Video Article

Composition and Properties of Aquafaba: Water Recovered from Commercially Canned Chickpeas

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URL: https://www.jove.com/video/56305
DOI: doi:10.3791/56305

Keywords: Environmental Sciences, Issue 132, Chickpea, emulsifiers, stabilizers, thickeners, manufacturing, egg/gluten-free (vegan) products

Date Published: 2/10/2018

Citation: Shim, Y.Y., Mustafa, R., Shen, J., Ratanapariyanuch, K., Reaney, M.J. Composition and Properties of Aquafaba: Water Recovered from Commercially Canned Chickpeas. *J. Vis. Exp.* (132), e56305, doi:10.3791/56305 (2018).

Abstract

Chickpea and other pulses are commonly sold as canned products packed in a thick solution or a brine. This solution has recently been shown to produce stable foams and emulsions, and can act as a thickener. Recently interest in this product has been enhanced through the internet where it is proposed that this solution, now called aquafaba by a growing community, can be used a replacement for egg and milk protein. As aquafaba is both new and being developed by an internet based community little is known of its composition or properties. Aquafaba was recovered from 10 commercial canned chickpea products and correlations among aquafaba composition, density, viscosity and foaming properties were investigated. Proton NMR was used to characterize aquafaba composition before and after ultrafiltration through membranes with different molecular weight cut offs (MWCOs of 3, 10, or 50 kDa). A protocol for electrophoresis, and peptide mass fingerprinting is also presented. Those methods provided valuable information regarding components responsible for aquafaba functional properties. This information will allow the development of practices to produce standard commercial aquafaba products and may help consumers select products of superior or consistent utility.

Video Link

The video component of this article can be found at https://www.jove.com/video/56305/

Introduction

Increasingly vegetarian products are being developed that mimic the properties of meat, milk, and eggs. The functional properties of pulses are important in their current uses in food applications and their properties are being explored in the development of replacements for animal protein. For example, dairy alternatives sales were $8.80 Billion USD in 2015 and this market is growing rapidly. This market is projected to grow to $35.06 billion by 2024. Moreover, the upward trend in demand for plant-based milk substitutes is, in part, a result of consumer health concerns regarding cholesterol, antibiotics, and growth hormones often used in milk production[1]. Similarly, vegetable protein and hydrocolloid egg replacer markets are rapidly expanding and a compound annual growth rate of 5.8% is expected for these materials over the next 8 years with sales of $1.5 billion USD expected in 2026[2]. A growing number of consumers prefer vegan protein sources, allergen reduced diets and reduced carbon footprint for food products. Demand for pulse-based products, especially from lentil, chickpea, and faba bean are steadily growing due to the high protein content, dietary fiber and low saturated fat content of pulses[3]. Pulses also contain phytochemicals with potentially beneficial biological activity[4].

Commercial entities, scientists and private individuals have taken different approaches to communicate the quality properties of chickpea based egg and milk replacements. Gugger *et al.*[5] produced a milk-like product from high starch grains including adzuki bean and chickpea. In their described methods the proponents attempted to show that their product is unique and different from "aquafaba"[6]. In another commercial approach elucidated by Tetrick *et al.*[7] a plant-based egg substitute was developed. Their patent application describes methods of combining pulse flour with known thickeners that emulate the function of egg white in baked materials. Typical formulae include 80-90% pulse flour and 10-20% thickening additives.

Peer reviewed literature also indicates the functionality possible with chickpea and has demonstrated that albumin protein fractions obtained from kabuli and desi chickpea flour have good emulsification properties. They have also found a significant effect of chickpea source on the albumin yield and performance[8].

After the initial internet report describing "aquafaba" by French chef Joël Roessel, an open source movement is showing the utility of aquafaba as a replacement of egg white and dairy protein in many food applications. There are many highly viewed webpages and YouTube videos showing the incorporation of aquafaba in foods that emulate the qualities of ice cream, meringue, cheese, mayonnaise, scrambled eggs, and whipped cream. Most pioneers providing open source aquafaba applications (recipes) obtain their material by straining canned chickpeas and

using the liquid in their recipes. These individuals are mostly not trained scientists. Video comments sections indicate that the respondents have copied the recipes and some have failed to replicate the successes of the aquafaba advocates.

