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# Reducing microbial contamination risk and improving physical properties of plant-based mayonnaise produced using chickpea aquafaba

[1,4]Muhialdin, B. J., [2]Mohammed, N. K., [1]Cheok, H. J., [3]Farouk, A. E. A. and [1,4]*Meor Hussin, A. S.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Food Science, Faculty of Agriculture, Tikrit University, Tikrit, Iraq*
[3]*Molecular Biotechnology Research Unit, Department of Biotechnology, Faculty of Science, Taif University, Alhawiya 888, Saudi Arabia*
[4]*Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

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**Abstract**

Aquafaba is obtained by soaking different types of legumes and utilised in the production of several plant-based foods due to its functional properties. The present work aimed to evaluate the effects of aquafaba produced by soaking chickpeas at different durations on the safety, physicochemical properties, and consumer acceptability of plant-based mayonnaise. The chickpea aquafaba was prepared by soaking chickpea at different durations of 12, 18, and 24 h, and later boiled for 35 min at 120°C. The results demonstrated significantly ($p < 0.05$) higher TSS (5.89 ± 0.00), viscosity (7.23 ± 0.06), and peptide content (0.463 ± 0.001) in chickpea aquafaba soaked for 24 h. The mayonnaise prepared with 24 h chickpea aquafaba showed improved firmness (129.47 ± 9.64 g) and consistency (860.26 ± 26.52 g/s). The bacterial load was observed to decrease in 24 h chickpea aquafaba mayonnaise (3.857 ± 0.948 log CFU/g), and increased by one-fold in 12 h chickpea aquafaba mayonnaise (4.672 ± 0.588 log CFU/g) after 35 days at 28 ± 2°C. The consumer acceptability evaluation showed no significant difference ($p > 0.05$) among all the tested attributes with the 24 h aquafaba mayonnaise received the highest score for taste (6.80 ± 1.38). The aquafaba produced from chickpeas soaked for 24 h and boiled for 35 min has high potential for applications in plant-based mayonnaise production to enhance safety and improve physicochemical and consumer acceptability.



## Introduction

Healthy eating is the trend among modern consumers who are aware of the relations between healthy foods and their role to prevent several diseases. Plant-based foods and food ingredients have high potential to be used as alternatives to animal-based ingredients (Mohamed *et al.,* 2017). Egg, due to its characteristics and preferred taste, is the most common animal-based emulsifier used in mayonnaise production (Ma and Boye, 2013). Egg contains high number of Gram-positive heat-resistant enterococci, and it could be a source for cross-contamination in chilled food products such as mayonnaise (Techer *et al.,* 2015). In addition, several studies have reported the contamination of eggs with *Salmonella* Enteritidis that causes food-poisoning outbreaks in egg-based food products (Rodrigue *et al.,* 1990; Wan *et al.,* 2017). On the other hand, *Salmonella* has been observed to survive after mayonnaise preparation, even with the addition of vinegar and storage at 4°C (Zhu *et al.,* 2012). The ready-to-eat ingredients such as mayonnaise are not subjected to heat process before consumption, and presents high risk due to microbial contamination. The safety of mayonnaise was suggested to be enhanced by the treatment of raw materials such as eggs with different decontamination methods, including irradiation (Al-Bachir and Zeinou, 2006).

Recently, the demand for plant-based foods has directed the research to focus on finding alternatives to emulsifying, foaming, thickening, and gelling agents. Aquafaba, which is a viscous liquid produced during the cooking of legumes in water, has been suggested to replace eggs in the production of several foods (Starmer *et al.,* 2018). In previous study, aquafaba prepared by using haricot beans, garbanzo chickpeas, whole green lentils, and split yellow peas demonstrated a broad range of foaming ability (39 - 97%), which had correlation to their protein content (Stantiall *et al.,* 2018). Aquafaba's characteristics make it as a promising candidate to replace eggs in producing plant-based mayonnaise. Buhl *et al.* (2019) reported

*Corresponding author.
Email: shobirin@upm.edu.my

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that relevant food products from aquafaba could be vegan mayonnaises and salad dressings.

