± 3.0^{bcd}
P3:1	5	----	----	0.74 ± 0.1^{f}	27.2 ± 5.4^{g}	26.5 ± 7.0^{fg}
P3:1	5	HPMC	0.2	1.11 ± 0.1^{de}	44.1 ± 4.6^{ef}	29.2 ± 2.2^{efg}
P3:1	5	HPMC	0.4	1.54 ± 0.3^{bc}	60.7 ± 11^{bcd}	50.1 ± 12^{ab}
P3:1	5	HPMC	0.6	2.02 ± 0.2^{a}	78.4 ± 10^{a}	57.2 ± 6.5^{a}
P3:1	5	XG	0.2	1.28 ± 0.09^{cd}	48 ± 5.4^{def}	35.1 ± 3.3^{defg}
P3:1	5	XG	0.4	1.37 ± 0.05^{cd}	51.3 ± 1.5^{de}	37.5 ± 3.2^{cdef}
P3:1	5	XG	0.6	1.35 ± 0.08^{cd}	52.5 ± 2.1^{de}	38.2 ± 1.5^{cdef}
EW[3]	----	----	----	1.48 ± 0.1	54.2 ± 5.5	59.7 ± 2.5

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1

[2]Mean values (mean value ± standard derivation, n = 3) in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Table 3.4. Apparent viscosity and flow behavior index (n) of the power law model ($R^2 > 0.99$) of egg white and aquafaba samples with 86.4 g sugar and added xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC).

Sample[1]	pH	Gum	Level (%)	Apparent Viscosity[2] (mPa·s)	Flow Behavior Index[2] (n)
B3:1	5	----	----	137 ± 13^{c}	0.89 ± 0.04^{a}
B3:1	5	HPMC	0.2	202 ± 9.9^{c}	0.91 ± 0.006^{a}
B3:1	5	HPMC	0.4	200 ± 71^{c}	0.90 ± 0.05^{a}
B3:1	5	HPMC	0.6	198 ± 12^{c}	0.91 ± 0.02^{a}
B3:1	5	XG	0.2	734 ± 230^{bc}	0.72 ± 0.03^{bc}
B3:1	5	XG	0.4	1240 ± 420^{b}	0.68 ± 0.005^{bc}
B3:1	5	XG	0.6	2820 ± 440^{a}	0.68 ± 0.07^{bc}
P3:1	5	----	----	170 ± 52^{c}	0.91 ± 0.03^{a}
P3:1	5	HPMC	0.2	366 ± 43^{c}	0.93 ± 0.003^{a}
P3:1	5	HPMC	0.4	342 ± 29^{c}	0.88 ± 0.01^{a}
P3:1	5	HPMC	0.6	207 ± 65^{c}	0.91 ± 0.02^{a}
P3:1	5	XG	0.2	417 ± 90^{c}	0.78 ± 0.05^{b}
P3:1	5	XG	0.4	1230 ± 570^{b}	0.69 ± 0.02^{bc}
P3:1	5	XG	0.6	3330 ± 330^{a}	0.65 ± 0.02^{c}
EW[3]	----	----	----	561 ± 390	0.95 ± 0.03

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1

[2]Mean values (mean value ± standard derivation, n = 3) in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Figures

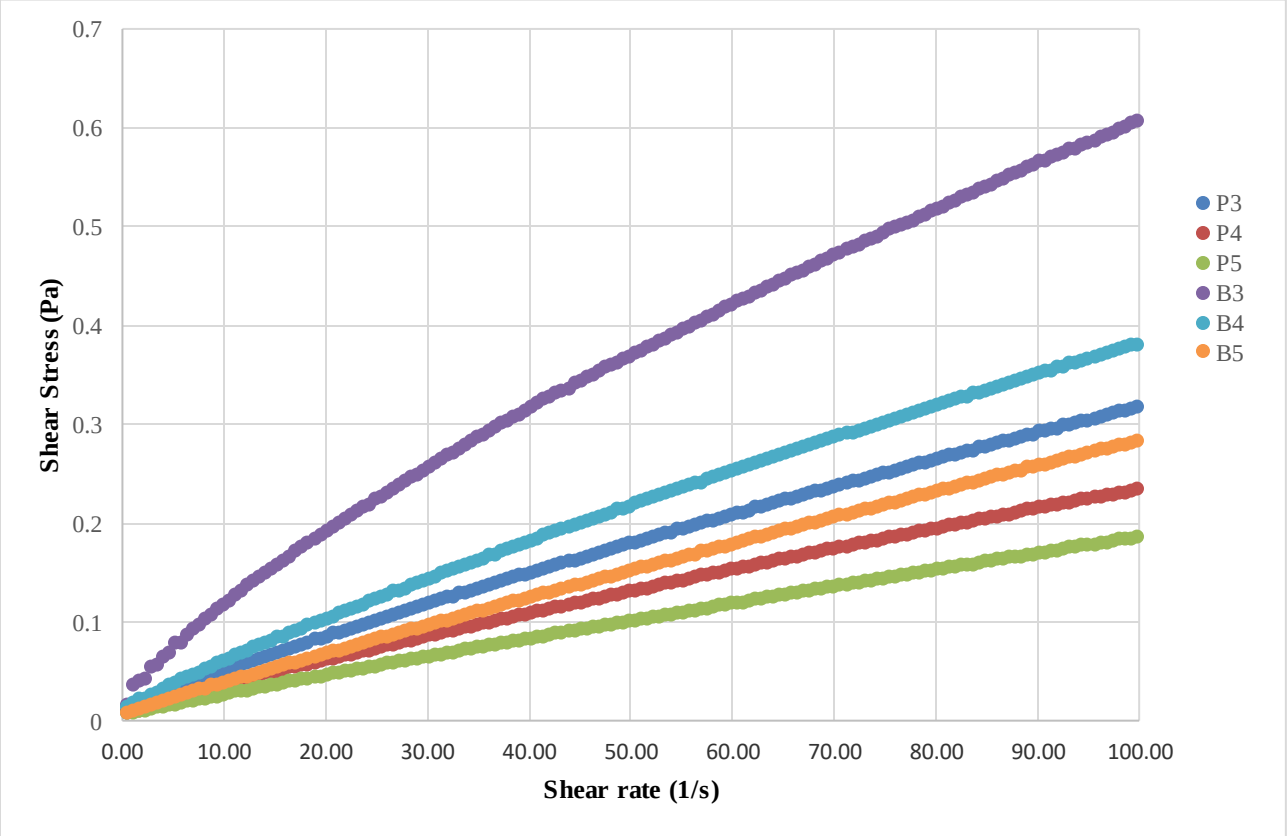

Figure 3.1. Shear stress versus shear rate of aquafaba samples. 'B': Boiled samples 'P': Pressure cooked samples. '5': Cooking ratio 5:1 (water to seed) '4': Cooking ratio 4:1 (water to seed) '3': Cooking ratio 3:1 (water to seed).

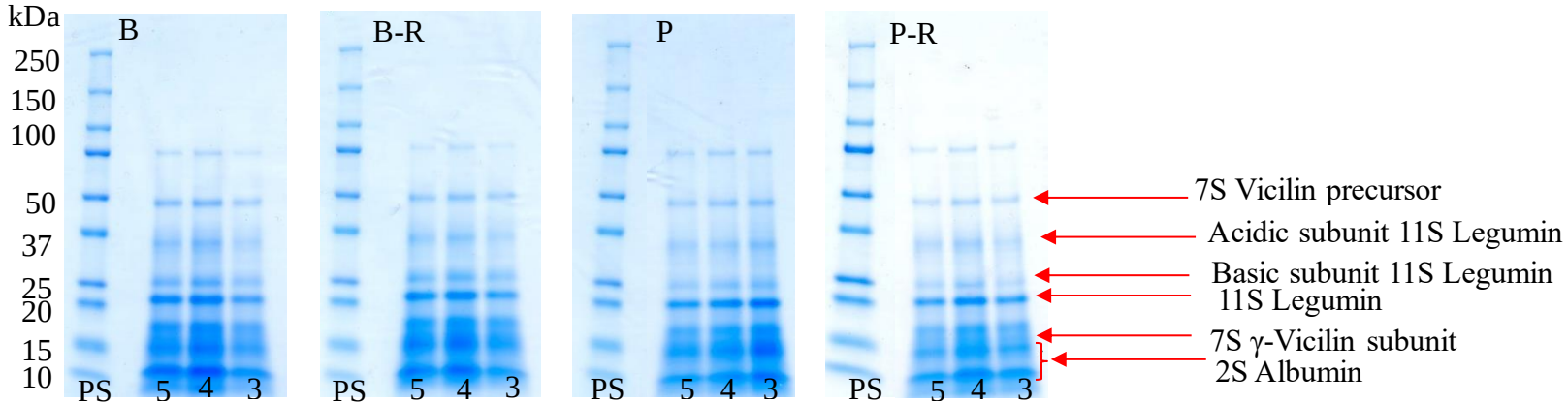

Figure 3.2. SDS-PAGE gels of aquafaba samples. 'B': Boiled samples 'P': Pressure cooked samples. '-R': Reducing conditions. 'PS': Protein standard . '5': Cooking ratio 5:1 (water to seed) '4': Cooking ratio 4:1 (water to seed) '3': Cooking ratio 3:1 (water to seed).

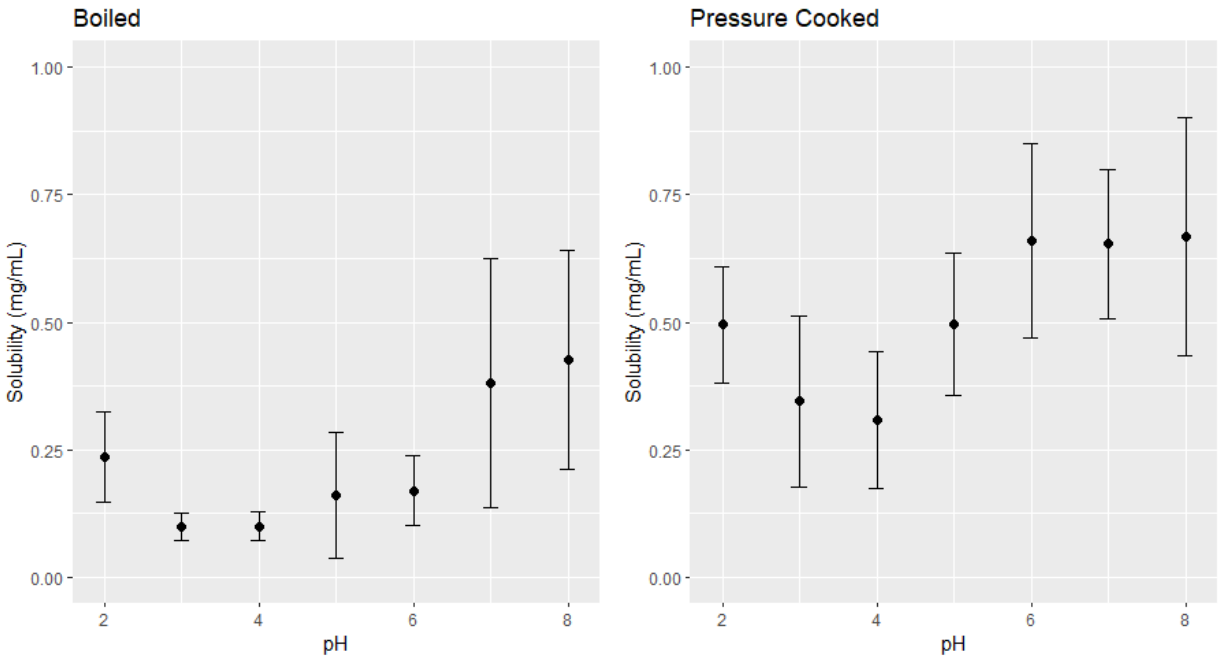

Figure 3.3. Protein concentration of pressure cooked and boiled aquafaba from pH 2 to 8. Error bars represent ± 3 standard deviations from the mean.