All three approaches (corporate, scientific and open source) to developing egg and milk replacements have merit but are missing an important dimension. Applied scientists, basic scientists and individuals promulgating pulse-based products have incompletely characterized and standardized their input material. Standardization of a product for a specific use is a normal industrial practice. Chickpea cultivars have not been standardized for aquafaba quality and industrial canning practices are standardized to produce consistent chickpeas not aquafaba.

Based on studies of other commodities, it is predictable that both genotype and environment will contribute to pulse aquafaba quality. It is known that both genotype and environment affect kabuli chickpea canning properties[9]. Typically, genotypic effects are large between related species and smaller within members of a species. Variation in physical and chemical properties can be minimized through identity preservation that allows the selection of cultivars with desired properties. Environmental effects can also be large and are managed by quality evaluation and blending to standard performance in specific tests[10].

There are many genetically distinct cultivars of chickpea in commercial production. For examples, the University of Saskatchewan Crop Development Centre, a major source of commercial chickpea germplasm, has released 23 chickpea cultivars since 1980 of which 6 are currently recommended for cultivation in Canada. While scientific manuscripts often describe the cultivar used in a study, the patents and internet pages that were surveyed did not indicate the cultivar used or the provenance of the chickpeas. The standardization of cultivars and handling could help users increase their success in using chickpea but this information is not available on canned chickpea products.

The objective of this research is to determine the aquafaba components that contribute foaming properties. Here,the rheological properties of aquafaba from commercial chickpea brands were compared and the chemical properties were studied by NMR, electrophoresis, and peptide mass fingerprinting. To our knowledge, this is the first research which describes the chemical composition and the functional properties of the aquafaba viscosifier components.

Protocol

Separation of Aquafaba from Chickpeas

1. Obtain cans of chick peas from local grocers and open with a manual can opener.
2. Label cans from A to J.
3. Separate chickpeas from aquafaba using a stainless steel meshed kitchen strainer and weigh the separated chickpeas and aquafaba.

Obtain a Representative Sample of Chickpeas and Aquafaba for Chemical Analysis.

4. Randomly select ten chickpeas after draining the aquafaba to determine moisture content.
5. Place the selected chickpeas in a drying tin and heat at 100 ± 2 °C for 16-18 h in a drying oven.
6. After drying, grind the chickpeas to a powder for further use (*e.g.* protein and carbon content analysis).
7. Freeze aquafaba and aquafaba foam from each source to -20 °C and then dry in a freeze dryer. The dried aquafaba will be used for nitrogen and carbon content determination.

Aquafaba Functional Properties

8. Determine aquafaba viscosity at 25 °C using a No. 2 shell cup.
    1. Immerse the shell cup in the aquafaba.
    2. Record the time required to drain the cup[11]. A timer is started when the cup was lifted from the aquafaba and stopped when the stream leaving the cup stops.
    3. The aquafaba viscosity can be ascertained by a chart supplied with the cup that relates viscosity and drainage time.
9. Foaming capability
    1. Blend the aquafaba solution (100 mL) in a stainless steel bowl with a hand mixer at speed setting of 10 for 2 min.
    2. Record the foam volume immediately after blending 100 mL of the aquafaba solution as V_{f100} by placing the foam in a graduated measuring cup.
    3. Allow the foam to stand and dry over time to observe foam stability and obtain a sample of dried foam.

Color Parameters of Chickpea Seed

10. Randomly select chickpea seed from each can for color determination.
11. Calibrate the Hunter lab colorimeter using white, black and reference standards prior to measurements.
12. Color coordinate values are determined by the CIE Lab system[12], including the *L* (positive represents white and 0 represents dark), *a* (positive is red and negative is green), and *b* (positive is yellow and negative is blue) in triplicate with day light 65°.

Protein and Carbon Contents

13. Determine protein and carbon contents by combustion using an elemental analyzer[13]. Protein content is estimated as nitrogen content multiplied by 6.25[14].

