Evaluation and optimization of functional and antinutritional properties of aquafaba

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Abstract
Egg protein is responsible for the second most serious of all food allergens, which affects predominantly the children. Therefore, a new type of vegan ingredient called “aquafaba,” is getting recognized as a plant-based emulsifier in many bakery product preparations instead of the conventionally used egg white and is emerging in the consumer market. It is the residue water from cooked chickpeas. In this study, an I-optimal mixture experimental design is combined with a response surface methodology to evaluate the chickpeas cooking process for obtaining aquafaba. The following variables were used: chickpea to cooking water ratio (CPCWR; 1:2, 1:4, and 2:3) and cook time (15, 30, 45, and 60 min). The principal goal was to maximize the functional properties and protein content, while minimizing tannin and phytate contents of aquafaba. The results showed that both CPCWR and cooking time had significant effect on the responses. Emulsion properties were the maximum at 2:3 CPCWR and cooking time of 60 min. Foaming capacity was highest (120%) at 2:3 CPCWR cooked for 30 min, whereas the foam was most stable (57 min) at 1:2 CPCWR with 45 min cooking. Water holding capacity reached the maximum level when cooked for 15 min, and oil holding capacity maximum was obtained after 60 min cooking. Polynomial models were developed for all 11 responses. Optimal results were achieved under the following conditions: 1.5:3.5 CPCWR and 60 min cook time, and the overall desirability fraction was 0.81. Validation tests confirmed these results.

KEYWORDS
antinutritional factors, aquafaba, functional properties, hydrophobicity

INTRODUCTION

Egg white is one of the most common emulsification agents used in cakes and many other bakery products. Interestingly, allergies to egg proteins are widely recognized and considered the second most serious of food allergens predominantly affecting children (Ruscigno, 2016). Concerned with adverse effects of meats, the consumer preference for vegetarian diets is increasing worldwide reaching 12% in Canada, 11% in Australia, 30% in India, 18% in Sweden, 14% in Switzerland, 20% in the United Kingdom, and 14% in Taiwan. There are many plant protein-based food products introduced by Beyond Meat®, which mimic the traditional meat, milk, and egg products.
Eggless cakes are now appearing in market for those who are allergic to egg and those who are considered vegans and opting only for plant-based foods. Pulses have been recognized to have the best functional properties for food applications and have been used to replace animal proteins. Tetrick et al. (2012) suggested a plant protein as a substitute to egg as an emulsifier and described methods where pulse flour was combined with other thickeners to simulate the emulsification properties of egg white.

There is also a new type of plant-based protein used to replace for egg white as an emulsifier and is called “aquafaba” which, in Latin, means bean water. It is simply the residual water obtained after cooking chickpea in water. It contains different levels of soluble carbohydrates, proteins, and saponins that leach out during soaking and cooking. These compounds can achieve good foaming and gelling abilities (Stantiall, Dale, Calizo, & Serventi, 2017). Alajaji and El-Adawy (2006) demonstrated that stable foams could be achieved from the water soluble polysaccharides and/or proteins present in chickpea flour. Albumin protein fractions obtained from chickpeas have also been reported to have good emulsification properties (Singh, Wani, Kaur, & Sogi, 2008). There are many webpages that show how to use aquafaba in a variety of food products to replicate the unique properties of several dairy-based products. Many sources provide aquafaba recipes using the water from canned chickpeas.

Aquafaba quality will vary depending on chickpea soaking and cooking time (Shim, Mustafa, Shen, Ratanapariyanuch, & Reaney, 2018); therefore, they affect the resulting functional properties. Most recipes used and studies carried out previously use aquafaba from canned chickpeas and not the residual water of home cooked chickpeas. It was observed that there are only very limited number of papers dealing with aquafaba (Buhl, Christensen, & Hammershøj, 2019; Lafarga, Villaró, Bobo, & Aguiló-Aguayo, 2019; Meurer, de Souza, & Marczak, 2020; Mustafa, He, Shim, & Reaney, 2018; Shim et al., 2018; Stantiall et al., 2017), and in fact, three of them use aquafaba as the drained water from chickpeas cans.

Further, the majority of previous studies just use aquafaba in food applications. They did not elucidate the quality aspects of aquafaba. The only study, which carried out a more detailed investigation on aquafaba, is Shim et al. (2018), and they mostly dealt with the composition of aquafaba from canned chickpeas and not aquafaba from freshly cooked chickpeas. As a result, there is no major study on aquafaba prepared from fresh chickpeas cooked under carefully controlled conditions, and there are no detailed studies on its functional properties. It is important to evaluate antinutritional factors such as phytic acid and tannin contents because they reduce protein digestibility and form complexes with minerals that reduce their absorption. In addition, legumes have higher amounts of those components than other plants. Studying the influence of protein is useful because the quality of many food formulations such as foam and emulsion creation and their stability are dependent on the properties of protein. Examples include formation good whipped toppings and desserts, emulsification to form/stabilize fat emulsions in soups and cakes, and water binding capacity to entrap water in bread and ice creams and oil binding used in doughnuts and other desserts.

Therefore, this study was carried out to evaluate the different ways of cooking of chickpeas for obtaining aquafaba by varying the chickpeas to cook water ratio (CPCWR) and cook time using a statistical sound experimental design. The influence of process variables and obtained optimal cooking conditions based on functionally important output parameters was assessed using a response surface methodology (RSM).