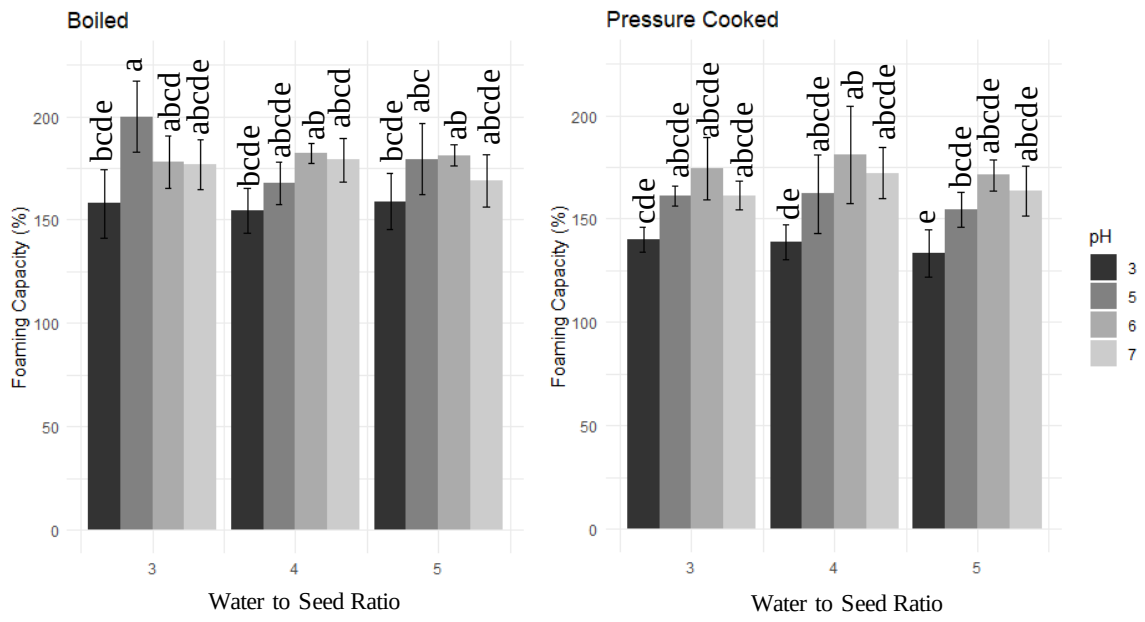

Figure 3.4. Foaming capacity of aquafaba samples at pH 3, 5, 6, and 7. Water to seed ratios indicated by 3 (3:1), 4 (4:1), and 5 (5:1). Bar colors represent pH values 3,5,7 and inherent pH of samples (~6). Error bars represent ± 3 standard deviations from the mean.

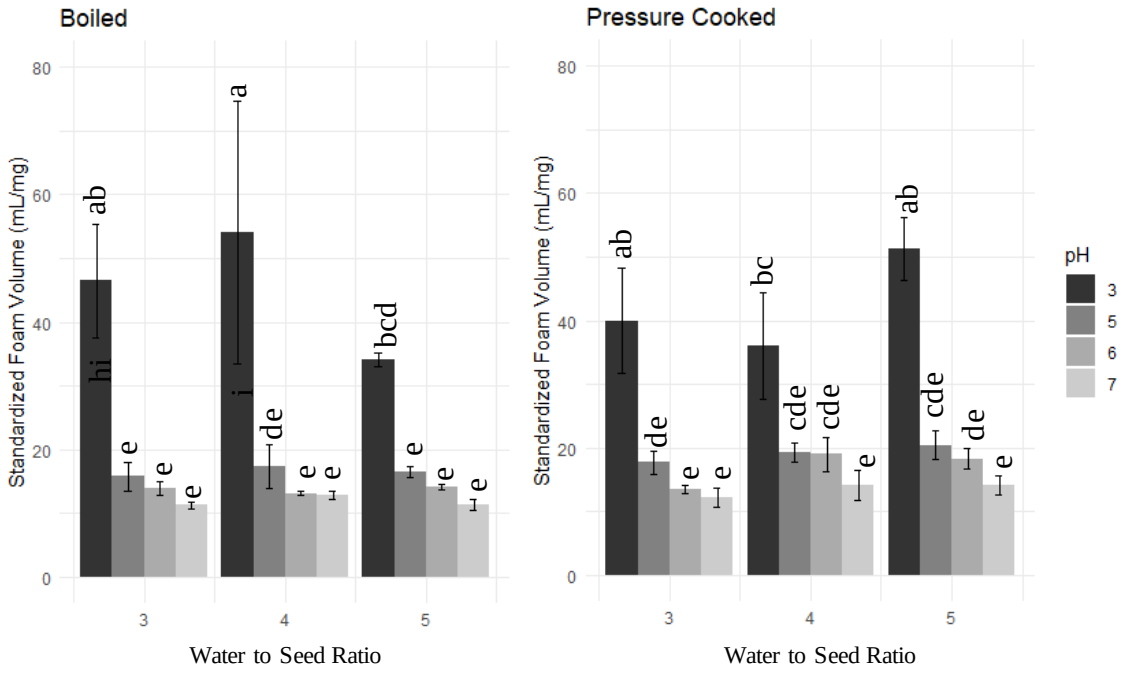

Figure 3.5. Standardized foam volume (mL/mg) of aquafaba samples at pH 3, 5, 6, and 7. Water to seed ratios indicated by 3 (3:1), 4 (4:1), and 5 (5:1). Bar colors represent pH values 3,5,7 and inherent pH of samples (~6). Error bars represent ± 3 standard deviations from the mean.

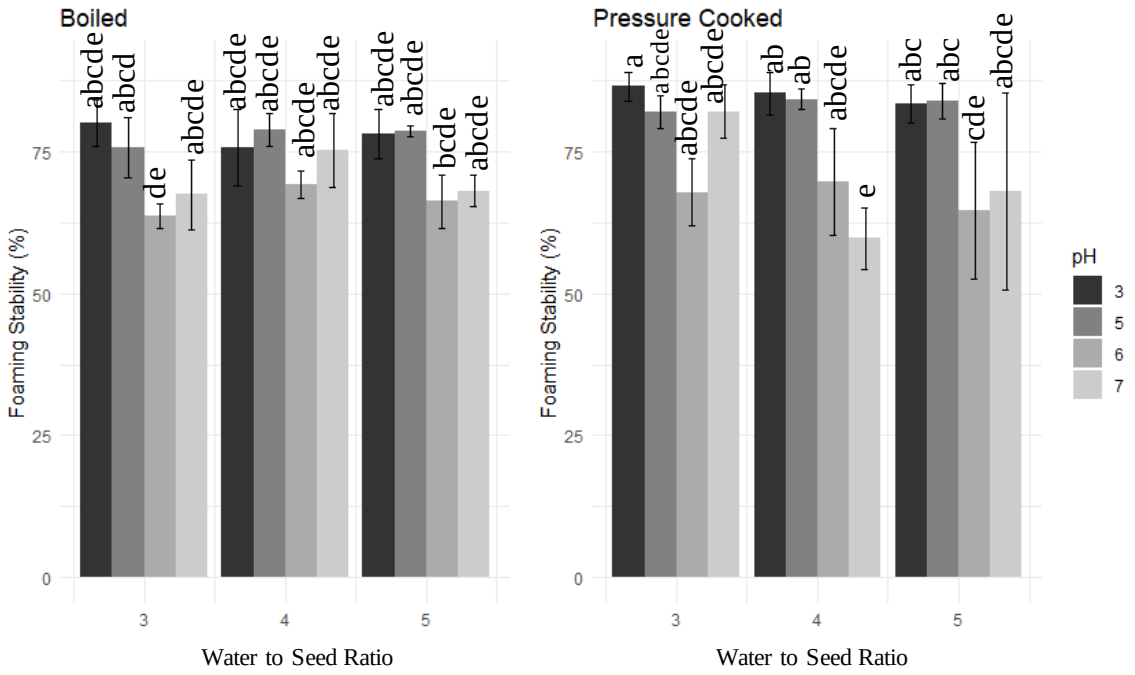

Figure 3.6. Foaming stability of aquafaba samples at pH 3, 5, 6, and 7 at 30 min. Water to seed ratios indicated by 3 (3:1), 4 (4:1), and 5 (5:1). Bar colors represent pH values 3,5,7 and inherent pH of samples (~6). Error bars represent ± 3 standard deviations from the mean.

