Aquafaba from commercially canned chickpeas as potential egg replacer for the development of vegan mayonnaise: recipe optimization and storage stability

Running title: Aquafaba as egg replacer in mayonnaise

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Summary Aquafaba**,** the viscous liquid recovered from canned chickpeas, was used as egg replacer for the development of vegan mayonnaise. The textural, microstructural and physicochemical properties of mayonnaise were determined during cold storage to optimize the aquafaba to oil ratio (A/O) of the formulation (15-25/80-70%). Aquafaba was capable to form a stable emulsion with an average value of droplet size distribution below 4μm. The physical stability determined by the Turbiscan Stability Index (TSI) was unaffected by the A/O ratio during 21 days of storage at 4 °C. The lowest droplet size distribution was obtained for samples with a low A/O ratio (15/80%). Firmness, adhesive force and adhesiveness decreased ($p<0.05$) with increasing the A/O ratio, whereas consistency remained unaffected. The oxidative stability of the oil phase was similar for all formulations and remained unaffected during storage. Aquafaba can be used effectively to replace egg in mayonnaise formulations containing oil at standard levels.

Keywords: emulsion, reformulation, food waste, texture, oxidation

Introduction

Mayonnaise is a semi-solid, acidic condiment which is widely appreciated for adding texture and flavor to other foods such as salads and sandwiches. In its traditional recipe, it contains 70-80% vegetable oil, egg yolk, salt, sugar, vinegar and spices, typically mustard (Depree and Savage, 2001). Mayonnaise is a colloidal system from a structural perspective, formed by emulsified oil droplets of spherical shape in a homogeneous aqueous phase. The oil droplet stability is mediated primarily by the emulsifying action of granular micro-particles formed from the phosphoprotein and low-density lipoprotein constituents of egg yolk (Laca *et al*., 2010).

Numerous attempts have been made in recent years to reformulate mayonnaise in order to meet consumer demands for a low-fat product with improved lipid profile. The primary objective of re-designing the recipe of mayonnaise focused on reducing the amount or altering the type of fat which, if overly consumed may be harmful for the onset and development of chronic diseases (Worrasinchai *et al*., 2006; Liu *et al.,* 2007; Di Mattia *et al*., 2015). Other potentially harmful ingredients for human health have also been targeted for reduction in the recipe, such as yolk cholesterol and salt. Selective egg yolk proteins are also considered responsible for triggering adverse immunological reactions in infants, young children and to a lesser extent the adult population (Caubet and Wang, 2011).

During the last years there has been considerable effort from the food industry to remove egg yolk from the formula of mayonnaise. This stems from health-related as well as sustainability issues associated with the consumption of animal products and is manifested as a market-driven preference towards the development of healthier, "free-from" and "natural" products. Furthermore, an egg-free mayonnaise may also be more cost effective from a manufacturer's perspective since pasteurization will no longer be a requirement during production. One of the main challenges encountered in the process of developing an egg-free mayonnaise is to identify suitable ingredients to replace egg yolk from the traditional recipe, without impairing stability, taste and color. Emulsifiers of animal or plant origin have been employed for this purpose (Riscardo *et al*., 2003; Herald *et al*., 2009; Nikzade *et al*., 2012). White lupin protein, soy milk, wheat germ protein isolate, chia mucilage, Durian seed gum and modified potato starch have been tested so far for their ability to replace egg yolk and the main challenge was to generate a stable emulsion structure of fine oil droplets capable to prevent coalescence and flocculation for prolonged periods of storage (Cornelia et al., 2015; Fernandes & Mellado, 2018; Ghazaei et al., 2015; Rahbari et al., 2015; Rahmati et al., 2014; Raymundo et al., 2002).

"Aquafaba" is the term used to describe the viscous liquid formed during cooking of legume seeds (typically chickpeas) or the one encountered in canned products of the same origin. The exact composition of aquafaba depends on the legume and is a mixture of carbohydrates, proteins and water (Shim *et al*., 2018). This liquid, which is usually discarded as food waste, is also a source of phenolic compounds and saponins (Damian *et al*., 2018). Research has indicated aquafaba as a valuable ingredient with desirable functional properties (i.e. foaming, emulsifying and gelling) which can be used in various formulations to replace eggs and milk in vegan products (Serventi *et al.*, 2018; Shim *et al.*, 2018; Stantiall *et al.*, 2018; Mustafa *et al.*, 2018).