2 MATERIALS AND METHODS

2.1 Materials

Dry Canadian Kabuli chickpeas (CLIC brand) packed in heat-sealed clear plastic bags in 407 g portions were purchased from Provigo Distribution Centre Outlet (Montreal) and stored at room temperature until use for experiments (time span less than a month). Canned Canadian Kabuli chickpeas (CLIC brand) was also obtained from the same store and used as a commercial control sample.

2.2 Sample preparation

Chickpeas obtained were soaked in water at 40°C for 2 hr, as determined by a preliminary study for optimizing chickpeas hydration and then placed in a classic pressure cooker (Hawkins brand) with different CPCWRs and cooked for different times according to a statistical design. Canned samples were used to compare the results of all responses used in the design. After cooking, the aquafaba was drained and separated from cooked chickpeas, weighed, and used for analysis. All test samples were analyzed in triplicate for various output parameters except when stated otherwise.

2.3 Aquafaba yield and protein content

Aquafaba yield was determined from the quantity of aquafaba obtained after cooking and quantity of chickpeas before cooking and expressed as g aquafaba per 100 g of chickpeas:

\[
\text{Yield} = \frac{\text{Quantity of aquafaba (g)}}{\text{quantity of raw chickpeas (g)}} \times 100.
\]  

Bradford technique (Bradford, 1976) was used to estimate the crude protein content of aquafaba. In this method, standard curve was first prepared from the absorbance data at 595 nm plotted against bovine serum albumin protein (as a standard) concentration (0–2 mg/ml). Unknown protein samples were diluted to obtain absorbance equivalent of 0.125 to 2 mg protein. For testing, 40 μl of sample solution was mixed with 2 ml Bradford reagent in cuvette tube, and the absorbance was measured at 595 nm after incubating for 5 min at room temperature.

2.4 Color

Color parameters, L*, a*, and b* values, of all aquafaba samples were measured in using a Minolta Chroma Meter (Minolta Corp., Ramsey,
Foam stability of aquafaba was measured by allowing the foam to stand in the graduated cylinder over time that was recorded as the bubbles broke down and the level decreased.

Water holding capacity (WHC) and oil holding capacity (OHC) of aquafaba were determined according to the methods detailed in Sathe and Salunkhe (1981). As suggested, 1 g of freeze dried aquafaba was mixed with 10 ml distilled water or oil and vortexed for 30 s. Samples were then allowed to stand at room temperature for 1 hr, centrifuged at 6,000 rpm for 30 min, and then weighed. The difference in weight between the empty tube and the one after centrifugation was recorded as water or OHC, respectively. Samples were analyzed in triplicate.

2.7 | Tannin

Tannin content of aquafaba was evaluated using the method detailed in Khandelwal, Udipi, and Ghugre (2010). The suggested method was based on reacting condensed tannins with vanillin in the presence of acid to produce red color. For this, 1 g of freeze-dried aquafaba samples was extracted with 20 ml of 1% HCl (ACROS ORGANICS, NJ, USA) in methanol (LC-MS Grade, EMD Millipore Corporation, USA) for 20 min in water bath at 30°C. After centrifuging the samples at 2,000 rpm for 4 min, an aliquot of 1 ml supernatant was mixed with 5 ml vanillin solution (0.5% vanillin [99% pure, ACROS ORGANICS, NJ, USA] and 2% HCl in methanol) and incubated for 20 min at 30°C. Four percent HCl in methanol was used instead of vanillin reagent to be used as a blank. All absorbance readings were taken at 500 nm in a UV/VIS spectrophotometer (VWR, Model V-3100PC). A standard curve was prepared with catechin (TRC Canada, Toronto, ON, Canada) from which the tannin content in the sample was calculated and expressed as mg CE/100 g.

2.8 | Phytatic acid

Phytic acid content of aquafaba was measured in freeze-dried samples according to McKie and McCleary (2016) using a Megazyme phytic acid (Phytase/Total phosphorus) assay kit (#K-PHYT, Megazyme International Ireland). As detailed in the assay kit, the methodology is based on the phytase and alkaline phosphatase hydrolysis of phytic acid to myo-inositol (phosphate) and inorganic phosphate (Pi). The released Pi then reacts with ammonium molybdate, which is reduced later to molybdenum blue in acidic conditions. Absorbance of molybdenum blue was measured at 655 nm in a UV/VIS spectrophotometer (VWR, Model V-3100PC) and was related to the amount of Pi presents in the sample through a standard curve.

2.9 | Hydrophobicity

Surface hydrophobicity (S0) of aquafaba was determined using a fluorescent probe 8-anilino-1-naphthalenesulfonic acid as described.
by Kato and Nakai (1980). A fluorescence spectrophotometer (Spectra Max i3x, Molecular devices, USA) was used with the excitation at 390 nm and emission wavelength at 470 nm.

2.10 Emulsion particle size

The particle size of fresh aquafaba emulsion was measured in a dynamic laser scattering particle size analyzer (Brookhaven Instrument 90 Plus Particle Size Analyzer, NY, USA) and Brookhaven Instrument-90 Plus Particle Size Sizing Software. Fresh aquafaba samples were diluted 1:8 using distilled water to which canola oil was added 1:1 to create an emulsion by homogenizing for 3 min, and finally diluted 1:10 water for the particle size analysis. The measurement was carried out in triplicate.