Moisture Content

14. Determine seed and aquafaba moisture contents by heating samples at 100 ± 2 °C for 16-18 h in a drying oven[15].

NMR Spectrometry

15. Sample preparation
    1. Prior to spectrometry, centrifuge aquafaba samples at 9200 × g for 10 min. After centrifugation, pass the supernatant through a 0.45 µm syringe filter (25 mm syringe filter with polytetrafluoroethylene (PTFE) membrane). Transfer the aquafaba sample (0.4 mL) into a NMR tube wuth 50 mg added deuterium oxide inside and subject the sample for NMR analysis.
    2. For the dried foam sample previously described, the sample (25 mg) is in deuterium oxide (500 mg) and the solution is subjected to NMR analysis.
    3. Place all samples for ^{13}C-NMR and ^{1}H-NMR in capped 5 mm NMR tubes. Add deuterium oxide and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) to each NMR tube to provide a solvent lock signal and act as an internal standard, respectively.

16. NMR conditions
    1. Acquire proton NMR spectra with a 500 MHz NMR or similar high field NMR spectrometer) with at least 16 scans per spectrum using a water suppression program such as the double pulse field gradient spin echo proton NMR (DPFGSE-NMR) pulse sequence[16].
    2. Adjust the spectral shift to place the TMSP peak at 0 ppm then use the integration software to determine relative amounts of compounds present.

Electrophoresis

NOTE: For this step, aquafaba that yielded the most stable foam (brand H) was selected. This brand did not contain salt.

17. Sample preparation
    1. Introduce aquafaba to the upper reservoir of three centrifugal regenerated cellulose ultrafiltration devices each with different molecular weight cut offs (MWCOs) of 3, 10, or 50 kDa.
    2. Place the centrifugal filter units in a suitable centrifuge at 4 °C and centrifuge at 3900 × g for 2 h to obtain supernatant and retentate fractions. The supernatant fractions were used for ^{1}H-NMR analysis of smaller molecules in the absence of higher molecular weight (MW) species. The 3 kDa membrane retentate fraction was used for electrophoretic separation as it was believed that this membrane would retain most proteins.
    3. Estimate the protein contents of both supernatant and retenate using a modified Bradford method using bovine serum albumin as a standard[17].
    4. Place samples of supernatant and retentate fractions in Eppendorf tubes and subject these fractions to shaking at a frequency of 25 per s (10 min) in a suitable shaker[18]. Observe the shaken solution to determine if a foam has formed from shaking.
    5. Dissolve the retentate by adding 2.0 mL of distilled water to the ultrafiltration device for a second centrifugation treatment to achieve diafilteration. Subject the ultrafiltration device to a second centrifugation at 4 °C and 3900 × g for 2 h.
    6. Mix the retentate (0.025 g) with 1 mL of 0.02 M Tris-HCl pH 7.4 or phosphate-buffered saline (PBS) pH 7.4 to dissolve protein.
    7. Centrifuge the mixture at 21000 x g for 1 min.
    8. Use the supernatant to determine protein content using the modified Bradford as previously mentioned.

18. Electrophoretic separation
    NOTE: The 3 kDa MWCO membrane retentate (described above) is subjected to electrophoretic separation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
    1. Prepare polyacrylamide gels using a 15% polyacrylamide resolving gel and a 5% polyacrylamide stacking gel.
    2. Apply a sample of 20 µg protein to one lane of the gel and PageRuler Prestained Protein Ladder with a range of 10-170 kDa to a separate polyacrylamide gel lane.
    3. Subject the gels to an electrical current in a Mini-Protein Tetra Cell system as modified from Laemmli (1970)[19]. For electrophoresis operating conditions, gel staining, and destaining follow Ratanapariyanuch *et al.* (2012)[20].

Peptide Mass Fingerprinting

NOTE: Cut bands from the SDS-PAGE gel of 3 kDa retentate (MWs of approximately 8, 10, 13, 14, 15, 20, 22, 31, 37, 55, and 100 kDa) for trypsin digestion according to Ratanapariyanuch *et al*. (2012)[20] and perform mass spectral analysis.

19. In-gel digestion
    1. De-stain the bands twice by immersing in 100 µL of 200 mM ammonium bicarbonate (NH_4HCO_3) in 50% acetonitrile (ACN) and incubate at 30 °C for 20 min.