To the best of our knowledge, there are no studies to investigate the potential of aquafaba as an egg yolk replacer for the development of vegan mayonnaise. The aim of the present study is to develop and optimize the recipe of mayonnaise using aquafaba from chickpeas. Formulation effects on texture and physicochemical properties of mayonnaise are determined during cold storage to assess the applicability of this secondary food ingredient as an emulsifier and stabilizer of high-fat colloidal systems.

Materials and methods

Materials

Canned chickpeas (Lot FDI CC26 6 LJ085), rapeseed oil, table salt, sugar and white wine vinegar were purchased from the local supermarket (Tesco, UK). The average chickpea to aquafaba weight ratio was 1.68. Folin-Denis' reagent and Nile red were supplied by Sigma Aldrich (St Louis, MO, USA). All standards and reagents used were of analytical grade.

Preparation and storage of mayonnaise

The formulation contained the following ingredients on % weight basis: 80% oil, 15%, aquafaba, 4% vinegar, 0.5% sugar, 0.5% salt. For reduced-fat mayonnaise (70%-75%), oil was replaced by an equal amount of aquafaba (20%-25%). A coarse emulsion was formed by adding the oil gradually to the aqueous mixture (aquafaba, vinegar, sugar and salt) and mixing for 10 min with a Russell Hobbs hand blender (Argos, UK). Mayonnaise was then homogenized with a T25 digital Ultra-turrax® homogenizer at 13500 rpm for 2 min (IKA® England Ltd, Oxford, UK). 500 gr of mayonnaise were prepared for each batch and for each formulation three batches were prepared. Mayonnaise was aliquoted and stored at 4 °C until further analysis at weekly intervals.

Proximate composition and determination of total phenols, tocopherols and carotenoids in aquafaba

Energy, moisture, ash, fat, carbohydrates, total sugars, and dietary fiber in the samples were determined according to the standard AOAC (1990) official methods. Protein content was determined by combustion according to the Dumas principle. Carbohydrates were determined by subtracting the sum of moisture, protein, fat, and ash percentages from 100%. Total phenols were determined using the Folin-Ciocalteu (F-C) colorimetric method according to Raikos *et al.* (2014) and results are expressed as mg GAE/g of dried aquafaba. A reverse-phase HPLC method was employed to quantify carotenoids and tocopherols and samples were analyzed in duplicates (Hess *et al.*, 1991).

pH and color determination

pH was recorded using a portable food and dairy pH meter (Hanna Instruments Ltd., Leighton Buzzard, UK) and color was determined by a Konica Minolta CR1 10 colorimeter (Konica Minolta Solutions. Ltd., Basildon, UK). ΔE* (total colour change) of mayonnaise samples during cold storage was calculated from the following equation:

$$\Delta E^* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

Lipid extraction from mayonnaise

Twenty-gram portions of mayonnaise were poured into 50 ml polypropylene centrifuge tubes and were frozen at -20 °C for 24 h according to the procedure of Lagunes-Galvez *et al.* (2002). After storage samples were thawed at room temperature until the emulsion was broken and oil phase was collected with centrifugation at 2,400 x g for 5 min with an Eppendorf 8810R apparatus (Eppendorf UK Ltd, Stevenage, UK).

Oxidation stability testing

The oxidative stability of mayonnaise was determined by a 743 Rancimat device (Metrohm Ltd., Herisau, Switzerland) according as described by Raikos *et al.* (2016)

with slight modifications. Three grams of extracted oil was poured into the reaction tubes, samples were exposed to an air flow of 20L/h at 120 °C and the induction time (IP) was calculated by the 743 Rancimat software 1.1.