2.11 Experimental design

An I-optimal combined mixture-process design was used to evaluate the effect of two main factors: the mixture composition (the ratio of chickpeas [parameter A] and cooking water [parameter B]) and the cooking time (C) on 14 output responses. Fifteen different combinations were selected as suggested by the experimental design as shown in Tables 1 and 2. Another sample included was the drained water from commercial canned chickpeas for comparison.

2.12 Statistical analysis

Data were analyzed using the StatEase Design Expert 10.0.5 statistical software (StatEase Inc., Minneapolis, USA). In the procedures employed, the software was used to analyze the test data obtained through experiments by least square multiple regression analysis. Different models like linear, quadratic, cubic functions, and interactions tested and their suitability were evaluated based on the analysis of variance and associated F values. The significance was tested at 5% probability level. The generated statistical parameters were used to assess the validity of generated models.

2.13 Optimization and validation

The surface-response plots were used to assess the influence of process variables on the various outcomes. The optimization process was carried out by the software based on multiresponse analysis and desired function methodology (software generated). The general approach of this desirability function was to transform all responses into dimensionless individual desirability functions (G) between 0 and 1 to describe their desirability. The Design Expert software was then used to maximize the G. Additional experiments in triplicate were carried out at the suggested optimal conditions, and the experimental data were compared with the predicted ones.

3 RESULTS AND DISCUSSION

3.1 Effect of variables on responses

The mixture-process design employed to investigate the influence of process variables (chickpea to cooking water ratio and cooking time) on functional properties, protein, tannins, and phytic acid contents of aquafaba obtained after pressure cooking are summarized in Tables 1 and 2. Seven polynomial models were fitted to the experimental data, and the results are summarized in Table 3.

3.1.1 Aquafaba yield and protein content

The yield had a quadratic (chickpea: cooking water) × cubic (cooking time) model with an $R^2$ value of .999 and insignificant lack of fit as shown in Table 3. The yield was calculated as the amount of liquid aquafaba per 100 g chickpeas. Figure 1a shows that the yield increased with an increase in water proportion and decrease in chickpeas' proportion, that is, chick peas to cook water ratio increased. Because the proportions (A, chickpea and B, cooking water) are significant model terms, the components’ coefficients did not show a specific trend for the yield. The same figure showed the highest yield obtained with 1:4 chickpeas: water ratio (CPCWR) cooked for 15 min and the lowest yield with 2:3 CPCWR cooked for 60 min. A middle-ranged yield resulted in 1:2 CPCWR no matter what the cooking time it was. Higher CPCWR increased the yield because of the ability to diffuse into chickpeas and extract more, and second, it also represented conditions with higher amount of water. As a result, higher amounts of water-soluble carbohydrates such as sugars, soluble fibers, and proteins leached into the water (Han & Baik, 2006; Johnny, Razavi, & Khodaei, 2015; Sayar, Turhan, & Gunasekaran, 2001; Zhong et al., 2018). The yield based on solids content would have given the opposite trend because the higher ration would have lot more aqueous phase relative to solids. Because many of the functional properties were based on the liquid aquafaba, the wet basis approach was used in this paper. The solids content is listed in the tables, and hence one can convert one form of unit to the other.