2. After the completion of de-staining, treat the gel samples with ACN (100 µL) for 10 min and then dry under vacuum, using a centrifugal vacuum evaporator, for 15 min at room temperature.
3. Immerse the dried gel samples in 100 µL of 10 mM dithiothreitol (DTT) in 100 mM NH_4HCO_3 solution and incubate at 56 °C for 1 h.
4. Remove excess reducing buffer and replace it with 100 µL alkylating buffer [100 mM iodoacetamide in mass spectrometry (MS) grade water].
5. Incubate the gel samples at room temperature in the dark for 30 min.
6. Wash gel samples twice with 200 mM NH_4HCO_3 (100 µL), shrink the gels by immersing them in ACN (100 µL), re-swell the gels with 200 mM NH_4HCO_3 (100 µL) and again shrink by treating with ACN (100 µL).
7. Drain the ACN and dry the gel samples under vacuum in a centrifugal vacuum evaporator for 15 min.
8. Re-swell dried gel samples using 20 µL trypsin buffer (50 ng/µL trypsin in 1:1,200 mM NH_4HCO_3: Trypsin stock solution) followed by addition of 30 µL of 200 mM NH_4HCO_3 to each sample.
9. Incubate samples on a Thermomixer overnight at 30 °C with shaking at 300 rpm.
10. Stop the trypsin digestion reaction by adding 1/10 of the final volume (volume of mixture after step 8) 1% trifluoracetic acid and extract the tryptic peptides from gel samples using 100 µL of 0.1% trifluoracetic acid in 60% ACN and dry under vacuum using a centrifugal vacuum evaporator.
11. Store tryptic peptides in -80 °C prior to mass spectrometric analysis.

20. Mass spectrometry
    1. Reconstitute tryptic peptides by adding 12 µL of MS grade water: ACN:formic acid (97:3:0.1, v/v) and then vortex for 1 to 2 min to dissolve peptides.
    2. Centrifuge the resulting solution at 18,000 g for 10 min at 4 °C.
    3. Transfer solution aliquots (10 µL) to MS vials for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis on a quadrupole time-of-flight (QTOF) mass spectrometer equipped with a liquid chromatography instrument and a LC-MS interface.
    4. Perform chromatographic peptide separation using a high-capacity HPLC-Chip: consisting of a 360 nL enrichment column and a 75 µm × 150 mm analytical column, both packed with C18-A, 180 Å, 3 µm stationary phase.
    5. Load samples onto the enrichment column with 0.1% formic acid in water at a flow rate of 2.0 µL/min.
    6. Transfer samples from the enrichment column to the analytical column.
    7. Peptide separation conditions are: a linear gradient solvent system consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in ACN). The linear gradient begins with 3% solvent B that increases to 25% solvent B over 50 min. Subsequently solvent B increases from 25 to 90% over 10 min at a flow rate of 0.3 µL/min.
    8. Acquire positive-ion electrospray mass spectral data using a capillary voltage set at 1900 V, the fragment ion set at 360 V, and the drying gas (nitrogen) set at 225 °C with a flow rate of 12.0 L/min.
    9. Collect spectral results over a mass range of 250-1700 (mass/charge; *m/z*) at a scan rate of 8 spectra/s. Collect MS/MS data over a range of 50-1700 *m/z* and a set isolation width of 1.3 atomic mass units. Select the top 20 most intense precursor ions for each MS scan for tandem MS with a 0.25 min active exclusion.

21. Protein identification
    1. Convert spectral data to a mass/charge data format using Agilent MassHunter Qualitative Analysis Software.
    2. Process data against the UniProt *Cicer arietinum* (chickpea) database, using SpectrumMill as the database search engine.
    3. Include search parameters such as a fragment mass error of 50 ppm, a parent mass error of 20 ppm, trypsin cleavage specificity, and carbamidomethyl as a fixed modification of cysteine.

Representative Results

Each can of chick peas is labeled to indicate the ingredients added during canning. Ingredients included water, chick peas, salt, and disodium ethylenediamine tetraacetic acid (EDTA). In addition, two cans were labeled as "may contain calcium chloride". Three distinct lining colours were observed; white, clear yellow and metallic (**Table 1**).

Brand code	Salt	Disodium EDTA	Calcium chloride	Can lining
A	+	–	–	White
B	+	–	–	White
C	+	+	–	Yellow
D	–	–	–	Metallic
E	+	+	–	White
F	+	+	–	Yellow
G	+	+	+/–	White
H	–	–	–	Yellow
I	+	+	–	Yellow
J	+	+	+/–	Yellow

Table 1: Additives and can lining.