However, replacing mayonnaise ingredients can cause significant changes to the physical properties and consumers' preferences (Ma and Boye, 2013). Worrasinchai *et al.* (2006) studied the possibility to use brewer's yeast β-glucan to replace the fat content in mayonnaise and simultaneously function as an emulsion. β-glucan fat was successfully applied to replace 50% of the total oil in mayonnaise. However, β-glucan demonstrated negative effect on the sensory property of mayonnaise and downgraded the quality of the product. On the other hand, oat dextrin was used to replace 30% of vegetable oil in mayonnaise, but the texture and taste exhibited low scores for sensory quality (Shen *et al.,* 2011). Cornelia *et al.* (2015) used the extract of durian seed gum as an egg replacer in vegan mayonnaise. It was found that 4% of the gum seed extract was the best concentration as an egg replacer in mayonnaise, which resulted in a good sensory quality and gained good emulsion stability. The approach of using Arabic gum powder as egg replacer in vegan 'egg-free' mayonnaise in different percentages of 25, 50, 75, and 100% was found to be a good choice for those who are unable to eat egg-based foods, in terms of their physicochemical and sensory properties (Ali and El Said, 2020).

Despite the utilisation of the bio-products of durian seed, the extraction process involved many steps which are costly and thus not preferred by manufacturers. Aquafaba has been recommended to replace egg white and functions as emulsifier in bakery and in the making of puddings and ice creams for its low cost in mass production and functional properties such as foaming, emulsifying, binding, and thickening (Mustafa *et al.,* 2018). Buhl *et al.* (2019) reported that aquafaba can replace egg for food forms and emulsions, and demonstrated high stability in the presence of salt which is preferred in the production of mayonnaise.

Unfortunately, there are limited studies on the effects of the aquafaba production conditions and its application in mayonnaise production. Therefore, the aim of the present work was to determine the effects of chickpea aquafaba prepared by soaking it in water for 12, 18, and 24 h, on the safety, physiochemical properties, and consumer acceptability of plant-based mayonnaise.

## Materials and methods

### *Materials and chemicals*

The ingredients used for the preparation of aquafaba mayonnaise were of food-grade, and purchased from a nearby market (TESCO, Selangor). These included soybean oil, lemon juice, sugar, vinegar, salt, and chickpeas. On the other hand, xanthan gum, modified starch (octenyl succinic anhydride), and mustard were purchased from a local bakery shop located in the state of Selangor.

### *Preparation of aquafaba*

The chickpeas were soaked in brine at different durations to determine the effects of soaking time on the produced aquafaba. A total of 200 g of dry chickpeas were washed twice with tap water, and soaked for 12, 18, and 24 h with 700 mL brine salt (7.5%, w/v). The soaked chickpeas were then boiled for 35 min at 120°C using a pressure cooker.

### *Determination of TSS, viscosity, and peptides*

The total soluble solid (TSS) of aquafaba was measured using a hand refractometer (Atago N1, Tokyo, Japan). The viscosity (mPas) of aquafaba was evaluated based on the method described by Jung *et al.* (2011) with modification. Briefly, 10 g of aquafaba samples were placed in cone plates, and their viscosity was measured by using a rheometer (model AR-G2; TA Instruments, USA) attached with 4 cm cone plate geometry with a cone angle of 2°, and the gap set at 0.055 mm. The peptide content of aquafaba was determined by using the *o*-phthalaldehyde (OPA) spectrophotometric assay as described by Goodno *et al.* (1981) with some modifications. Briefly, 36 µL of each sample was mixed with 270 µL of OPA reagent in 96-wells microtiter plate, and incubated for 2 min at room temperature. The absorbance at 340 nm was measured by using an ELISA reader (Labomed, model UVD-2950, Culver City, CA, USA). The standard curve was prepared by using glutathione at different concentrations (0.1 - 2 mg/mL), and the test was carried out in triplicate.