Protein content also resulted in a quadratic (chickpea: cooking water) × cubic (cooking time) model. Figure 1b shows that 1:2 and 2:3 CPCWR cooked for 60 min had the highest protein content (1%). The longer cooking time resulted in higher protein content except for 1:4 CPCWR it decreased after cooking for 60 min. Although functionally important, the protein content of liquid aquafaba was very low (0.5%-1%), and the variation between the test runs was also low, and as a result, the analysis could not determine the significance of the model terms. Water diffusion into chickpeas to extract more water-soluble proteins such as albumin with longer cooking time and higher chickpeas ratio that leached out into the water has been reported earlier (Chigwedere, Njoroge, Van Loey, & Hendrickx, 2019; Güzel & Sayar, 2012; Sayar, Turhan, & KÖKSEL, 2011).
| Run | A Chickpea | B Water (min) | C Cooking time | Aquafaba yield (%) | Solid content (%) | Color (L*) | Color (a*) | Color (b*) | Turbidity (%) | Tannins (MG CE/100 g) | Phytates (g/100 g) (%) | Protein content (%) |
|-----|------------|---------------|----------------|-------------------|------------------|------------|------------|------------|--------------|----------------------|------------------------|----------------------|
| 1   | 1          | 2             | 45             | 1.49 ± 0.07       | 10.0 ± 0.3       | 16.6 ± 0.6 | 2.5 ± 0.1  | 14.3 ± 0.2 | 99.7 ± 0.01  | 1.92 ± 0.3           | 0.00 ± 0.01            | 1.00 ± 0.0            |
| 2   | 1          | 4             | 30             | 3.68 ± 0.21       | 10.0 ± 0.0       | 16.0 ± 0.3 | 0.8 ± 0.1  | 13.8 ± 0.7 | 75.4 ± 0.00  | 10.25 ± 0.4          | 0.049 ± 0.03           | 0.70 ± 0.0             |
| 3   | 1          | 4             | 15             | 4.28 ± 0.13       | 8.0 ± 0.5        | 19.2 ± 0.1 | 0.2 ± 0.3  | 10.8 ± 1.3 | 33.2 ± 0.19  | 11.80 ± 0.4          | 0.068 ± 0.01           | 0.50 ± 0.0             |
| 4   | 2          | 3             | 45             | 0.48 ± 0.17       | 6.3 ± 0.8        | 16.8 ± 0.1 | 3.5 ± 0.1  | 14.4 ± 0.4 | 99.7 ± 0.01  | 0.64 ± 0.8           | 0.00 ± 0.04            | 0.80 ± 0.0             |
| 5   | 1          | 4             | 45             | 2.65 ± 0.03       | 11.0 ± 0.5       | 16.1 ± 0.1 | 1.5 ± 0.1  | 11.2 ± 0.8 | 94.1 ± 0.04  | 9.79 ± 0.4           | 0.051 ± 0.01           | 0.87 ± 0.0             |
| 6   | 1          | 2             | 15             | 2.34 ± 0.04       | 7.0 ± 0.8        | 16.8 ± 0.2 | 1.1 ± 0.4  | 13.9 ± 1.5 | 93.2 ± 0.02  | 5.93 ± 0.3           | 0.021 ± 0.01           | 0.60 ± 0.0             |
| 7   | 1          | 4             | 60             | 1.82 ± 0.02       | 15.0 ± 1.0       | 15.2 ± 0.2 | 1.7 ± 0.1  | 12.8 ± 0.2 | 99.2 ± 0.01  | 9.53 ± 0.3           | 0.024 ± 0.02           | 0.84 ± 0.0             |
| 8   | 2          | 3             | 15             | 1.03 ± 0.22       | 3.5 ± 0.5        | 16.6 ± 0.6 | 1.0 ± 0.2  | 10.7 ± 1.2 | 76.9 ± 0.04  | 3.72 ± 0.3           | 0.002 ± 0.00           | 0.71 ± 0.0             |
| 9   | 1          | 2             | 30             | 1.78 ± 0.01       | 7.5 ± 0.0        | 17.0 ± 1.4 | 2.1 ± 0.0  | 12.4 ± 0.7 | 99.6 ± 0.04  | 3.39 ± 0.4           | 0.011 ± 0.01           | 0.80 ± 0.0             |
| 10  | 2          | 3             | 60             | 0.26 ± 0.03       | 8.7 ± 1.2        | 20.1 ± 0.1 | 4.1 ± 0.1  | 17.5 ± 0.1 | 99.8 ± 0.04  | 0.54 ± 0.8           | 0.000 ± 0.05           | 1.00 ± 0.0             |
| 11  | 1          | 2             | 15             | 2.38 ± 0.04       | 6.5 ± 0.0        | 17.2 ± 0.2 | 0.4 ± 0.4  | 11.0 ± 1.5 | 93.7 ± 0.04  | 3.10 ± 0.3           | 0.012 ± 0.01           | 0.60 ± 0.0             |
| 12  | 2          | 3             | 30             | 0.71 ± 0.08       | 5.0 ± 0.8        | 17.4 ± 0.2 | 1.5 ± 0.1  | 10.7 ± 0.2 | 99.4 ± 0.02  | 0.98 ± 0.3           | 0.000 ± 0.00           | 0.77 ± 0.0             |
| 13  | 1          | 2             | 30             | 1.75 ± 0.01       | 8.0 ± 0.5        | 14.3 ± 1.4 | 2.1 ± 0.0  | 13.7 ± 0.7 | 98.0 ± 0.00  | 4.40 ± 0.8           | 0.010 ± 0.01           | 0.80 ± 0.0             |
| 14  | 1          | 2             | 45             | 1.48 ± 0.07       | 10.3 ± 0.6       | 17.8 ± 0.6 | 2.7 ± 0.1  | 13.9 ± 0.2 | 99.7 ± 0.03  | 1.98 ± 0.3           | 0.000 ± 0.03           | 1.00 ± 0.0             |
| 15  | 1          | 2             | 60             | 1.14 ± 0.12       | 11.3 ± 0.5       | 14.9 ± 0.1 | 2.9 ± 0.1  | 12.9 ± 0.9 | 99.8 ± 0.04  | 0.91 ± 0.8           | 0.000 ± 0.3            | 1.00 ± 0.0             |
| Canned | -         | -             | -              | -                | 5.5 ± 0.3        | 16.8 ± 0.3 | 0.1 ± 0.0  | 12.1 ± 0.7 | 97.2 ± 0.02  | 0.49 ± 0.4           | 0.057 ± 0.6            | 1.00 ± 0.0             |
| Run | A Chickpea | B Water | C Cooking time (min) | Emulsion capacity (ml) | Emulsion stability (ml) | Foaming capacity (%) | Foam stability (min) | Water holding capacity (g) | Oil holding capacity (g) | Hydrophobicity ($S_0 \times 10^3$) | Emulsion particle size (μm) |
|-----|------------|---------|----------------------|-----------------------|------------------------|---------------------|----------------------|--------------------------|--------------------------|-----------------------------|-------------------------|
| 1   | 1          | 2       | 45                   | 5.7 ± 0.2             | 5.0 ± 0.2              | 100.2 ± 1.1         | 55 ± 2.2             | 1.9 ± 0.3                 | 2.2 ± 0.7                 | 250 ± 1.2                   | 4.1 ± 0.4                |
| 2   | 1          | 4       | 30                   | 1.7 ± 0.2             | 1.7 ± 0.2              | 43.4 ± 0.2          | 18 ± 1.8             | 2.0 ± 0.2                 | 1.6 ± 0.9                 | 199 ± 1.4                   | 2.0 ± 0.3                |
| 3   | 1          | 4       | 15                   | 0.5 ± 0.3             | 0.1 ± 0.3              | 40.1 ± 3.1          | 7 ± 2.7              | 2.5 ± 0.4                 | 0.9 ± 1.8                 | 164 ± 1.8                   | 1.8 ± 0.2                |
| 4   | 2          | 3       | 45                   | 7.1 ± 0.2             | 5.0 ± 0.6              | 70.2 ± 1.9          | 34 ± 3.5             | 1.9 ± 0.2                 | 3.5 ± 0.2                 | 279 ± 1.3                   | 3.1 ± 0.7                |
| 5   | 1          | 4       | 45                   | 3.3 ± 0.1             | 3.2 ± 0.5              | 77 ± 2.2            | 28 ± 3.6             | 1.8 ± 0.4                 | 2.8 ± 0.7                 | 219 ± 1.4                   | 1.8 ± 0.4                |
| 6   | 1          | 2       | 15                   | 0.9 ± 0.3             | 0.5 ± 0.3              | 50.4 ± 2.8          | 7 ± 2.9              | 2.7 ± 0.1                 | 1.2 ± 0.9                 | 250 ± 1.9                   | 3.5 ± 0.5                |
| 7   | 1          | 4       | 60                   | 2.3 ± 0.2             | 1.6 ± 0.2              | 113.5 ± 1.4         | 32 ± 4.2             | 1.7 ± 0.3                 | 3.5 ± 1.2                 | 345 ± 1.0                   | 2.3 ± 0.4                |
| 8   | 2          | 3       | 15                   | 1.2 ± 0.3             | 0.8 ± 0.3              | 120.2 ± 2.6         | 30 ± 4.6             | 2.3 ± 0.5                 | 2.9 ± 1.0                 | 204 ± 1.6                   | 2.5 ± 0.2                |
| 9   | 1          | 2       | 30                   | 3.1 ± 0.2             | 2.2 ± 0.4              | 62.5 ± 1.3          | 19 ± 3.7             | 1.9 ± 0.3                 | 1.6 ± 1.0                 | 240 ± 1.5                   | 4.2 ± 2.3                |
| 10  | 2          | 3       | 60                   | 7.3 ± 0.4             | 6.9 ± 0.5              | 87.7 ± 1.1          | 37 ± 2.4             | 1.7 ± 0.2                 | 3.5 ± 0.6                 | 277 ± 1.0                   | 2.9 ± 0.3                |
| 11  | 1          | 2       | 15                   | 1.1 ± 0.1             | 1.0 ± 0.3              | 50.6 ± 3.0          | 14 ± 2.9             | 2.2 ± 0.1                 | 1.5 ± 0.9                 | 249 ± 1.7                   | 3.3 ± 0.5                |
| 12  | 2          | 3       | 30                   | 5.6 ± 0.4             | 4.1 ± 0.3              | 120.3 ± 1.7         | 33 ± 2.9             | 1.9 ± 0.3                 | 3.2 ± 0.9                 | 208 ± 1.1                   | 2.2 ± 0.6                |
| 13  | 1          | 2       | 30                   | 2.8 ± 0.3             | 2.3 ± 0.4              | 70.4 ± 1.3          | 15 ± 1.8             | 1.4 ± 0.4                 | 1.3 ± 1.3                 | 242 ± 1.5                   | 4.7 ± 2.3                |
| 14  | 1          | 2       | 45                   | 5.1 ± 0.2             | 4.2 ± 0.2              | 110.2 ± 1.1         | 57 ± 2.2             | 1.5 ± 0.3                 | 3.2 ± 1.4                 | 249 ± 1.2                   | 4.0 ± 0.4                |
| 15  | 1          | 2       | 60                   | 6.4 ± 0.4             | 5.5 ± 0.4              | 90.8 ± 2.4          | 52 ± 3.4             | 1.9 ± 0.2                 | 3.4 ± 0.8                 | 325 ± 1.0                   | 1.9 ± 0.1                |
| Canned | -      | -       | -                    | 7.0 ± 0.3             | 6.0 ± 0.3              | 290.1 ± 2.7         | 58 ± 4.1             | 0.2 ± 0.0                 | 3.9 ± 0.6                 | 188 ± 1.1                   | 2.8 ± 0.7                |
### TABLE 3  Model statistics and adequacy of the models for all responses