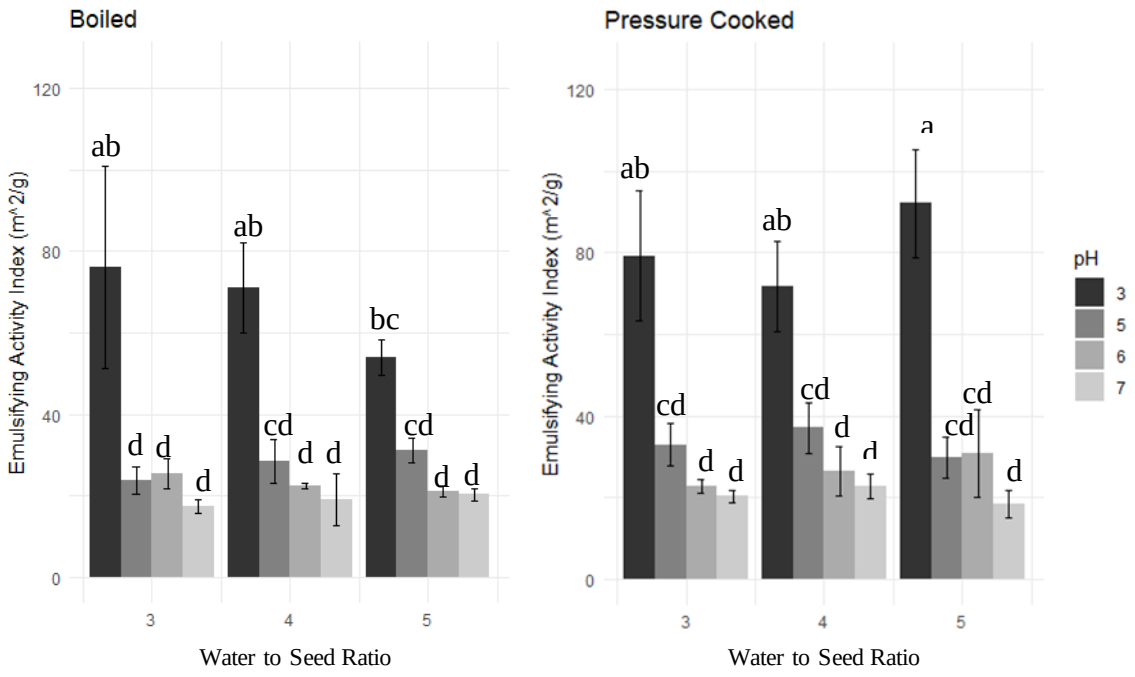

Figure 3.7. Emulsifying activity index of aquafaba samples at pH 3, 5, 6, and 7. Water to seed ratios indicated by 3 (3:1), 4 (4:1), and 5 (5:1). Bar colors represent pH values 3,5,7 and inherent pH of samples (~6). Error bars represent ± 3 standard deviations from the mean.

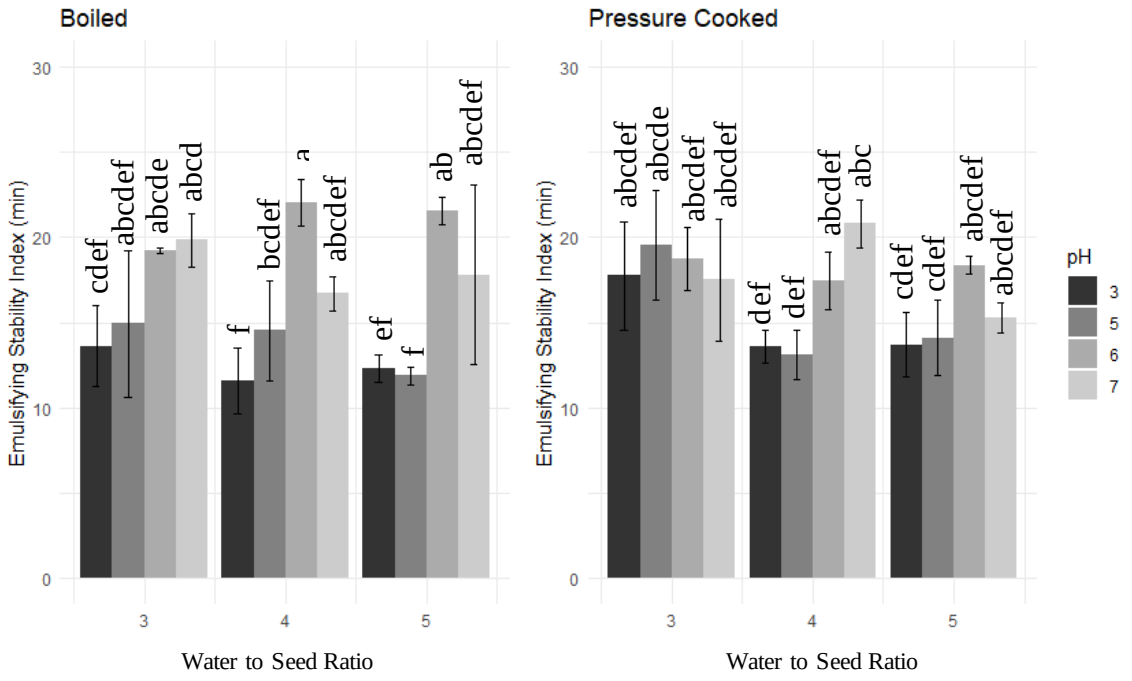

Figure 3.8. Emulsifying stability index of aquafaba samples at pH 3, 5, 6, and 7. Water to seed ratios indicated by 3 (3:1), 4 (4:1), and 5 (5:1). Bar colors represent pH values 3,5,7 and inherent pH of samples (~6). Error bars represent ± 3 standard deviations from the mean.

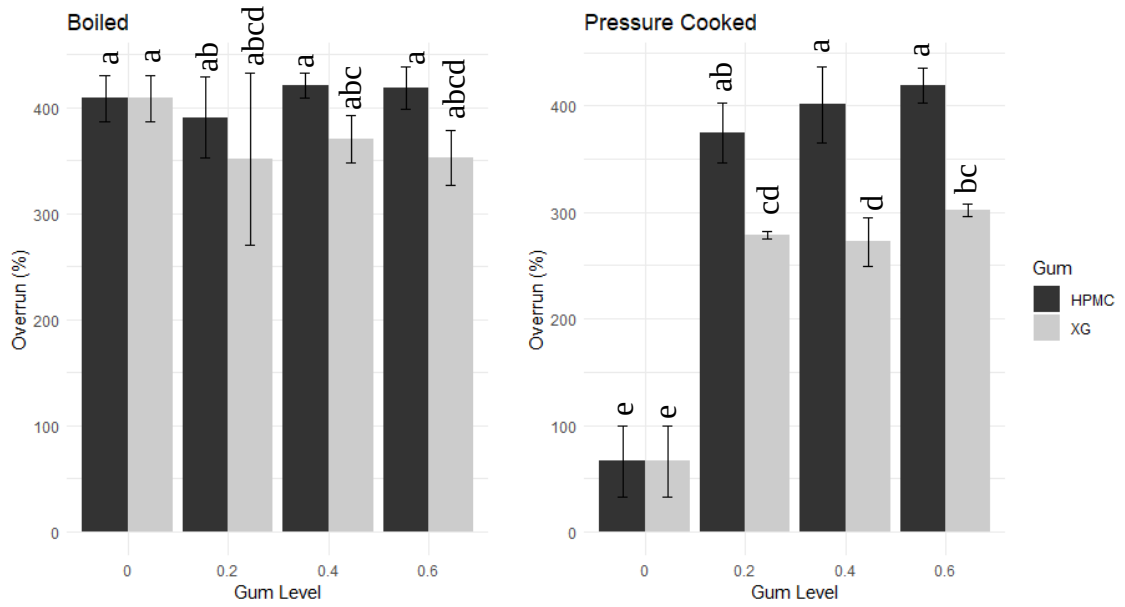

Figure 3.9. (a): Foam overrun of aquafaba with added xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC). Error bars represent ± 3 standard deviations from the mean.

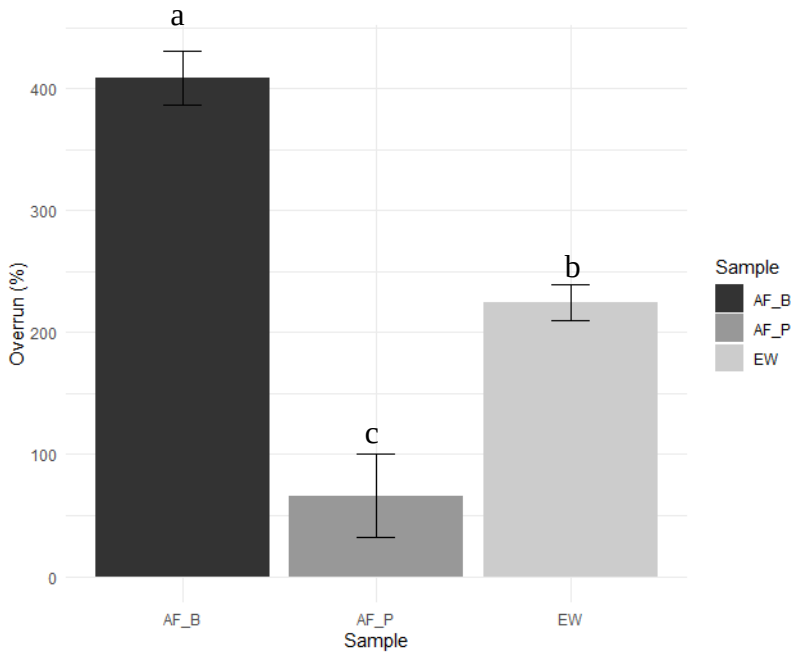

Figure 3.9. (b): Foam overrun of egg white and aquafaba produced by boiling or pressure cooking with no gum added. Error bars represent ± 3 standard deviations from the mean.

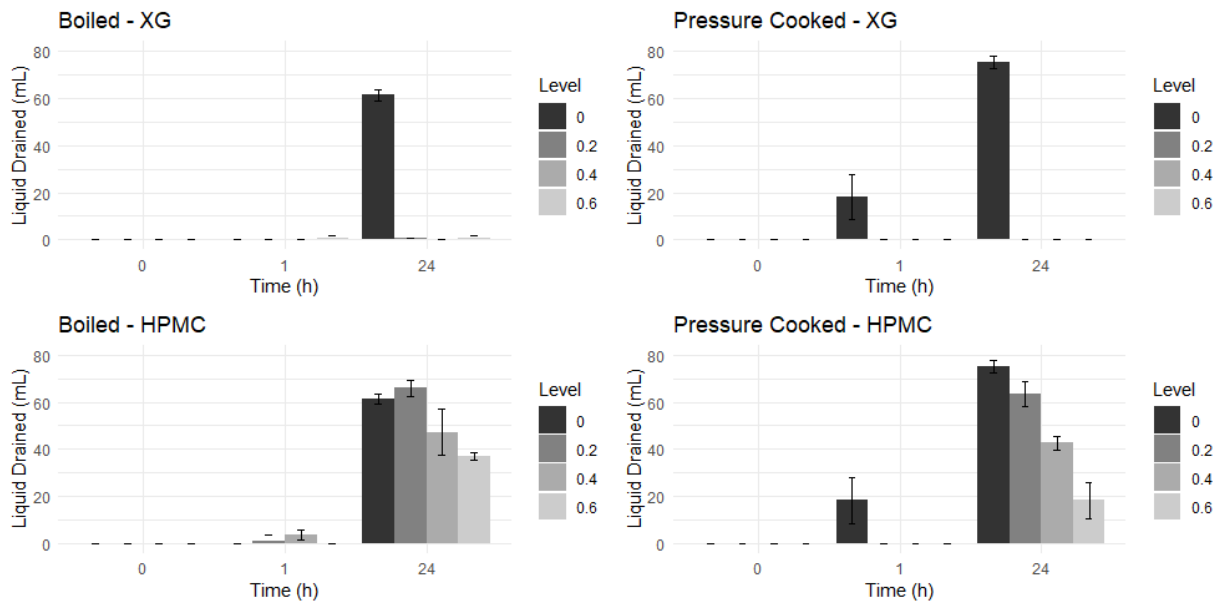

Figure 3.10. (a): Foam liquid drainage of aquafaba with added xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) at 0 h, 1 h, and 24 h. Error bars represent ± 3 standard deviations from the mean.