### *Production of plant-based mayonnaise*

Plant-based mayonnaise was produced following the method described by Basuny and Al-Marzooq (2011) with slight modifications (Table 1). First, aquafaba was thoroughly blended at a medium speed. The mustard paste, salt, sugar, vinegar, and lemon juice were added to the mixing bowl. The mixture was blended for 3 min until it turned pale yellow. After 5 min, 17% of the oil was gradually added and blended at a medium speed. For the next 3 min, another 50% of oil was added and blended with a medium speed. Further, the modified starch was added to form a paste in order to give it a creamy texture, while xanthan gum was added to give a better texture and stability, facilitate processing, and

Table 1. The ingredients of aquafaba mayonnaise.

| Ingredient | Percentage (%) |
|---|---|
| Soybean oil | 67.90 |
| Aquafaba | 17.00 |
| Lemon juice | 5.70 |
| Sugar | 5.70 |
| Vinegar | 1.90 |
| Salt | 0.80 |
| Xanthan gum | 0.60 |
| Modified starch | 0.31 |
| Mustard | 0.09 |

generally improve overall quality of mayonnaise. The produced mayonnaise was immediately analysed when the texture was formed. The samples were prepared in triplicate and mayonnaise samples were stored in plastic containers (250 g) at room temperature (28 ± 2°C) for further analyses.

*Determination of pH and texture*

pH was determined by using a pH meter (3505 pH meter, Jenway, UK) at a room temperature. The texture of the plant-based mayonnaise was determined by using a texture analyser (XT2i, Surrey, UK) following the method of Borneo and Aguirre (2008) with slight modification. A total of 0.5 kg for each sample was placed in round plastic containers at a depth of 30 mm. The texture was the determined by using a P/35-cylinder probe (Stable Micro System, Surrey, UK), and the force was measured in compression mode at a fixed 75% strain. The testing conditions were 10 mm penetration, 1 mm/s pre-test speed, 1 mm/s test speed, and 10 mm/s.

*Determination of bacterial content*

The bacterial content was analysed for a prolonged period of storage at 28 ± 2°C. The bacterial content in mayonnaise samples was determined following the method described by Azanza (2005) with slight modification. Mayonnaise samples (25 g) were diluted in 225 mL peptone water (0.1%), and homogenised using a stomacher. The serial dilution was carried out by using 9 mL of peptone water in each Universal bottle, up to $10^{-5}$. Then, 100 µL of each dilution was spread on Plate Count Agar (PCA), and plates were incubated at 37°C for 24 h. The test was carried out in triplicate, and results were recorded as log CFU/g for each sample.

*Consumer acceptability*

The consumer acceptability was carried out by 69 random consumers to determine the appearance, colour, aroma, texture, and taste as described by Liu *et al.* (2007). The hedonic 9-point scale was applied, where 1 = the least/lowest liked, and 9 = the most/highest liked. Mayonnaise samples were coded with random numbers (MYN177, MYN188, and MYN199 for 12, 18, and 24 h aquafaba mayonnaise, respectively) and presented to the panellists in individual booths. The untrained panellists were given a briefing on the sensory evaluation procedures. Mayonnaise samples (10 g) were served without carrier, and water was provided to cleanse the palate to avoid inaccuracy in sensory testing.

*Statistical analysis*

Data collected were subjected to one-way analysis of variance (ANOVA) by using Minitab (Version 17, Minitab Pennsylvania, USA) statistical software. The test result was expressed as the mean value ± standard deviation (SD).