| Response          | Model (mix × process) | Lack of fit | $R^2$   | Adjusted $R^2$ | Std. dev. | $F$ value | $P$ value |
|-------------------|------------------------|-------------|---------|----------------|-----------|-----------|-----------|
| Yield             | Quadratic × Cubic      | 0.0119      | .9999   | .9997          | 0.021     | 3,684.24  | <.0001    |
| Color (L*)        | Quadratic × Linear     | 0.4991      | .5516   | .3025          | 1.51      | 2.21      | .1417     |
| Color (a*)        | Linear × Linear        | 0.2826      | .8982   | .8705          | 1.12      | 26.18     | .0003     |
| Color (b*)        | Linear × Linear        | 0.4971      | .5633   | .4442          | 1.88      | 1.36      | .2684     |
| Turbidity         | Quadratic × Cubic      | 0.0155      | .9997   | .9985          | 17.81     | 857.51    | <.0001    |
| Protein content   | Quadratic × Cubic      | -           | 1.0000  | 1.0000         | 0.00      | -         | -         |
| Emulsion stability| Linear × Linear        | 0.1060      | .8928   | .8635          | 2.07      | 22.10     | .0006     |
| Emulsion capacity | Linear × Quadratic     | 0.0735      | .9557   | .9310          | 2.38      | 38.79     | <.0001    |
| Foaming capacity  | Quadratic × Cubic      | 0.0243      | .9926   | .9653          | 28.08     | 36.44     | .0065     |
| Foam stability    | Quadratic × Cubic      | 0.0242      | .9907   | .9567          | 16.28     | 29.09     | .0090     |
| Water holding capacity | Mean × Quadratic  | 0.9423      | .5774   | .5070          | 0.47      | 8.20      | .0057     |
| Oil holding capacity | Quadratic × Linear    | 0.8526      | .9214   | .8777          | 0.99      | 21.09     | <.0001    |
| Hydrophobicity    | Quadratic × Cubic      | 0.0001      | .9999   | .9999          | 1000      | 2823      | <.0001    |
| Emulsion particle size | Quadratic × Quadratic | 0.2930      | .9598   | .9063          | 2.68      | 17.92     | .0012     |
| Tannins           | Quadratic × Linear     | 0.8819      | .9648   | .9452          | 0.92      | 49.27     | <.0001    |
| Phytates          | Quadratic × Linear     | 0.6177      | .9673   | .9492          | 0.004     | 53.31     | <.0001    |