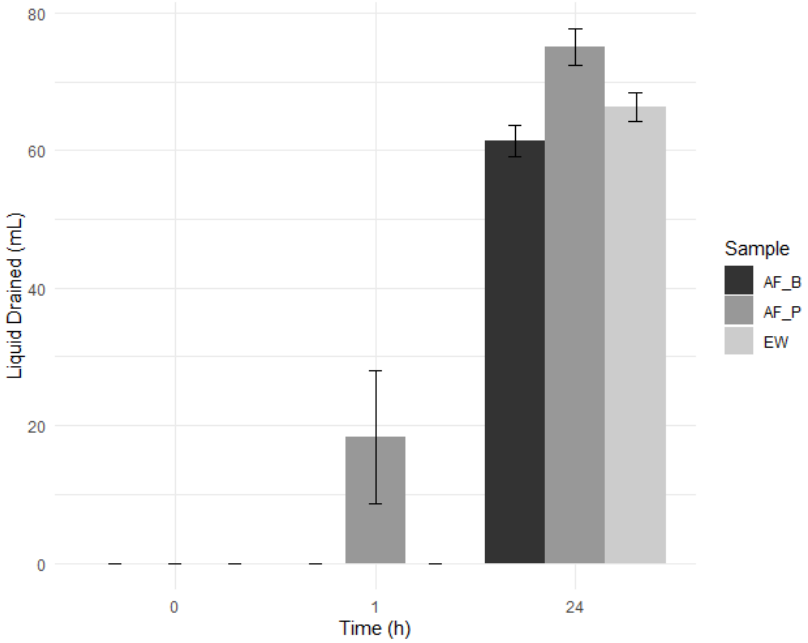

Figure 3.10. (b): Foam liquid drainage at 0 h, 1 h, and 24 h of egg white and aquafaba produced by boiling or pressure cooking. Error bars represent ± 3 standard deviations from the mean.

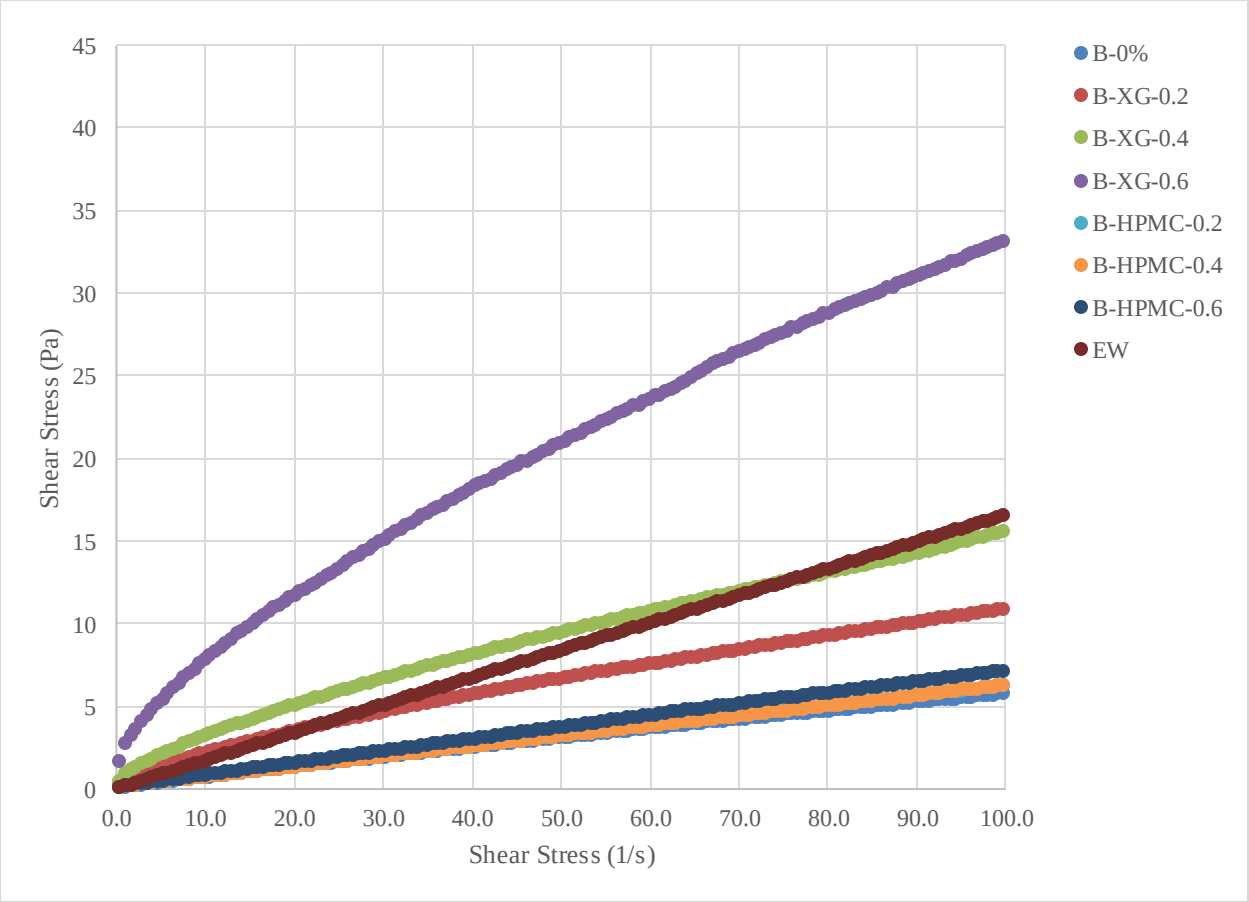

Figure 3.11 (a): Shear stress versus shear rate of egg white (EW) with sugar and boiled (B) aquafaba samples with added gum (XG: Xanthan Gum, HPMC: Hydroxypropyl methylcellulose) and sugar.

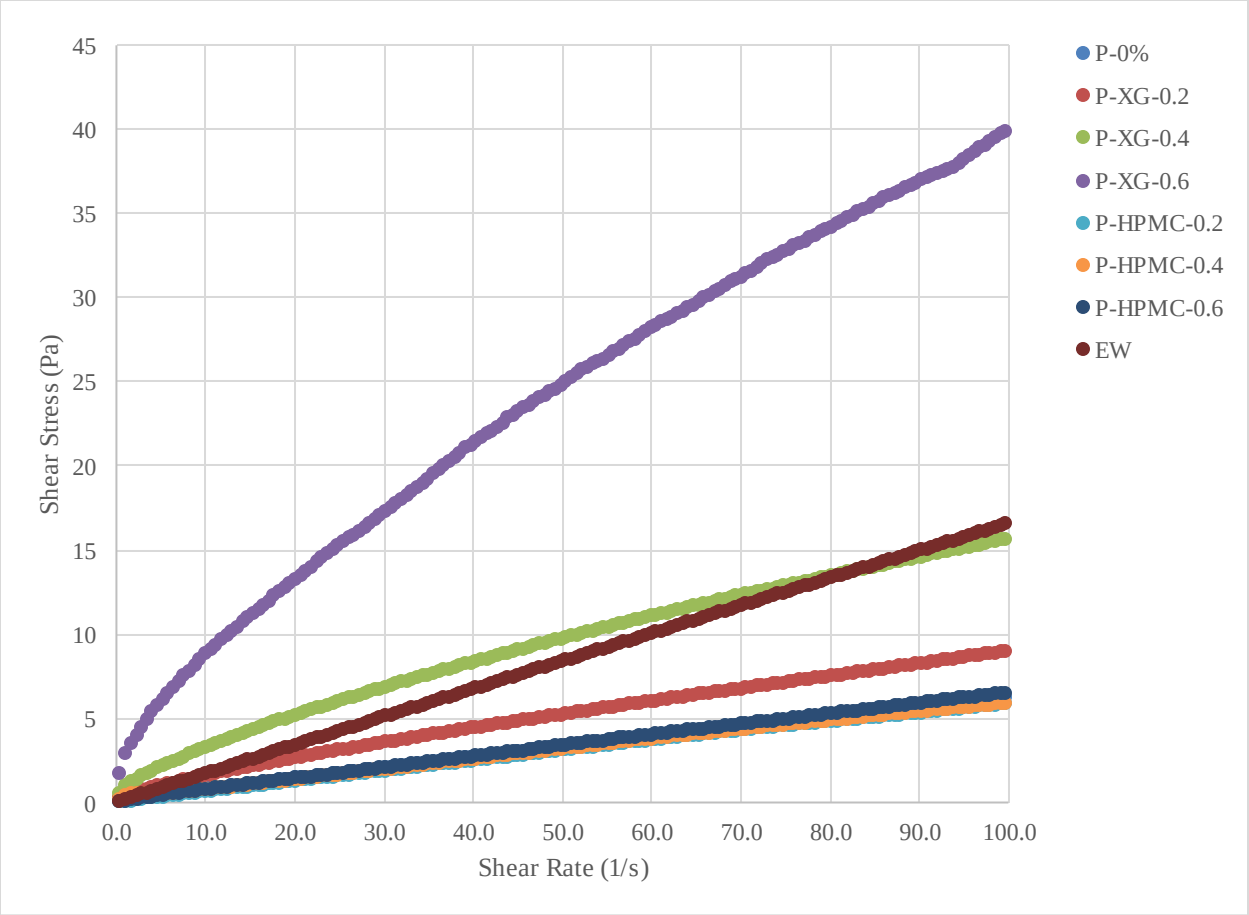

Figure 3.11 (b): Shear stress versus shear rate of egg white (EW) with sugar and pressure-cooked (P) aquafaba samples with added gum (XG: Xanthan Gum, HPMC: Hydroxypropyl methylcellulose) and sugar.