Emulsion stability

The physical stability of mayonnaise samples was monitored using a Turbiscan MA2000 apparatus (Formulaction, RamonvilleSt. Agne, France) as described by Raikos *et al.* (2017) with slight modifications. The sample in the cell was scanned every 5 min for 30 min at 40 °C and the changes in the intensity of the backscattered light (ΔBS) in unit time was taken as a measure of the stability of the emulsions. The particle size (mean spherical equivalent diameter) was computed from the ΔBS values based on the refractive index of particles and continuous phase, and the volume fraction (φ) of particles in the sample (Mie theory). The volume fraction was adjusted at 80%, 75% or 70% depending on the oil added for each mayonnaise formulation and the refractive indices for particle size calculation were 1.47 for the dispersed phase (rapeseed oil) and 1.33 for the continuous phase. The Turbiscan stability index (TSI) was calculated from ΔBS that indicate the particles aggregation and migration by Turbisoft 2.0.

Texture analysis

Texture measurements were performed using a CT3 Texture Analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA) attached with a 10 kg load cell and equipped with a special mayonnaise mesh probe (TA-MP, length:3.8cm, width:3.4cm, mesh size:0.4mm) A two cycle Texture Profile Analysis compression test (TPA) was performed with the following TPA settings: pre-test speed: 2 mm/s; test speed: 1 mm/s; return speed: 1 mm/s; trigger load: 10 g; target mode distance: 20 mm; data acquisition rate: 10 points/s. Mayonnaise samples (200 g) were carefully scooped into 250-mL Corning® polypropylene cone beakers (Sigma-Aldrich, St. Louis, MO) and the mesh probe compressed the sample at a constant crosshead speed of 1 mm/s twice to a depth of 20mm of the initial height at room temperature. Data were recorded using Texture

Proc CT V1.3 Build 15 software (Brookfield Engineering Laboratories Inc.) and the parameters determined were hardness (firmness), adhesive force - adhesiveness (stickiness) and cohesiveness (consistency). Hardness was calculated from the load detected at highest peak during compression, adhesive force from the peak negative value, adhesiveness from the area under the negative peak and cohesiveness from the ratio of the areas under the compression stoke of the second and first cycles.

Microstructure analysis

Mayonnaise microstructure was analyzed with a confocal laser scanning microscope (CLSM) (Carl Zeiss Ltd, Cambridge, UK) according to the method of Raikos et al. (2019). Nile red dye was used to stain the fat globules and observations were performed at 543 nm using a 63x oil immersion objective. Images were captured at a resolution of 1024 x 1024 pixels.

Statistical analysis

The data are reported as means±standard error (SE) for duplicate measurements from 3 batches (n=6) unless otherwise stated. Analysis of variance (SPSS for Windows 22, SPSS Inc., Chicago, IL) were conducted to identify differences among the means by the *Tukey's* post hoc test. Statistical significance was set at $p < 0.05$.

Results and discussion

Proximate and chemical composition of aquafaba

The proximal composition of aquafaba from commercial canned chickpeas is presented in Table 1. The values obtained for protein, moisture, ash and simple and complex carbohydrates agree with previously published data (Mustafa et *al.,* 2018). Fat was not detected in measurable levels which agrees with published literature (Mustafa et *al.,* 2018; Stantiall *et al*., 2018). The total phenolic content was lower, and the sodium content was higher compared to the data from Damian *et al*. (2018). The observed differences can be due to pulse compositional differences or to degradation effects from different processing methods (Shim *et al*., 2018). The vitamin C and tocopherol content of raw and processed chickpea seeds from previously published research indicates that this legume is a fair source of tocopherols and the main tocopherol detected was the γ-isomer (7.7 mg/100 g of dry matter) (Fernandez-Orozco *et al*., 2009). To the best of our knowledge, the vitamin and carotenoid content of aquafaba remains largely unknown. Our data indicated that although tocopherols (mainly γ-tocopherol) were detected in aquafaba, these were at very low levels. Non-surprisingly, only traces of carotenoids were detected which is due to the absence of fat from the proximal composition of the viscous liquid. It is therefore assumed that their nutritional and functional contribution to the properties of mayonnaise is negligible.