Brands E and D had the highest (607 g) and lowest (567 g) total mass (chickpeas plus aquafaba). The coefficient of variation (CV) of total mass per can was just 2%. Brand H contained the highest chickpea mass (488 g). Brand J, with the lowest chickpea mass (335 g), was 31% lighter than brand H and had the lowest number of seeds (244) in each can (**Table 2**). Brand D contained the lowest juice volume in the can (110 mL) while the highest juice volume was observed for brand E (225 mL). The highest viscosity observed, for brand H (114.2 cP), was 4.3 to 20 times greater than observed for other brands (5.7-26.4 cP). A number of significant correlations were observed that might predict the usefulness of canned product for producing aquafaba. Chickpea fresh weight was negatively correlated with juice volume and positively correlated with juice viscosity. Whipping 100 mL of aquafaba uniformly increased the foam by about 5 fold (V_{f100} = 470) immediately after blending with a CV of just eight percent. Brands D, E and G produced the lowest amount of foam from 100 mL of aquafaba. Aquafaba viscosity was negatively correlated with total foam volume per can (V_{fcan}) (**Table 3**). Juice density was the least variable parameter CV of 4.6% while juice viscosity was the most variable 160%. The CV of peas per can was 22%.

Brand code	Can content (g)	Chickpea fwt (g)	Seeds/can	Juice volume (mL)	Juice density (kg/m^3)	Juice viscosity (cP)[1]	V_{f100} (mL)[2]	V_{fcan} (mL/can)[3]
A	588	392	454	170	1067	8.75 ± 0.27^{bc}	500	850
B	581	355	404	200	1100	8.34 ± 0.17^{abc}	500	1000
C	590	389	289	175	1112	10.50 ± 0.18^{c}	470	823
D	567	428	423	110	1180	26.41 ± 1.14^{e}	410	451
E	607	364	300	225	1066	6.21 ± 0.24^{ab}	405	911
F	602	364	304	220	1048	6.32 ± 0.10^{ab}	480	1056
G	598	349	280	220	1103	5.70 ± 0.13^{a}	430	946
H	595	488	429	125	1160	114.2 ± 4.29^{f}	500	625
I	599	385	323	200	1009	16.12 ± 0.64^{d}	500	1000
J	584	335	244	220	1109	5.79 ± 0.30^{a}	510	1122
Mean	591	385	345	186.5	1095.4	20.84	470	878.4
SD	12	44	74.72	41.17	50.83	33.44	40	204.63
CV[4]	2	12	21.66	22.07	4.64	160.48	8.5	23.29

[1]Each value is presented as the mean ± SD (n = 3). Values followed by different letters are significantly different ($p < 0.05$).

[2]Percent volume increase from whipping 100 mL of chickpea juice.

[3]Total foam volume per can

[4]Coefficient of variation (%)

Table 2: Quantitative values of chickpea can.

	Can content (g)	Chickpea fwt (g)	Seeds/ can	Juice volume (mL)	Juice density (kg/m^3)	Juice viscosity (cP)	V_{fcan} (mL/can)[1]
Can content (g)	1						
Chickpea fwt (g)	–0.172	1					
Seeds/can	–0.442	0.664**	1				
Juice volume (mL)	0.600	–0.878**	–0.739**	1			
Juice density (kg/m^3)	–0.647**	0.519	0.339	–0.705**	1		
Juice viscosity (cP)	–0.002	0.890**	0.474	–0.657**	0.512	1	
V_{fcan} (mL/can)[1]	0.483	–0.818**	–0.639**	0.927**	–0.728**	–0.568	1
[1]Total foam volume from a can of product.							
Note: There were no significant correlations relating V_{f100} to other parameters.							

Table 3: Correlation coefficient.

The functional properties of aquafaba from these 10 commercial products, especially foam volume and foam stability, varied significantly (**Figure 1**). The highest V_{fcan} occurred in brands B, F, I and J with volumes over 1,000 mL. The highest juice density and the lowest volume increase ratio were observed in brand D. Despite the uniform yield of foam per 100 mL of aquafaba in all materials the stability of the foam varied greatly. After 1 h liquid had separated from all products except from brand H (**Figure 1**). After an additional 14 h storage the foam was largely gone from all brands except D and H. Interestingly brands D and H had a number of distinguishing properties including: 1) the highest chickpea mass per can; 2) the lowest aquafaba yield per can; 3) the highest aquafaba density; and 4) the highest aquafaba viscosity. The labels on products D and H indicated that no additives were included other than water and chickpeas.