CHAPTER 4

ANALYSIS OF TEXTURE, COLOR, AND CRUMB STRUCTURE OF CAKE PREPARED WITH AQUAFABA[2]

[2]Crawford, K.E. and W.L. Kerr. To be submitted to *Food Hydrocolloids.*

Abstract

Pressure-cooked and boiled chickpea aquafaba was prepared at a cooking ratio of 3:1 water to seed and pH 5 and compared with egg white for use in a hi-ratio cake. Xanthan gum or hydroxypropyl methylcellulose were added at 0, 0.2, 0.4 and 0.6% to aquafaba samples in order to increase air volume fraction in foams as well as improve foam stability. Measurements of cake size, symmetry, moisture loss, crumb structure, texture, and color of were evaluated. Aquafaba cakes had high standard deviations for multiple measured qualities, with most samples regardless of gum type or level not being significantly different from each other. Egg white cakes had a harder crumb than all aquafaba cakes but lost almost 3% less moisture than aquafaba cakes. Egg white cakes were also less dense than all aquafaba cakes but had a similar volume index to cakes made with pressure-cooked aquafaba with 0.2% HPMC. Egg white cake had a similar colored crumb to aquafaba cakes, but with a darker and more orange colored crust.

Introduction

A UK poll showed a 350% increase in the number of people identifying as vegan, and consumers are putting the pressure on businesses to offer products free of animal byproducts (Hodson & Earle, 2018). There are many reasons consumers may want to avoid eggs in a product such as cake including egg allergies, health concerns, dietary restrictions, and ethical reasons. Plant proteins have been used as an egg substitute in cakes with moderate success, with textural and sensorial issues named as the primary challenges to consumer acceptance (Aslan & Ertaş, 2020, Bravo-Núñez & Gómez, 2021). Chickpea aquafaba has been suggested as a possible egg white substitute in cake, as it has shown similar foamability to egg white (Buhl et al., 2019; Meurer et al., 2020). Two studies that investigated the use of chickpea aquafaba in cake

successfully substituted whole egg and egg white in sponge cake, but with the cakes exhibiting flat and slightly collapsed structures (Aslan & Ertaş, 2020; Mustafa et al., 2018).

Existing studies on the use of legume proteins in cake have not specifically investigated chickpea protein. One study, which analyzed how chickpea flour affected sponge cake, found that cake hardness increased, and volume decreased (Bravo-Núñez & Gómez, 2021). That same study also found chickpea variety significantly influenced sponge cake volume and protein content had some correlation to improved cake volume (Bravo-Núñez & Gómez, 2021). Aslan & Ertaş's (2020) use of chickpea aquafaba showed similar results, with increasing percentages of aquafaba substitution for whole egg resulting in harder cakes with a lower volume index. Another study which used chickpea aquafaba in cakes and compared them to cake made with egg white showed a significantly lower volume index in aquafaba cakes, however, there was no statistical difference in crust or crumb hardness (Mustafa et al., 2018).

There have been some studies to investigate the effects of hydrocolloids on sponge cakes. For sponge cakes with whole egg, xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) added at a 1% flour basis resulted in significantly lowered cake volume, and XG produced harder cakes than those with HPMC or no gum (Noorlaila et al., 2020). In eggless cakes, XG addition produced similar volume and crumb firmness to control cakes, while HPMC addition produced a higher volume and decreased crumb firmness (Ashwini et al., 2009). Eggless cakes that used pea protein isolate as a substitute showed significantly lower specific volume and increased hardness, but 0.1% XG addition led to increased specific volume and improved moisture content (Lin et al., 2017a). A similar study done with soy protein isolate did not show improved volume or moisture with 0.1% XG addition (Lin et al., 2017b).

The purpose of this research was to determine if hydrocolloid addition to optimized chickpea aquafaba could improve the color, crumb structure, size properties, baking loss, and texture of aquafaba cake as compared to cakes made with egg white. Cakes were prepared with aquafaba produced by boiling or pressure-cooking and with the addition of 0.2, 0.4 or 0.6% by mass of either XG or HPMC, and measurements of cake crumb and cake physical properties was done to evaluate cake quality. A critical attribute of cakes is the volume attained during baking and the density of the final product, and this was measured through displacement methods. Image analyses was used to assess overall cake contour and symmetry, as well as to examine the porous crumb structure. In addition, the amount of moisture lost during baking was determined gravimetrically, as it determines the final perceived moistness of the cake. Finally, instrumental methods were used to evaluate important cake textural properties such as hardness, adhesiveness, and consistency.

Materials and Methods

Sponge cakes were prepared to compare the effects of egg white or aquafaba as a foaming agent, and with added hydrocolloids xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) to thicken the batter and potentially reinforce the foam. Hi-ratio cake flour (Ardent Mills, Denver, CO, USA) and fine (6x) sugar (Domino, Yonkers, NY, USA) were purchased from U.S. Foods (Atlanta, GA). Liquid egg whites were from Publix (Athens, GA, USA), XG from Now Foods (Bloomingdale, IL, USA), and HPMC from Modernist Pantry (Eliot, ME, USA). Aquafaba was produced by boiling or pressure-cooking of chickpeas as described in Chapter 3.

Batter and Cake Preparation

Cake batter was made using a modified recipe by Pycarelle et al. (2019) with a multi-stage mixing method. Cake formulations are outlined in Table 4.1. The batter contained sieved hi-ratio cake flour (145.7 g), fine granulated sugar (126.72 g), egg white or aquafaba (88 mL), tap water (45.3 g), and baking powder (2.9 g). A KitchenAid stand mixer (KitchenAid, St. Joseph, MI, USA) with a 5-quart stationary bowl was used for ingredient mixing. First 88 mL of EW or pH 5 AF was whipped using a wire whip attachment for 7 min (speed 10). For samples with xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC) levels, the gums were added prior to whipping and mixed in at speed 1. After the 7-min whip time, 126.72 g of fine granulated sugar was added slowly while whipping for 1 min at speed 3. In a separate mixing bowl, 40 g sifted hi-ratio cake flour and 45.3 g tap water were whisked with a hand whisk until mixed. The resulting mixture was then blended with the sugar and foam mixture for 30 s at speed 1. The remaining flour (105.7 g) and baking powder (2.9g) were added and mixed with a flat beater for 20 s at speed 1, thereafter the sides and base of the bowl was scraped with a spatula to ensure thorough blending and the batter was then mixed for 15 s at speed 1. Each batter was prepared in triplicate.

Two rectangular (6.5 cm x 13 cm x 5.5 cm) cake pans (Chicago Metallic) were filled with 150 g of each cake batter batch. The batter was baked at 160°C for 30 min in a CTP7-20 commercial Combitherm oven (Alto-Shaam, Menomonee Falls, WI, USA). Cakes were removed from pans and cooled on a wire rack for 30 min at room temperature before subsequent analyses.

Cake Density and Specific Volume

Cake density and specific volume were determined using the seed displacement method (AACC method 10-05.01) and described by Rahmati & Tehrani (2014). Each cooled cake was placed in a 2 L container, rapeseeds were added to fill container and then leveled off. The mass of the container, seeds, and cake as well as volume of seeds added to container were measured. Cake density was calculated using equation 1, 2 and 3:

$$W_{seeds} = W_{total} - W_{cake} - W_{container} \quad (1)$$

$$V_{cake} = V_{container} - V_{seeds} \quad (2)$$

$$Cake\ density = W_{cake}/V_{cake} \quad (3)$$

where W represents the mass in grams, and V represents the volume in cm^3. Specific volume was calculated using equation 4:

$$Specific\ volume = V_{cake}/W_{cake} \quad (4)$$

with W representing mass in grams, and V representing volume in cm^3.

Volume Index, Cake Contour, and Symmetry

The volume index, cake contour, and symmetry were measured using AACC method 10-91 and described by Rahmati & Tehrani (2014) with modification. After cooling, the cakes were cut through the center and scanned using an EPSON scanner (Epson, Los Alamitos, CA, USA). The cake images were then analyzed using ImageJ software 1.50i version (National Institute of Health, Rockville, MD) at three points (B, C, and D) of the two cross sectioned slices. The volume index, cake contour, and symmetry were calculated using equations 6, 7 and 8 below:

$$Volume\ Index = B + C + D, \quad (5)$$

$$Contour = (2C - B - D), \quad (6)$$

$$Symmetry = |B - D| \quad (7)$$

where C is the height of the cake at the center, and B and D are the heights of the cake 1.5 cm away from the center to the left and right of C.

Moisture Loss

Moisture loss was calculated using the equation described by Rahmati & Tehrani (2014):

$$Moisture\ Loss\ (\%) = \frac{W_1 - W_2}{W_1} * 100 \quad (8)$$

where W_1 is the weight of cake batter in each cake pan (150 g) and W_2 is the weight of the baked cake after 30 min of cooling at room temperature.

Crumb Structure

Crumb structure was analyzed in triplicate according to Banjade et al. (2019) using two slices from the center of each cake (n=6). Again, the slices were scanned using an Epson scanner (Epson, Los Alamitos, CA, USA) with the 8-bit grayscale, 600 dpi setting. The resulting images were analyzed with ImageJ 1.50i version (National Institute of Health, Rockville, MD, USA). Images were converted from greyscale to binary using the Otsu clustering algorithm, and three 2 x 2 cm squares from the top center, lower left and lower right corner of the slices were analyzed. Gas cell size range was analyzed from 0.0001 to infinity and cell count (number of cells per selected area), cell area (area covered by cells in selected area), and average cell size (average area per cell) were measured (n=18).

Texture Analysis

Textural attributes of crumb (hardness, consistency, adhesiveness) were measured using a TA-XT2 texture analyzer with exponent software (Stable Microsystems Ltd., Godalming, Surrey, UK) according to Rahmati & Tehrani (2014) derived from AACC Method 2000. A 2 x 2 cm cube was cut from the center of the cake sample and tested by a two-cycle compression test performed with a 35mm diameter cylindrical probe with 25% deformation. Hardness was measured as the peak force value, consistency as the positive area under the force-deformation curve, and adhesiveness as the negative area under the curve. Samples were tested in triplicate.

Color Analysis

The color of the crust and crumb was measured using L*a*b* values using an Aeros colorimeter (HunterLab, Reston, Virginia, USA) standardized with white and black tiles. Each replicate value was an average of 25 measurements taken while the sample platform was rotated, with five measurements per second taken.

Statistical Analysis

All samples were tested in triplicate (n=3). Statistical analysis was conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Three-way analysis of variance (ANOVA) was used for analyses with three factors (cooking method, gum type, gum level). Tukey's honest significant difference (HSD) test was also performed for significant difference at $p<0.05$.