Microstructure and textural properties of mayonnaise

The main objective of this study was to determine the efficiency of aquafaba to form a stable colloidal dispersion of oil droplets in mayonnaise structure. As there is no existing literature to provide preliminary data, different formulations with respect to aquafaba to oil ratios were tested. Mayonnaise is a semisolid food which exhibits pseudoplastic and time-dependent behavior (Olsson *et al.*, 2018). The microstructure and texture of mayonnaise relate to its viscoelastic properties, which in turn determine product quality and acceptability. The microstructure of mayonnaise is determined by different factors including the type and concentration of emulsifiers used to form the emulsion, the viscosity of the aqueous phase, the oil content and the droplet size (Laca *et al*., 2010). The microstructure of the freshly formed mayonnaise with different aquafaba to oil ratios were analyzed by CLSM (Fig. 1). Microstructural analysis revealed that all mayonnaises consisted of finely dispersed, spherical oil droplets in the aqueous medium. Due to the high oil content (>60%), the droplets are densely packed and show a certain degree of polydispersity (different size). The close packing of the droplets, also denoted as droplet density, favors inter-droplet interactions and is at least partially responsible for the stability of the mayonnaise structure (Depree & Savage, 2001). The appearance and mean spherical equivalent diameter (3.4-4.1 μm) of the droplets is comparable to the microstructure of traditional mayonnaise made from egg yolk (Patil & Benjakul, 2019).

The textural parameters of the freshly prepared mayonnaise samples are presented in Table 2. Hardness, adhesive force and adhesiveness are significantly affected by the aquafaba to oil ratio. Hardness (or firmness) indicates the force required to compress food between molar teeth, adhesive force represents the force required to overcome the attractive forces between mayonnaise and the surface of other materials and adhesiveness is the energy required to separate mayonnaise from the spoon or knife (Chandra & Shamasundar, 2015; Raikos *et al.,* 2016). Cohesiveness (or consistency), which indicates the strength of internal bonds within mayonnaise and the degree to which it can be deformed before rupture, was not affected by the aquafaba to oil ratio. Increasing the oil to aquafaba ratio resulted in significant increases in hardness, adhesiveness and adhesive force. The observed increase in textural parameters is reflective of the increase in viscosity due to higher oil content. The larger the amount of oil added, the greater the number of droplets formed and thus emulsion viscosity increases (Fernandes & Mellado, 2017). According to Liu *et al.* (2007), viscosity can at least partially determine the textural profile of mayonnaise. Data from previous research also indicates that firmness and adhesiveness of mayonnaise are affected by changes in viscosity when the protein, hydrocolloid or oil content of the formulation is modified (Nikzade *et al*., 2012; Raymundo *et al*., 2002)

Physicochemical stability of mayonnaise during cold storage

Mayonnaise in its traditional recipe is an acidic emulsion with a long shelf-life (up to 6 months) under refrigerated temperatures (Herald *et al.,* 2009). The long-term stability of mayonnaise is attributed to the high fat content which results in a highly dense packing of droplets with limited space to move and to the presence of highly efficient emulsifiers (i.e. egg lecithin) capable to reduce the interfacial tension between the dispersed and continuous phases and form a strong viscoelastic film around the oil droplets which is electrically charged (McClements, 2009; Mun *et al*., 2009). Although there have been considerable efforts to (at least) partially replace egg yolk from the traditional formulation of mayonnaise, long-term stability of the reformulated product has been compromised in most attempts. The long-term stability of mayonnaise is associated, among other factors, with the mean particle size and particle-size distribution of the oil droplets (Yildirim *et al.,* 2016). All mayonnaise samples from three independent batches were acidic (pH ranging from 3.45 to 3.67) and pH values of three aquafaba to oil formulations did not differ significantly ($p>0.05$). The change of particle size (mean spherical equivalent diameter) and Turbiscan Stability Index (TSI) were used to evaluate the emulsion stability of mayonnaise during refrigerated storage (Fig. 2). TSI is a stability specific parameter that can be used for the determination of emulsion stability and is obtained as the sum of all destabilization phenomena taking place during the monitoring process. High TSI values indicate decreased stability of the system (Sun *et al.,* 2015). As shown in Fig. 2, the mean spherical equivalent diameter remained unaffected during 21 days of refrigerated storage for all 3 formulations. At the end of the storage period, the formulation with 15% aquafaba had the smallest particle size (3.22 μm), which suggests that the amount of emulsifier present in the lowest aquafaba to oil ratio (15:80) is adequate to form a stable emulsion. The emulsifying ability of aquafaba is attributed to the protein and carbohydrate content (Table 1). Both macromolecular components should contribute to the formation and stabilisation of the emulsion structure. Previous research has shown that proteins and polysaccharides can interact to form a thick interfacial film surrounding the oil droplet, which results in a stable emulsion (Ghoush *et al.,* 2008). Although the chemical composition of aquafaba is not standardised or known in detail so far, there is evidence to suggest that it is a good source of storage proteins (albumins and globulins) and complex, digestible and non-digestible carbohydrates (Shim *et al*., 2018). Aquafaba is known to contain saponins, amphiphilic glycosides which can act as surfactants and thus may also contribute to the high emulsifying ability of the viscous liquid from canned chickpeas (Damian *et al*., 2018).