## Results and discussion

*Aquafaba characteristics*

The preparation of aquafaba heavily depends on the soaking time of chickpeas due to the precipitation of soluble solids into brine. In the present work, chickpeas were soaked in brine for 12, 18, and 24 h, and boiled for 35 min at 120°C. The results demonstrated significantly higher viscosity ($p$ > 0.05) for the sample soaked for 24 h (Table 2). Moreover, the TSS and peptide contents were found to have increased during prolonged soaking time as a result of the accumulation of water-soluble materials. According to Tixier *et al.* (2003), the viscosity has strong correlation to the TSS, and increasing the TSS will increase the viscosity. Moreover, viscosity has a strong correlation with the protein content of the legumes used in the preparation of aquafaba (Stantiall *et al.,* 2018). The results of the present work agree with the previous studies — the viscosity was significantly low in comparison to the previous studies due to the short soaking period and cooking time. The prolonged soaking time is not suitable for food industry due to the risk of microbial contamination that may lower the pH and possibly produce certain toxins. In addition, the prolonged cooking time will increase the cost of producing aquafaba for commercial purposes.

*Mayonnaise physiochemical properties*

The aquafaba produced from each soaking

Table 2. Effect of chickpea soaking time (h) on the aquafaba characteristics.

| Soaking time (h) | Brix | Viscosity (mPas) | Peptides (mg/mL) |
|---|---|---|---|
| 12 | 5.09 ± 0.00[b] | 5.13 ± 0.12[b] | 0.208 ± 0.103[c] |
| 18 | 5.22 ± 0.00[b] | 5.73 ± 0.06[b] | 0.369 ± 0.038[b] |
| 24 | 5.89 ± 0.00[a] | 7.23 ± 0.06[a] | 0.463 ± 0.001[a] |

Values are mean ± SD of triplicates ($n = 3$). Different letters indicate significant differences ($p < 0.05$) within a column.

time was used to replace eggs in the production of plant-based mayonnaise. The results of the pH analysis demonstrated no significant difference among the three mayonnaise samples (Table 3). The pH value of mayonnaise was determined for its critical role in mayonnaise production. The pH of mayonnaise must remain acidic, and is preferred to be between pH 3 and 4.5 to prevent spoilage and pathogenic microorganisms (Ma and Boye, 2013). In the previous study, mayonnaise (pH 3.8), due to its low acidity, was applied on ham samples and found to control the growth of *Listeria monocytogenes*. In the present work, plant-based mayonnaise had a slightly lower pH due to the formulation that included lemon juice (citric acid) and vinegar (acetic acid), in which the combination of both acids could lower the pH of mayonnaise. The pH value is highly crucial for the structure and stability of mayonnaise due to the formation of protein network, which is highly affected by the isoelectric point (Depree and Savage, 2001). The raw egg products, such as mayonnaise, are recommended to have pH below 4.2 to control the microbial growth, especially that of *Salmonella* (Jung and Beuchat, 2000).

The texture of mayonnaise samples was evaluated to determine its firmness, consistency, and cohesiveness. The mayonnaise produced by using the 24 h aquafaba sample showed good characteristics necessary for firmness and consistency (Table 3 and Figure 1). The cohesiveness for 24 h sample (-61.42 ± 2.11) demonstrated no significant differences to the other two mayonnaise samples. Liu *et al.* (2007) studied the texture properties of mayonnaise modified by using whey protein and determined the

Figure 1. Aquafaba mayonnaise produced from different soaking times of aquafaba, (A) 12 h, (B) 18 h, and (C) 24 h.

effects on firmness, consistency, and cohesiveness of mayonnaise samples. The results showed a low consistency (2785.35 ± 8.89 to 3654.78 ± 9.34), high cohesiveness (-120.45 ± 1.23 to -198.45 ± 2.73), and high firmness (256.56 ± 2.19 to 349.35 ± 2.67). Moreover, as mayonnaise had weak gels as determined by oscillatory shear test, it was acceptable for commercialisation. In the present work, the 24 h aquafaba mayonnaise exhibited higher values for consistency in comparison to mayonnaise modified with whey protein. According to Liu *et al.* (2007), cohesiveness refers to stickiness for mayonnaise samples, and higher negative results indicate that samples are stickier. The 24 h sample had a low cohesiveness value (-61.42 ± 2.11 g) thus indicating a low stickiness. In another study, mayonnaise prepared by using chia seeds as alternative emulsifier showed good physical properties where firmness, consistency, and cohesiveness were 127.53 ± 7.40 g, 4538.57 ± 214.82 g/s and -131.56 ± 7.64 g, respectively (Fernandesa and Mellado, 2018). Moreover, the mayonnaise modified with chia seeds showed solid properties due