Results and Discussion

Cake Density and Specific Volume

Table 4.2 lists the cake density and specific volume of egg white cakes and boiled and pressure-cooked aquafaba cakes with and without xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC). Cake density ranged from 0.34 to 0.39 g/cm^3, however, density values were not significantly different amongst samples. In contrast, the egg white cake was less dense (0.14 g/cm^3) with a value that was almost half that of the cakes made with aquafaba. The specific volume of cakes ranged from 2.6 to 2.9 cm^3/g. As specific volume is a different representation of the mass and size of the cakes, as with density, the average specific volume of all the cakes were not statistically different. This also meant that the egg white cake specific volume was more than double than that of aquafaba cakes. A study which replaced portions of wheat flour with pea protein isolate in sponge cake made with commercial liquid whole egg had cake density values that directly correlated to batter density as well as the air volume fraction in the batter (Assad Bustillos et al., 2020). Air volume fraction in the batter does not appear to correlate as well in full egg replacement however, as this experiment had significantly different % overrun between aquafaba samples, gum addition type, and gum level, yet density and specific volume were not significantly different. This suggests that the other important cake crumb structure building processes, such as starch gelatinization and protein networking and hydration, are the limiting factors in aquafaba cake density and specific volume.

Volume Index, Cake Contour, and Symmetry

The volume index, cake contour, and symmetry of egg white cakes alongside boiled and pressure-cooked aquafaba cakes (with and without XG and HPMC) are listed in Table 4.2.

Images of the sliced cakes are shown in Figure 4.1. The volume index of aquafaba cakes ranged from 91.7mm to 120mm. The cake with the smallest volume index was made from pressure-cooked aquafaba with no added gum. This is also observable in Figure 4.1 (b) where the pressure-cooked cake with no gums is considerably smaller than other cake cross-sections. The cake with the greatest volume index was the pressure-cooked sample with 0.2% HPMC. This can also be observed in Figure 4.1 (b) where the cake cross section is taller than most of the cake cross sections as well as having a domed top. The volume of cake made from pressure-cooked aquafaba and 0.2% HPMC was not different from cakes made with 0.4% HPMC, however. The egg white cake had a volume index of 126 mm, close to that of the cake with the pressure cooked aquafaba sample with 0.2% HPMC. Despite the addition of different hydrocolloids, the cake volumes observed in this research align with those of sponge cakes made using canned chickpea aquafaba and boiled aquafaba, which had a volume index of ~111.3mm and 128mm respectively (Aslan & Ertaş, 2020; Mustafa et al., 2018). In addition, Mustafa et al. (2018) used similar ingredients for the egg white control cake and reported a volume index of 125.7 mm, quite close to the 126 mm value observed in our work.

As many of the aquafaba cakes had some degree of collapsed centers, many of the cake contour values were negative (see Equation 6). The contour values ranged from -7.66mm to 1.61 mm (Table 4.2). The sample with the lowest contour value was the cake with the boiled aquafaba sample with 0.6% XG. As seen in Figure 4.1, this cake indeed had a pronounced downward indentation in the central region. Of the aquafaba cakes, the highest contour value was observed for those from boiled aquafaba and no gum; the only other aquafaba cake with a positive contour value was made from pressure-cooked aquafaba and 0.2% HPMC. Cake made with egg white had a slightly greater contour at 2.76 mm. The egg white cake contour was also affected by the

cake splitting across the top. Aquafaba cakes by Mustafa et al. (2018) also experienced cake collapse, and this was attributed to the "heat-stable" proteins delaying the denaturation and coagulation of proteins that made it unable to stabilize the existing air cells. The results of this experiment could suggest that protein quantity and competition at the interface with hydrocolloid addition limits the amount of air aquafaba proteins can stabilize, especially with the added interactions between dispersed gluten.

As symmetry is the measure of the difference in heights a given distance on each side of the cake center (Equation 7), a lower value indicates greater symmetry. The cake symmetry values from aquafaba samples ranged from 0.36 to 1.3 mm, but high levels of variance in the samples led to the means not being statistically different. Egg white cakes had a cake symmetry value in the center of the range of values for aquafaba cakes at 0.77.

Moisture Loss

Table 4.2 also lists the moisture loss of egg white cakes and cakes made with boiled or pressure cooked aquafaba samples with and without XG or HPMC. Moisture loss was calculated as the percent difference between the mass of the cake batter and the mass of the baked cakes. The moisture loss from the EW cake was 13.5%, while the aquafaba cakes experienced a loss ranging from 15.9 to 17.5%. The moisture loss in most aquafaba samples were not different from each other, except for the cake made with pressure-cooked aquafaba and no gum added which had the highest moisture loss at 17.5%. The 17.5% loss was not statistically different than values observed for cakes with boiled or pressure-cooked aquafaba that had 0.2 and 0.4% XG. Cake made with egg white lost almost 3% less moisture than aquafaba cakes. This aligns with current literature, where baking loss/moisture loss is often higher for total egg-replacement compared to

control cakes (Yazici & Ozer, 2021). One investigation of partial and full replacement of egg in sponge cake by boiled aquafaba did not result in significant baking loss values in comparison to whole egg control, but cake ingredients also included shortening, milk powder, DATEM, and xanthan gum which likely contributed to the lack of significant difference (Aslan & Ertaş, 2020).

Crumb Structure

Table 4.3 lists the ImageJ crumb structure profile of egg white cakes and cakes made with boiled and pressure cooked aquafaba samples with and without XG and HPMC. The crumb structure of the two innermost slices of the cakes were analyzed to determine the cell count (number of cells per area selected), the average cell size (average size of cells per selected area), and the percent area (area covered by cell per area selected). A porous cake structure, which in this experiment would be indicated by larger average cell size and a smaller cell count, is usually associated with softer and lighter cake crumb (Yazici & Ozer, 2021). Despite significant differences in the aquafaba foams (Figure 3.9 and Table 3.3), the final set crumb structure had high standard deviations and thus there were no significant differences in average cell size or area amongst the aquafaba cakes. The only samples that were significantly different in crumb cell count were cakes made with boiled aquafaba and 0.4% HPMC which had the lowest cell count (33.6), and the cakes made with pressure-cooked aquafaba with no gum that had the highest cell count (65.8). Egg white cakes had a cell count of 41.2, similar to that of cake made with boiled aquafaba and 0.2% XG. Egg white cakes also had a cell average size similar to that of cakes with 0.4 and 0.6% HPMC, and a cell % area similar to that of cake made with pressure-cooked aquafaba with 0.4% XG.

Texture Analysis

Table 4.4 lists the hardness, adhesiveness, and consistency of the cake crumb of EW and aquafaba cakes with and without XG and HPMC. The hardness value (N) is the measurement of the maximum force exerted during compression. Consistency (N·s) is the positive area from the compression while adhesiveness (N·s) is the negative area.

The hardness of aquafaba cake samples ranged from 0.63 N to 2.36 N. Amongst the aquafaba cakes, the hardest crumb was from cakes made with boiled aquafaba with no added gum, while the softest samples (0.63 and 0.69 N) came from cakes made from boiled aquafaba with 0.2 and 0.4% XG. All other cakes made from aquafaba were not significantly different from each other or the hardest and softest samples. Cake crumb from cakes made with egg white had a hardness of 2.53 N, harder than all the aquafaba cake crumb samples. Aquafaba sponge cakes made by Mustafa et al. (2018) had a crumb hardness (14.2 N) that was not significantly different than their egg white control (15.2 N). Their crumb hardness values are higher than those obtained in this experiment, in part due to their sample compression of 50% strain versus the 25% strain used here. Aquafaba cakes made by Aslan & Ertaş (2020) under 25% strain showed increased firmness (from 4.98 to 6.44 N) with 100% aquafaba substitution of whole egg, with their cake crumb likely exhibiting greater firmness then the cake samples in Table 4.4 due to additional ingredients like shortening.

Adhesiveness values of aquafaba cake crumb ranged from 0.12 to 0.23 N·s and were not found to be significantly different from one another. Adhesiveness of cake crumb made from egg white was 0.23 (N·s). Consistency of aquafaba cake crumb ranged from 1.5 to 6.5 N·s. Cakes made with boiled aquafaba and no added gum had the highest consistency, while cakes made with boiled aquafaba with 0.4% XG had the lowest consistency. As with the other textural

properties of aquafaba cake crumb, high standard deviations meant most samples were not significantly different from each other. Egg white cake had a high value of consistency at 7.3 (N·s). As the egg white cake had higher values of hardness (maximum force to compress the cake) and consistency (total work to compress the cake), it is likely that those cakes would be judged as firmer by consumers.

Color Analysis

Table 4.5 lists the L* a* b* color values egg white cake and aquafaba cakes made with aquafaba with and without XG or HPMC. For the aquafaba cake crust, L* values ranged from 74.0 to 78.8 but the sample means were not significantly different. In comparison, the egg white cake crust had a lower L* value at 65.1, indicating a darker crust. The a* and b* values for the aquafaba cakes were indicative of an orange hue with more yellow than red, and modest color saturation. The positive a* values of the aquafaba cake crust ranged from 6.27 to 9.70 and were also not significantly different due to different gum types and levels. The egg white cake crust was slightly redder than the aquafaba cake crusts, with an a* value of 14.8. For the aquafaba cake crusts b* values ranged from 23.2 to 34.0. The least yellow saturated cake crust was from the pressure-cooked aquafaba with 0.6 HPMC, while the most yellow saturation occurred with boiled and pressure-cooked aquafaba and 0.2% HPMC. The egg white cake crust was slightly more red and yellow than aquafaba cakes. In contrast to these results, Mustafa et al. (2018) saw significantly lighter and less red crusts in egg white cakes when compared to canned chickpea aquafaba cakes, as well as a slight decrease in yellowness. The color of cake crust can be dependent on the raw material color of ingredients and the browning caused by Maillard

browning and caramelization, which is further influenced by pH and water affinity of ingredients that factor into color-forming reactions (Yazici & Ozer, 2021).

For the aquafaba cake crumb L* values ranged from 76.0 to 83.5, and as expected the crumbs were lighter than the crusts. The cake with the lightest crumb was prepared using boiled aquafaba and 0.6% HPMC and the darkest crumb was from the cake prepared using pressure-cooked aquafaba. The egg white cake crumb had a similar L* value (82.8) to most of the aquafaba crumb L* values. The a* values of the aquafaba cake crumb were also not significantly different due to gum types and levels. The a* range was 0.46 to 1.2, such that most of the color differences fell along the blue-yellow axis. The a* value for egg white cake (0.37) was only slightly lower than that for the aquafaba cakes. For the aquafaba cake crumb, b* values ranged from 16.1 to 20.0. There were no real differences in color, however, save for the boiled aquafaba cake crumb with no gum (b*=20.0) and the pressure-cooked crumb with 0.2% HPMC (b*=16.1). The egg white cake crumb b* values (18.5) were similar to that of aquafaba crumbs. Aquafaba cake crumb and egg white cake crumb L* a* b* values and their differences were comparable to that of Mustafa et al. (2018), except their a* indicate that their cake crumbs were more red. These differences could be attributed to different equipment or technique, or they could indicate differences in the composition of canned chickpea aquafaba in comparison to prepared aquafaba that affect the color-forming reactions within cake crumb.