### FIGURE 1  3-D graphs corresponding to models fitted for (a) aquafaba yield, (b) protein content, (c) tannins, and (d) phytates
3.1.2 | Color and turbidity

Changes with respect to the three color parameters, $L^*$, $a^*$, and $b^*$, as well as the turbidity are summarized in Table 1. In general, the variation in color parameters between treatments was small ranging from 14.3 to 20.1 for $L^*$ value on a scale of 0–100; 0.1 to 2.9 for $a^*$ value on a scale starting from 0 on the positive side for redness and 10.8 to 17.5 for $b^*$ values on a scale starting from 0 for yellowness. These represented a small change in brightness, redness, and yellowness of aquafaba between the different treatment conditions (Figure 5). The resulting $L^*$ values were also around the same $L^*$ and $b^*$ values, and little more for $a^*$ value (redness) than the aquafaba from canned chick peas. The $\Delta E$ values, which were a combination of the three color parameters (Eq. 2), gave similar results (not shown). The turbidity values of aquafaba test samples were generally between 90 and 100 (except for #3 and #8 representing minimally cooked samples, with low yield). The aquafaba from canned chickpeas also had turbidity values in the same range.

Models were described in Table 3 for these parameters, and all of them were significant except for $L^*$ parameter for the color. For $a^*$ and $b^*$ parameters in addition to turbidity, all of them were influenced significantly by experimental variables. Increasing cooking time and CPCWR resulted higher $a^*$ and $b^*$ values representing more redness and yellowness, respectively. Longer cooking increases leaching out of many water soluble components and pigments in addition to the degradation of the latter, which reflected over all color. By comparing pressure-cooked aquafaba to canned sample, $L^*$ and $b^*$ values were in a similar range, but redness was significantly higher in pressure-cooked aquafaba. These four responses had the highest values with 2:3 CPCWR cooked for 60 min. The changes in these four responses were considered minor and not included in the optimization step, which was focused on functional properties not the visual appearance of aquafaba.

3.1.3 | Emulsion capacity and stability

Emulsion capacity was also fitted into Linear (chickpea: cooking water) × Quadratic (cooking time) model with an $R^2$ value of 0.956. This response had a maximum value of 7.3 ml after 60 min cooking with 2:3 CPCWR and the lowest when cooked for 15 min as illustrated in Figure 2a. On the other hand, the emulsion stability fitted most into Linear × Linear model where maximum value was at 60 min cooking as well with 2:3 CPCWR. Because both variables were significant for both models, they magnitude could not be predicted from the coefficients shown in Table 4. Longer cooking time denatured...
chickpea proteins, which led to higher emulsion properties because the hydrophobic areas got exposed as proved by the hydrophobicity experiment. Yanjun et al. (2014) supported our observations because high surface hydrophobicity enhances emulsifying properties by improving the film rigidity through hydrophobic interactions between protein molecules at the interface. Ma et al. (2011) reported that boiling chickpeas increases its emulsion activity, whereas Aguilera, Esteban, Benitez, Molla, and Martín-Cabrejas (2009) reported that soaking and cooking decrease emulsion capacity of chickpeas flour, hence there exists contradicting observations.