Mayonnaise is a high-fat food (70%-80% vegetable oil) and therefore is susceptible to oxidative deterioration through auto-oxidation of the unsaturated and polyunsaturated fats in the oil, which depending on the extent is likely to have a negative impact on flavor, aroma, colour and nutritional value of food (Depree & Savage, 2001). Several strategies can be effective against lipid oxidation of mayonnaise such as the addition of antioxidants or the use of a lipid source that is naturally rich in compounds with powerful antioxidant activity (Di Mattia *et al*., 2015; Li *et al*., 2015). The oxidative stability of the extracted lipid phase of mayonnaise samples during storage at 4 °C was determined by Rancimat analysis and is presented in Figure 3. The induction time, which is an indication of the ability of oil to resist oxidation under accelerated oxidation conditions, of all three formulations was similar and was not affected significantly after 21 days of storage. The formulation effect (oil to aquafaba ratio) was non-significant with respect to the oxidative stability of mayonnaise. The induction time of all samples ranged between 3.33 h to 3.55 h, which is comparable to previously published data for rapeseed oil (Maszewska *et al.* 2018). Data from colour properties of the mayonnaise samples also suggests that samples remained stable during storage as indicated by the total differences in colour (ΔE^*) (Table 3). There was a non-significant ($p<0.05$) incremental effect for all formulations at the end of the study period which was evident for all colour coordinates (L^*, a^*, b^*). The formulation effect (oil to aquafaba ratio) was observed only for b^* (yellowness), which non-surprisingly increased significantly for higher oil to aquafaba ratios.

Conclusions

This study aimed to evaluate the potential of aquafaba as an egg replacer for the development of a vegan mayonnaise formulation. Microstructural and light scattering data indicated that aquafaba can be effectively used to form a fine emulsion, which remained stable during cold storage for 21 days. The A/O ratio used for product development had a minor impact on colour properties and no significant effect on the physicochemical stability of the mayonnaise during cold storage. The A/O ratio significantly affected the textural properties and this effect was dependent on the oil contribution to the formulation. Increasing the aquafaba at levels above 15% had no beneficial effect on the long-term stability or the antioxidant properties of the formulation. Sensory evaluation of the mayonnaise made from aquafaba should be carried out to determine consumer acceptability. This study suggests that aquafaba is currently an underutilized secondary (waste) product which can have several applications in the food industry thanks to its nutritional, functional and health-related properties.

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Data Availability

Research data are not shared

Ethical Guidelines

Ethics approval was not required for this research

Conflicts of interest

The authors declare no conflicts of interest.

Figure captions

Graphical abstract The development of vegan mayonnaise from secondary products of food processing and its benefits

Figure 1 Confocal Laser Scanning Microscopic (CLSM) images of mayonnaise prepared with A: 15%, B: 20% and C: 25% aquafaba. Lipid droplets are stained with Nile red and scale bar equals to 20 μm.

Figure 2 Effect of aquafaba to oil ratio on A: the mean spherical equivalent diameter (d) and B: the physical stability of mayonnaise (TSI) during 21 days of storage period at 4 °C. Results are presented as means±SE. Different letters denote significant differences ($p<0.05$).

Figure 3 Effect of aquafaba to oil ratio on the oxidative stability (IP) of mayonnaise during 21 days of storage period at 4 °C. Results are presented as means±SE.

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