Thus, while the formulation did have some effect on cake crust color the impact was only minor on the crumb color. Overall, the egg white cake crusts were darker and with greater saturation along the yellow and red color axes, even when compared to the aquafaba cakes with no added gums. This suggests that the egg white cakes were more susceptible to non-enzymatic

browning during baking which might be related to more available reactive amine groups in the egg white proteins, or to some enhancement of caramelization attributed to the egg white.

Conclusion

Chickpea aquafaba was used to make a foam-based cake with and without the addition of the hydrocolloids xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC). Cakes made with egg white were less dense than aquafaba cakes and had the greatest volume overall. Substitution of egg white with chickpea aquafaba did produce cakes that were denser and less voluminous, and addition of either gum at any level led to a similar cake density. This indicates that regardless of how the hydrocolloid may affect the aquafaba foam, it does not appear to improve the volume and density of aquafaba cakes at the tested levels. However, while the egg white cake had the greatest volume, cake from pressure-cooked aquafaba with 0.2% HPMC was a close second. As measured by the 'contour', the egg white cake had the most rounded top. While most of the aquafaba cakes had indented tops, those from the pressure-cooked aquafaba with 0.2% HPMC and boiled aquafaba with no gum did not. This could suggest that a smaller level of certain gums than was tested may help produce desirable cake properties like volume, "contour" and perhaps density. It was noted that there was a great deal of variability in the cake size measurements, and this made it difficult to see many differences in the mean values. There was also little to be gleaned about differences in the porous structure of the cake crumbs. This could imply that aquafaba proteins could be more sensitive as an ingredient in a baked good and therefore less consistent in the outcome of the final product.

There were also few differences in the instrumental texture values amongst the cakes. Cakes made with boiled aquafaba with no added gum had a similar crumb hardness to that of egg

white cakes. When 0.4 and 0.6% XG was added a softer crumb resulted, this could imply that high levels of XG are affecting the internal cake structure or the moisture distribution between the crust and crumb. Additionally, the color of the crusts of egg white cake was darker and more orange than the aquafaba cakes. Which could be attributed to a difference in composition of egg white versus aquafaba (likely sugars). Lastly, future studies should investigate additional ingredients added to aquafaba cake recipes, such as added fats and flavorings, along with sensory testing. While studies so far have shown most aquafaba cakes are denser than their egg white cake counterparts, sensory studies with optimized and prepared aquafaba have the potential to have positive sensorial results.

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Tables

Table 4.1. Cake formulations for egg white or aquafaba[1] cakes. Aquafaba cakes were made with and without 0.2 to 0.6% hydrocolloid gums[2] of xanthan (XG) or hydroxypropyl methylcellulose (HPMC).

	Egg White Cake		Aquafaba Cakes		
	Wt (g)	%	0.2% Gum Wt (g)	0.4% Gum Wt (g)	0.6% Gum Wt (g)
Cake flour	145.7				
Aquafaba[1]	88	35.7	88	88	88
Liquid Egg White	88	-	-	-	-
Sugar	126.7	21.5	126.7	126.7	126.7
Water	45.3	31	45.3	45.3	45.3
Baking Powder	2.9	11.1	2.9	2.9	2.9
XG or HPMC	-	-	0.176	0.352	0.704
Total	408.6	-			

[1]Aquafaba includes samples prepared by boiling and pressure-cooking at a ratio of 3:1 water to seed, and adjusted to pH 5

[2] 0.2%, 0.4%, and 0.6% descriptors of cakes with hydrocolloid addition was in relation to aquafaba quantity

Table 4.2. Cake density, specific volume, volume index, contour, symmetry, and moisture loss of egg white and boiled and pressure cooked aquafaba samples with added gum. Mean values (mean value ± standard derivation, n = 3) [2]

Sample[1]	Gum	Level	Density (g/cm^3)	Specific Volume(cm^3/g)	Volume Index(mm)	Contour(mm)	Symmetry (mm)	Moisture Loss (%)
B3-5	----	----	0.39 ± 0.02^{a}	2.6 ± 0.2^{a}	102 ± 2.7^{de}	1.61 ± 0.76^{a}	0.55 ± 0.5^{a}	15.9 ± 0.36^{b}
B3-5	HPMC	0.2	0.36 ± 0.03^{a}	2.8 ± 0.3^{a}	111 ± 8.6^{bc}	-2.71 ± 5.6^{abcd}	0.94 ± 0.9^{a}	16.4 ± 0.26^{b}
B3-5	HPMC	0.4	0.35 ± 0.04^{a}	2.9 ± 0.3^{a}	109 ± 5.8^{bcd}	-6.11 ± 3.4^{bcd}	1.3 ± 0.3^{a}	15.9 ± 0.48^{b}
B3-5	HPMC	0.6	0.36 ± 0.03^{a}	2.8 ± 0.2^{a}	110 ± 2.4^{bc}	-5.99 ± 2.6^{bcd}	1.1 ± 0.9^{a}	16.3 ± 0.93^{b}
B3-5	XG	0.2	0.38 ± 0.02^{a}	2.7 ± 0.1^{a}	102 ± 2.5^{de}	-4.41 ± 7.0^{abcd}	0.58 ± 0.6^{a}	16.6 ± 0.70^{ab}
B3-5	XG	0.4	0.35 ± 0.01^{a}	2.8 ± 0.09^{a}	102 ± 1.2^{de}	-7.04 ± 2.5^{cd}	0.40 ± 0.3^{a}	16.7 ± 0.34^{ab}
B3-5	XG	0.6	0.38 ± 0.03^{a}	2.7 ± 0.3^{a}	102 ± 4.2^{de}	-7.66 ± 3.5^{d}	0.57 ± 0.3^{a}	16.4 ± 0.35^{b}
P3-5	----	----	0.38 ± 0.01^{a}	2.6 ± 0.09^{a}	91.7 ± 3.5^{f}	-0.035 ± 3.3^{ab}	0.36 ± 0.2^{a}	17.5 ± 0.41^{a}
P3-5	HPMC	0.2	0.34 ± 0.008^{a}	2.9 ± 0.07^{a}	120 ± 4.6^{a}	0.314 ± 2.3^{ab}	0.90 ± 0.7^{a}	16.0 ± 0.33^{b}
P3-5	HPMC	0.4	0.35 ± 0.03^{a}	2.9 ± 0.3^{a}	113 ± 3.5^{ab}	-4.14 ± 3.0^{abcd}	0.66 ± 0.5^{a}	16.3 ± 0.28^{b}
P3-5	HPMC	0.6	0.35 ± 0.04^{a}	2.9 ± 0.3^{a}	109 ± 3.1^{bcd}	-7.54 ± 0.88^{cd}	1.3 ± 0.8^{a}	16.1 ± 0.45^{b}
P3-5	XG	0.2	0.35 ± 0.003^{a}	2.8 ± 0.02^{a}	104 ± 0.89^{cde}	-0.681 ± 2.4^{abc}	1.22 ± 0.5^{a}	16.6 ± 0.55^{ab}
P3-5	XG	0.4	0.34 ± 0.006^{a}	2.9 ± 0.05^{a}	100 ± 2.4^{e}	-6.14 ± 3.1^{bcd}	0.36 ± 0.2^{a}	16.6 ± 0.22^{ab}
P3-5	XG	0.6	0.34 ± 0.006^{a}	2.9 ± 0.05^{a}	105 ± 1.9^{cde}	-4.46 ± 3.8^{abcd}	0.58 ± 0.5^{a}	16.4 ± 0.43^{b}
EW[3]	----	----	0.14 ± 0.004	7.00 ± 0.2	126 ± 4.9	2.76 ± 3.3	0.77 ± 0.5	13.5 ± 0.81

[1]Boiled aquafaba sample indicated by 'B'. Pressure-cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1, pH 5 indicated by '-5'

[2] Values in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Table 4.3. ImageJ crumb structure profile of egg white and aquafaba cake with and without added gum. Mean values (mean value ± standard derivation, n = 6)[2]

Sample[1]	Gum	Level	Cell Count	Average Size	% Area
B3-5	----	----	54.6 ± 24[ab]	0.077 ± 0.08[a]	74.0 ± 4.5[a]
B3-5	HPMC	0.2	44.6 ± 28[ab]	0.094 ± 0.05[a]	77.5 ± 3.6[a]
B3-5	HPMC	0.4	33.6 ± 17[b]	0.13 ± 0.09[a]	78.2 ± 4.0[a]
B3-5	HPMC	0.6	46.3 ± 30[ab]	0.13 ± 0.14[a]	79.0 ± 4.2[a]
B3-5	XG	0.2	41.3 ± 20[ab]	0.095 ± 0.06[a]	76.3 ± 2.6[a]
B3-5	XG	0.4	55.9 ± 30[ab]	0.081 ± 0.07[a]	75.2 ± 4.7[a]
B3-5	XG	0.6	62.7 ± 38[ab]	0.077 ± 0.08[a]	74.7 ± 7.1[a]
P3-5	----	----	65.8 ± 29[a]	0.061 ± 0.04[a]	76.2 ± 3.8[a]
P3-5	HPMC	0.2	51.3 ± 29[ab]	0.097 ± 0.09[a]	76.7 ± 4.2[a]
P3-5	HPMC	0.4	37.9 ± 22[ab]	0.13 ± 0.1[a]	78.3 ± 3.4[a]
P3-5	HPMC	0.6	44.6 ± 29[ab]	0.11 ± 0.08[a]	78.6 ± 5.3[a]
P3-5	XG	0.2	47.4 ± 24[ab]	0.084 ± 0.05[a]	77.4 ± 3.7[a]
P3-5	XG	0.4	43.6 ± 29[ab]	0.14 ± 0.2[a]	75.7 ± 4.3[a]
P3-5	XG	0.6	55.7 ± 28[ab]	0.066 ± 0.03[a]	74.3 ± 4.7[a]
EW[3]	----	----	41.2 ± 23	0.12 ± 0.1	75.9 ± 3.0

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1, pH 5 indicated by '-5'

[2] Values in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Table 4.4. Texture profile of egg white and aquafaba cake crumb. Shown are mean values (mean value ± standard derivation, n = 3)[2]