### 3.1.4 Foaming properties and hydrophobicity

Best model fitted for foaming capacity, foaming stability, and hydrophobicity was Quadratic (chickpea: cooking water) × Cubic (cooking time) model with and R² value of 0.991. Regarding foaming capacity as in Figure 3a, it was the highest (120%) with 2:3 CPCWR after 15 and 30 min cooking time then 110% and 100% with 1:2 CPCWR after 45 min and finally 113% after 60 min cooking with 1:4 CPCWR. Least response was 40% and 50% at 15 min with 1:4 CPCWR, respectively. It shows that CPCWR had a significant effect on foaming capacities for all combinations where the highest ratio resulted the highest capacity and vice versa. At the same time, cooking time increased the response linearly in the 1:4 ratio and decreased at 60 and 45 min cooking time for the 1:2 and 2:3 ratios, which indicated that the lowest ratio obtained the highest foaming capability at the longest cooking time. All models' terms were significant for all three responses.

Our findings were lower than the study conducted by Meurer et al. (2020) because they got 250% foaming capacity of aquafaba obtained from 1:3 CPCWR and pressure cooked for 20 min. Serventi, Wang, Zhu, Liu, and Fei (2018) reported that aquafaba from yellow soybean cooking water (65%) has better foaming ability.

Foaming stability increased linearly with cooking time for 1:4 and 2:3 CPCWR with higher stability for the latter one. The best foaming stability (57 min) was for 1:2 CPCWR for 45 min, which decreased slightly at 60 min. Stantial et al. (2017) reported that proteins are responsible of good foaming properties, which is a logic reason of the linear increase because longer cooking time can solubilize more water-soluble proteins. Also, aquafaba has high concentration of carbohydrates, and it would contribute to good foaming stability because it has been reported that polysaccharides and its cross-linking with proteins play a role in foam stability (Schramm, 2005). Also, the 2:3 ratio had the highest stability, which is significantly higher than those of other ratios in the first 15 min because of the greater protein content in aquafaba. It can be deduced that both chickpea and water ratios have an attribute to foaming stability because low chickpea content in the 1:4 ratio and low water in the 2:3 ratio could not obtain the best stability as in 1:2 CPCWR.

### 3.1.5 Emulsion particle size

Emulsion particle size has a Quadratic (chickpea: cooking water) × Quadratic (cooking time) model with an R² value of 0.960 where the mixture components have a significant effect on the responses, but the cooking time was insignificant. Figure 2c shows that particle size ranged between 1.8 and 4.7 μm, and the biggest size was at 1:2 CPCWR and the smallest at 1:4 CPCWR, but these did not have a
correlation with emulsion capacity and stability. Raikos, Hayes, and Ni (2019) found that aquafaba has the capability to form stable emulsions for up to 21 days with droplet size distribution <4 μm. In literature, it was reported that the smaller particle size, the better the emulsion stability because there would not be any emulsion flocculation or coalescence because of low attractive forces between droplets (Qian & McClements, 2011).

3.1.6 | Water and oil holding capacities

Regarding water and oil holding capacities, they had different models because the impact of variables was different for each response. Starting with WHC, it had a Mean (chickpea: water) × Quadratic (cooking time) model with a $p$ value of 0.0057. Cooking time was the only variable which had a significant impact in the model. It had a negative coefficient, which indicates that increasing cooking time reduces WHC, which was approved experimentally. Figure 4a illustrated that lowest WHC at 60 min cooking and highest WHC at 15 min cooking. Damian, Huo, and Serventi (2018) study agrees with our results because they found that WHC of aquafaba from chickpeas has 1.5 g/g.

On the other hand, OHC has a Quadratic (chickpea: cooking water) × Linear (cooking time) model with an $R^2$ value of 0.935. Both factors, mixture and process variable, had a significant effect on OHC. By looking at Figure 4b, it showed that lowest OHC was 0.9 g for the 1:4 ratio during the first 15 min cooking, and it increased with longer cooking time and higher mixture proportions till it reached the maximum (3.5 g) at the 2:3 ratio with 60 min cooking, which agrees with Damian et al. (2018) where they reported OHC of aquafaba is 3.2 g/g. Longer cooking time caused protein denaturation, which resulted in higher OHC and lower WHC because the hydrophobic areas got exposed as proved by the hydrophobicity experiment and supported by Yanjun et al. (2014). It has been mentioned by Xu et al. (2017) that pressure cooker is better than dry heat to cause protein dissociation, thereby exposing more water-/oil-binding sites and increases WHC and OHC.

**FIGURE 3** 3-D graphs corresponding to models fitted for (a) foaming capacity, (b) foaming stability, (c) hydrophobicity, and (d) turbidity
Antinutritional factors, tannin and phytic acid, both have a significant Quadratic (chickpea: water) × Linear (cooking time) model with $R^2$ values of 0.965 and 0.970 for tannin and phytic acid, respectively. Regarding the experimental variables, chickpeas ratio was significant for tannin content and insignificant in phytic acid content opposing to water ratio, which was significant for both responses. Cooking time was insignificant for tannin and significant for phytic acid.