Table 3. Effect of chickpea soaking time (h) on the aquafaba mayonnaise firmness, consistency, cohesiveness, and pH.

| Soaking time (h) | Firmness (g) | Consistency (g/s) | Cohesiveness (g) | pH |
|---|---|---|---|---|
| 12 | 81.06 ± 1.83[c] | 567.35 ± 42.86[c] | -55.56 ± 3.21[a] | 3.00 ± 0.01[a] |
| 18 | 101.86 ± 4.64[b] | 710.42 ± 20.64[b] | -64.06 ± 0.45[a] | 3.01 ± 0.01[a] |
| 24 | 129.47 ± 9.64[a] | 860.26 ± 26.52[a] | -61.42 ± 2.11[a] | 3.01 ± 0.01[a] |

Values are mean ± SD of triplicates ($n = 3$). Different letters indicate significant differences ($p < 0.05$) within a column.

to the high cohesiveness and consistency. The results of the present work are in agreement with the previous studies, and the plant-based mayonnaise was found to be less solid and highly viscose.

*Bacterial loads of mayonnaise*

Bacterial loads were measured using plate count in mayonnaise samples throughout 35 days of storage at 28 ± 2°C (Figure 2). The 24 h aquafaba showed the lowest bacterial loads; 2.544 log CFU/g after 7 days of storage, to 3.857 log CFU/g after 35 days of storage. No significant differences were observed in the bacterial loads between 18 and 24 h aquafaba mayonnaise samples after 7 and 35 days of storage. On the other hand, the mayonnaise prepared by using 12 h aquafaba showed a higher bacterial load of 4.672 log CFU/g after storage of 35 days. Mayonnaise is a ready-to-eat food that can be consumed without any further process, and the microbial content should be maintained below $10^5$ CFU/g (Faour-Klingbeil *et al.,* 2016). The results demonstrated a little growth in the 18 and 24 h aquafaba, and a higher growth in the 12 h aquafaba samples. Nevertheless, the bacterial loads in all samples were still within the acceptable limit for ready-to-eat food products. Xiong *et al.* (2000) reported the relationship between the pH, egg content, and safety of the mayonnaise, and recommended to store mayonnaise at low temperatures to prevent microbial contamination. The egg yolk is the main emulsifier used in the preparation of mayonnaise, and therefore recommended to be stored at low temperatures to reduce microbial contamination (Hwang, 2005) In addition, mayonnaise was found to be stored at room temperature in most food canteens, thus representing a risk to consumers in regard to the high content (1.1 × $10^2$ to 2.9 × $10^6$ CFU/g) of foodborne bacteria (Tayfur *et al.,* 2013). In the present work, the egg was replaced with aquafaba, and the effects were found to be good in terms of physical properties. On the other hand, the application of aquafaba (24 h aquafaba) showed low bacterial content and enhanced the safety of mayonnaise at higher storage temperature (28 ± 2°C). The stability and reduction of bacterial load in 24 h aquafaba mayonnaise may be due to low pH and high peptide content. Several studies have recommended the incorporation of pulse proteins, such as broad beans and chickpeas, to increase the functional properties of mayonnaise (Tharanathan and Mahadevamma, 2003; Roy *et al.,* 2010). Mayonnaise prepared from a mixture of protein isolates enhanced the antihypertensive, antidiabetic, antimicrobial activity, and thermal denaturation temperature properties (Alu'datt *et al.,* 2016).