Sample[1]	Gum	Level	Hardness (N)	Adhesiveness (N·s)	Consistency (N·s)
B3-5	----	----	2.36 ± 1^{a}	0.16 ± 0.02^{a}	6.5 ± 3.1^{a}
B3-5	HPMC	0.2	1.13 ± 0.09^{ab}	0.24 ± 0.05^{a}	2.9 ± 0.43^{ab}
B3-5	HPMC	0.4	1.12 ± 0.08^{ab}	0.17 ± 0.1^{a}	2.5 ± 0.17^{ab}
B3-5	HPMC	0.6	1.10 ± 0.2^{ab}	0.23 ± 0.07^{a}	2.6 ± 0.43^{ab}
B3-5	XG	0.2	0.69 ± 0.3^{b}	0.14 ± 0.03^{a}	1.7 ± 0.68^{b}
B3-5	XG	0.4	0.63 ± 0.1^{b}	0.12 ± 0.05^{a}	1.5 ± 0.25^{b}
B3-5	XG	0.6	0.87 ± 0.1^{ab}	0.19 ± 0.06^{a}	2.0 ± 0.18^{b}
P3-5	----	----	2.20 ± 0.9^{ab}	0.22 ± 0.16^{a}	5.1 ± 2.1^{ab}
P3-5	HPMC	0.2	1.03 ± 0.2^{ab}	0.12 ± 0.04^{a}	2.5 ± 0.52^{ab}
P3-5	HPMC	0.4	1.03 ± 0.02^{ab}	0.18 ± 0.07^{a}	2.4 ± 0.06^{ab}
P3-5	HPMC	0.6	0.97 ± 0.3^{ab}	0.13 ± 0.1^{a}	2.2 ± 0.7^{b}
P3-5	XG	0.2	1.10 ± 0.4^{ab}	0.12 ± 0.05^{a}	2.5 ± 0.97^{ab}
P3-5	XG	0.4	1.06 ± 0.3^{ab}	0.21 ± 0.05^{a}	2.6 ± 0.57^{ab}
P3-5	XG	0.6	0.95 ± 0.1^{ab}	0.16 ± 0.04^{a}	2.2 ± 0.32^{b}
EW[3]	----	----	2.53 ± 0.3	0.23 ± 0.2	7.3 ± 0.86

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1, pH 5 indicated by '-5'

[2] Values in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Table 4.5. L* a* b* color values of egg white and aquafaba cake crust and crumb. Shown are mean values (mean value ± standard derivation, n = 3)[2]

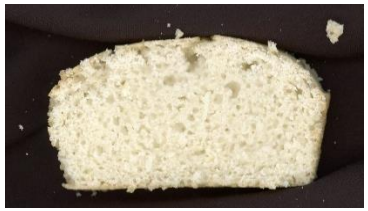
			Crust			Crumb		
Sample[1]	Gum	Level	L*	a*	b*	L*	a*	b*
B3-5	----	----	77.3 ± 1.6[a]	6.89 ± 0.95[a]	30.4 ± 1.4[ab]	79.8 ± 1.7[ab]	1.2 ± 0.7[a]	20.0 ± 2.5[a]
B3-5	HPMC	0.2	75.6 ± 0.78[a]	9.48 ± 0.15[a]	34.0 ± 2.6[a]	82.3 ± 1.9[ab]	0.64 ± 0.1[a]	17.5 ± 0.6[ab]
B3-5	HPMC	0.4	75.3 ± 3.0[a]	8.87 ± 1.44[a]	29.0 ± 3.5[ab]	83.4 ± 1.9[a]	0.69 ± 0.3[a]	17.2 ± 1.2[ab]
B3-5	HPMC	0.6	76.6 ± 1.0[a]	8.22 ± 0.75[a]	29.8 ± 1.5[ab]	83.5 ± 1.4[a]	0.78 ± 0.1[a]	16.2 ± 1.3[ab]
B3-5	XG	0.2	76.5 ± 2.6[a]	8.25 ± 0.46[a]	30.7 ± 0.90[ab]	79.9 ± 0.56[ab]	0.52 ± 0.2[a]	16.5 ± 1.22[ab]
B3-5	XG	0.4	76.4 ± 2.0[a]	7.90 ± 1.2[a]	26.8 ± 1.6[ab]	80.2 ± 3.7[ab]	0.61 ± 0.2[a]	16.3 ± 0.17[ab]
B3-5	XG	0.6	77.4 ± 3.6[a]	6.94 ± 1.2[a]	26.6 ± 2.7[ab]	82.9 ± 1.2[a]	0.46 ± 0.04[a]	16.4 ± 0.81[ab]
P3-5	----	----	74.0 ± 3.1[a]	6.27 ± 0.76[a]	29.2 ± 1.4[ab]	76.0 ± 1.8[b]	1.1 ± 0.1[a]	19.0 ± 1.1[ab]
P3-5	HPMC	0.2	76.5 ± 0.57[a]	9.70 ± 0.34[a]	33.5 ± 0.74[a]	80.1 ± 4.6[ab]	0.53 ± 0.06[a]	16.1 ± 1.4[b]
P3-5	HPMC	0.4	74.8 ± 1.0[a]	9.02 ± 1.3[a]	30.9 ± 1.8[ab]	82.4 ± 0.51[ab]	0.69 ± 0.07[a]	17.0 ± 0.28[ab]
P3-5	HPMC	0.6	78.8 ± 2.4[a]	6.76 ± 2.8[a]	23.2 ± 8.2[b]	83.1 ± 1.7[a]	0.63 ± 0.2[a]	16.6 ± 0.44[ab]
P3-5	XG	0.2	76.8 ± 2.4[a]	7.20 ± 0.32[a]	29.6 ± 1.6[ab]	81.3 ± 1.7[ab]	0.62 ± 0.2[a]	17.1 ± 0.56[ab]
P3-5	XG	0.4	76.0 ± 5.1[a]	7.32 ± 3.1[a]	25.2 ± 8.6[ab]	80.7 ± 3.4[ab]	0.58 ± 0.1[a]	16.7 ± 1.6[ab]
P3-5	XG	0.6	77.8 ± 2.0[a]	7.88 ± 0.53[a]	28.8 ± 0.59[ab]	83.1 ± 3.2[ab]	0.73 ± 0.1[a]	17.3 ± 0.49[ab]
EW[3]	----	----	65.1 ± 3.0	14.8 ± 0.98	35.1 ± 1.3	82.8 ± 4.8	0.37 ± 0.4	18.5 ± 1.2

[1]Boiled aquafaba sample indicated by 'B'. Pressure cooked aquafaba sample indicated by 'P'. Water to seed cooking ratio of aquafaba samples indicated by 3:1, pH 5 indicated by '-5'

[2] Values in columns not followed by the same superscripts are significantly different ($p<0.05$).

[3]Egg white (EW) values included for comparison.

Figures


0%


HPMC 0.2%


HPMC 0.4%


HPMC 0.6%

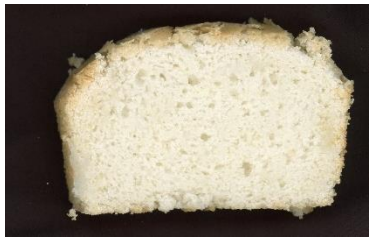

XG 0.2%


XG 0.4%

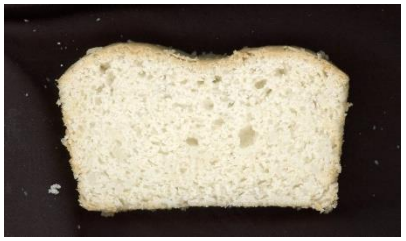

XG 0.6%

Figure 4.1 (a): Cross-sections of cake made with boiled aquafaba with or without xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC).

0%



HPMC 0.2%



HPMC 0.4%

HPMC 0.6 %

XG 0.2%

XG 0.4%

XG 0.6%

Figure 4.1 (b): Cross-sections of cake made with pressure-cooked aquafaba with or without xanthan gum (XG) or hydroxypropyl methylcellulose (HPMC).

Figure 4.1 (c): Cross-sections of cake made with egg white

CHAPTER 5

CONCLUSIONS

This study first aimed to optimize the functional properties of aquafaba, with a primary focus on foaming, by comparing cooking methods, cooking ratio (water to seed), and the influence of pH as well as examining how hydrocolloid addition affects meringue foam overrun, stability, and texture. The cooking method for preparing aquafaba, boiling versus pressure cooking, significantly affected protein concentration, apparent viscosity, foaming properties, and emulsifying properties as well as foam overrun and the texture of meringue foams. Boiled aquafaba had a greater influence than pressure-cooked aquafaba which was largely attributed to greater water loss in the boiling process leading to higher protein concentration. Cooking ratio was only a significant factor in apparent viscosity of aquafaba, with the lower water to seed ratio resulting in higher apparent viscosity. Future research should further investigate total soluble solids content and soluble/insoluble fiber content, which was not explored in this experiment, as it could be contributing more significantly to aquafaba viscosity. Lastly, the protein profile of aquafaba was not affected by cooking method or ratio and solubility was lowest around pH 4 regardless of cooking method, which aligned with reported values of the isoelectric point of chickpea protein isolate (4.5) and canned chickpea aquafaba (4.6).

In meringue aquafaba foams, gum type and level affected foam overrun, liquid drainage, foam texture, and apparent viscosity. Xanthan gum (XG) had a more pronounced effect on aquafaba viscosity. This translated to its effect on liquid drainage, where virtually no liquid drained out of aquafaba foams regardless of cook type, and foam overrun, where XG decreased

the air fraction in foams in comparison to hydroxypropyl methylcellulose (HPMC). HPMC significantly improved foam overrun of pressure-cooked samples and increased all analyzed textural properties of the foams. However, the texture of egg white meringue foam was closest to that of foam made with boiled aquafaba with 0.6% XG added by weight. This aligns with currently understood gelling and water-binding properties of XG at room temperature, as well as HPMC's surface activity. Overall, egg white meringue foams displayed liquid drainage and overrun between that of foams made with boiled and pressure-cooked aquafaba with no added gum despite a greater apparent viscosity.

The second focus of this study was to test if optimized aquafaba with and without hydrocolloid addition could be used as an egg-white substitute in a foam-type cake. Cakes made with pressure-cooked aquafaba and 0.2% HPMC addition did have a volume index similar to that of egg white cakes. Additionally, aquafaba crumb had similar color values to that of egg white cake and regardless of cooking method or gum addition at any level had a softer crumb than egg white. However, despite differences in the meringue foams with XG and HPMC addition at different levels, high standard deviations in most of the tested cake properties led to gum type and level not being statistically different from each other. Hydrocolloid addition did not improve specific volume/density of aquafaba samples or produce a crumb similar to that of egg white. Future studies could investigate other baked goods, like muffins or cupcakes, or cakes with different ingredient ratios than the foam-type cake. Use of aquafaba in cakes has potential, but hydrocolloid use in conjunction with aquafaba shows greater stability, foamability, and increased textural properties. Use of aquafaba with either hydrocolloid, XG or HPMC, could help in the development of cold foam food products comparable or better than egg white.