Tannin content was the highest for 1:4 CPCWR and the least for the 2:3 ratio, with a linear decrease in all proportions through longer cooking. Tannins are either hydrolysable or condensed, in which most of them are soluble in water (Kim, Silva, & Jung, 2011). As a result, longer time needed to allow more tannins to leach out to water, and the longer the time is, the more tannins would be destroyed either by hydrolyzing to tannic acid and carbohydrates or by polymerizing and become in soluble in water. Also, the reduction might be due to the formation of complexes with proteins and lower extractability as in our case for 1:2 and 2:3 CPCWR (Khandelwal et al., 2010; Sinha & Kawatra, 2003; Somsob, Kongsakuncharai, Sungpuag, & Charoenporn, 2008). On the other hand, phytic acid content was also the lowest (0–2 mg) for 2:3 CPCWR and the highest (68 mg) for the 1:4 ratio. It was decreasing linearly with cooking time to reach zero at 45 min cooking in the 1:2 and 2:3 ratios. Reduction of phytic acid might be due to low bioavailability when free phytic acid forms complexes with other proteins and minerals and then cannot be extracted by water (Urbano et al., 2000). It could also be because of the hydrolytic activity of phytase enzyme to penta and tetraphosphates (Deng, Padilla-Zakour, Zhao, & Tao, 2015 and Lopez-Martinez, Leyva-Lopez, Gutierrez-Grijalva, & Heredia, 2017).

3.1.8 General comparison with aquafaba from canned chickpeas

In this study, a comparison was made between aquafaba obtained from fresh chickpeas and aquafaba from canned chickpeas, which was shown in Tables 1 and 2. Aquafaba from canned chickpeas revealed higher foaming properties (Mustafa et al., 2018; Shim et al., 2018), OHC, phytic acid content, and lower emulsion properties and hydrophobicity than the one obtained from optimized cooking conditions. Generally, high functional properties might be due to higher protein content because aquafaba from canned chickpeas contained 6% as described by Buhl et al. (2019), whereas 1% from aquafaba obtained from chickpea cooking water based on wet basis. Hydrophobicity of aquafaba from canned chickpeas in our study did not correlate positively with emulsion properties as was mentioned by Buhl et al. (2019), but they supported our findings that emulsion particle size did not affect emulsion properties.

Statistical details of the various models generated by the software are detailed in Table 3. The associated high $R^2$ values, non significance of lack of fit, and the other analysis of variance parameters demonstrated a good performance of the generated models.

3.2 Optimization and validation

As detailed in the methodology, the software was used to generate optimum processing conditions for obtaining aquafaba through cooking in a pressure cooker to result in maximization of several desirable functional properties and protein contents while achieving minimization of the undesirable tannin and phytic acid contents and emulsion particle size. Factors yield, color parameters ($a^*$, $b^*$, and $L^*$), and turbidity were not included in the optimization step. Yield, for example, was not included in this study on a dry weight basis in relation to functional properties as the study was concerned with the fresh aquafaba rather than dried and reformulated. It was also necessary to minimize the number of factors to have better
desirability values in terms of functionality. Different polynomial models were first developed for each response and later utilized arrive at optimum conditions using the desirability function method. The optimum condition was obtained with 1.5:3.5 chickpea: water ratio and 60 min cooking time. This gave an overall maximized desirability value of 0.81. Three additional experiments were carried out at this optimum condition to verify it validity. The predicted and experimentally validated results are shown in Table 5.

Applying a mixture-process design from RSM enabled us to evaluate the factors affecting the quality of aquafaba. Diagnostic graphs help to check the model adequacy and effectiveness. Figure 6 (residuals plot) demonstrated that the developed models were adequate because the residuals of responses were within 5% limit. A few of the residuals in foaming capacity and stability were more than 5% (not shown). Overall, the models were accurate because the $R^2$ of all of them was 0.99, and the adjusted $R^2$ was lower than $R^2$ with no more than 0.20.

![Figure 5: 3-D graphs corresponding to models fitted for color (a) "L", (b) color "a", and (c) color "b".](image)

**TABLE 5** Predicted and experimental values of the optimum conditions

| Responses                  | Predicted | Experimental       |
|---------------------------|-----------|--------------------|
| Tannins (mg ce/100 g)     | 0.195     | 2.12 ± 1.50        |
| Phytates (g/100 g)        | 0.004     | 0.0053 ± 0.02      |
| Protein %                 | 1.0       | 1.0 ± 0.16         |
| Emulsion capacity (ml)    | 6.1       | 5.3 ± 0.3          |
| Emulsion stability (ml)   | 5.9       | 3.9 ± 0.8          |
| Foaming capacity (%)      | 86.9      | 88.3 ± 2.36        |
| Foaming stability (min)   | 51.0      | 55.0 ± 2.45        |
| WHC (g)                   | 1.8       | 3.3 ± 0.02         |
| OHC (g)                   | 3.4       | 3.9 ± 0.28         |
| Hydrophobicity ($S_0$)    | 312,637.9 | 329,489.5 ± 1.4 × 10³ |
| Emulsion particle size (μm)| 2.1       | 2.2 ± 0.59         |
This study was conducted by applying RSM-based design in combination with two factors to optimize the variables such as functional properties, tannin, phytate, and protein contents of aquafaba obtained from pressure cooker and compare the results to aquafaba from canned chickpeas. Results showed that chickpea to water ratio and processing time have a significant effect on most of the responses. I-optimal combined mixture-process design can be applied to develop mathematical models for predicting the optimal levels of variables for specific conditions within experimental range. The optimal conditions were 1.5:3.5 chickpea to water ratio cooked under specific conditions.
for 60 min. By applying optimal conditions, the experimental values were in agreement with predicted ones, therefore confirming the adequacy of the developed models.

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CONFLICT OF INTEREST
Authors confirm that there is no conflict of interest in the work.

DATA AVAILABILITY STATEMENT
The raw data cannot be shared at this time due to technical limitations, however, available on request.

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