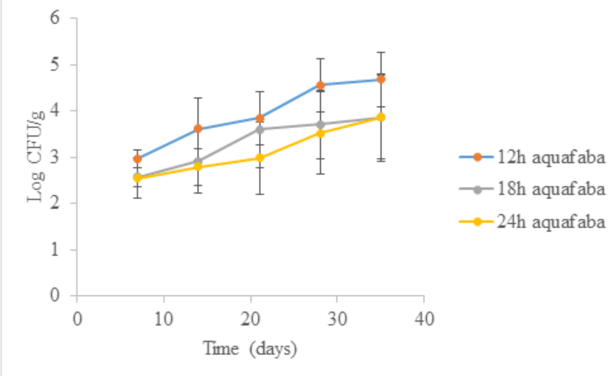


Figure 2. Effect of chickpea soaking time (h) on the aquafaba mayonnaise bacterial loads determined for 35 days by total plate count.

*Consumer acceptability*

The sensory analysis showed no significant difference in terms of the aroma, texture, and taste among the mayonnaise samples (Table 4). However, the 24 h aquafaba mayonnaise showed higher score for taste (6.80 ± 1.38), and was most acceptable, as determined by the consumers who participated in the experiment. On the other hand, 18 h mayonnaise exhibited the highest scores for appearance (6.97 ± 1.43), aroma (6.30 ± 1.72), colour (7.27 ± 1.52), and texture (6.80 ± 1.28). In a previous study, a mayonnaise sample was prepared by replacing egg white with modified potato starch, and the results

Table 4. Effect of chickpea soaking time (h) on the aquafaba mayonnaise sensory attributes.

| Attribute | Soaking time (h) | | |
|---|---|---|---|
| | 12 | 18 | 24 |
| Appearance | 5.60 ± 1.54$^b$ | 6.97 ± 1.43$^a$ | 6.60 ± 1.38$^a$ |
| Aroma | 6.45 ± 1.31$^a$ | 6.30 ± 1.72$^a$ | 6.27 ± 1.14$^a$ |
| Colour | 5.70 ± 1.59$^b$ | 7.27 ± 1.52$^a$ | 6.90 ± 1.65$^a$ |
| Texture | 5.90 ± 1.72$^b$ | 6.80 ± 1.28$^a$ | 6.67 ± 1.52$^{ab}$ |
| Taste | 6.57 ± 1.48$^a$ | 6.33 ± 1.91$^a$ | 6.80 ± 1.38$^a$ |

Values are mean ± SD of 69 untrained panellists. Different letters indicate significant differences ($p < 0.05$) within a row.

demonstrated a significant reduction in the overall acceptability (30%) (Ghazaei *et al.,* 2015). In another study, the sensory analysis of meringues demonstrated low acceptance due to the use of aquafaba from haricot beans and whole green lentils. In comparison, high acceptance was observed for meringues produced by using garbanzo chickpeas and split yellow peas (Stantiall *et al.,* 2018). In the present work, the replacement of eggs with chickpeas aquafaba maintained the organoleptic properties of mayonnaise, and it is thus recommended for the production of plant-based mayonnaise.

## Conclusion

Aquafaba was produced from chickpeas soaked for 12, 18, and 24 h in brine, and boiled for 35 min at 120°C. The 24 h aquafaba showed the highest total soluble solid content, peptide content, and viscosity. The plant-based mayonnaise prepared by using 24 h aquafaba showed an increase in the product safety, acceptable physicochemical properties, and good sensory properties. The 24 h aquafaba mayonnaise was preferred by consumers over 12 and 18 h, in terms of taste attribute. Further studies should be carried out to compare the acceptability and quality of aquafaba mayonnaise as vegan mayonnaises. In addition, the effects of the prolonged storage at different temperatures could be carried out to determine the maximum shelf life of aquafaba mayonnaise and its stability against microbial contamination.

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