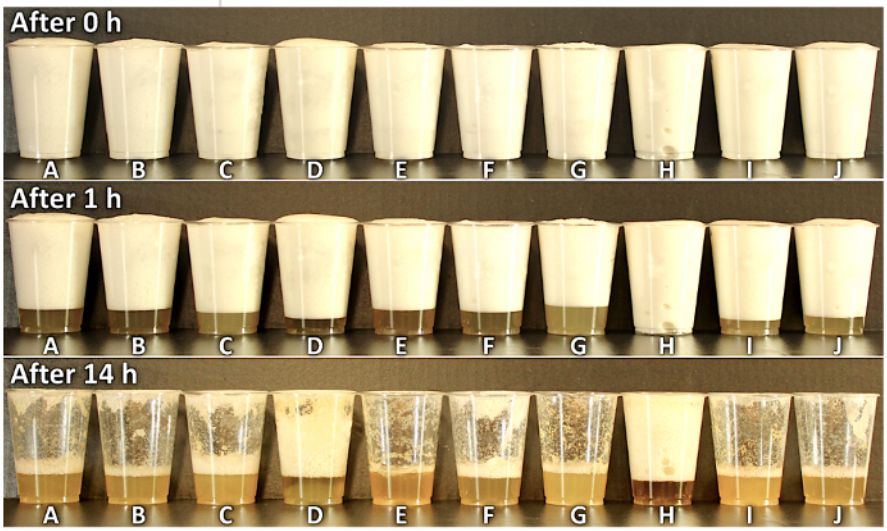


Figure 1: Aquafaba foam and juice from 10 commercial chickpeas. Please click here to view a larger version of this figure.

Chickpea seeds contained 63.2-69.9% moisture and 45.6-46.5% carbon (**Table 4**). The highest and lowest protein contents were observed in brands J (22.4%) and B (18.2%), respectively.

Brand code	Moisture (%)	Carbon (%)	Protein (%)
A	67.3 ± 0.2^{cd}	46.47 ± 0.01^{f}	19.04 ± 0.18^{b}
B	66.4 ± 0.4^{bc}	46.24 ± 0.00^{e}	18.24 ± 0.19^{a}
C	67.6 ± 0.2^{d}	45.73 ± 0.01^{b}	18.38 ± 0.07^{a}
D	69.9 ± 0.7^{e}	46.43 ± 0.02^{f}	21.99 ± 0.23^{f}
E	66.8 ± 0.5^{cd}	45.63 ± 0.04^{a}	20.71 ± 0.01^{d}
F	67.1 ± 0.2^{cd}	46.24 ± 0.00^{e}	22.05 ± 0.03^{fg}
G	63.2 ± 0.4^{a}	46.15 ± 0.01^{de}	19.53 ± 0.08^{c}
H	69.3 ± 0.3^{e}	46.49 ± 0.05^{f}	21.53 ± 0.28^{e}
I	66.9 ± 1.5^{cd}	46.09 ± 0.09^{cd}	19.82 ± 0.07^{c}
J	65.4 ± 0.8^{b}	46.04 ± 0.04^{c}	22.37 ± 0.11^{g}
Mean ± SD (CV)	67.0 ± 1.87 (2.79)	46.20 ± 0.29 (0.63)	20.40 ± 1.57 (7.70)
Each value is presented as the mean ± SD (n = 3). Values followed by different letters are significantly different ($p < 0.05$).			

Table 4: Composition of chickpea seed.

Brand E had the highest freeze dried mass (17.9 g) (**Table 5**). Aquafaba obtained from brand D had the highest protein (26.8%) and carbon (39.2%) contents and brand E contained the lowest protein content (23.3%).

Brand code	Freeze dried mass (g)	Moisture (%)	Carbon (%)	Protein (%)
A	12.4	94.39 ± 0.02^{bc}	35.46 ± 0.59^{de}	24.91 ± 0.55^{cd}
B	NA	94.06 ± 0.01^{abc}	ND	ND
C	15.7	94.41 ± 1.89^{bc}	35.09 ± 0.06^{cd}	22.65 ± 0.30^{a}
D	11	94.07 ± 0.47^{abc}	39.22 ± 0.02^{g}	26.83 ± 0.06^{e}
E	17.9	93.59 ± 0.01^{ab}	31.28 ± 0.12^{a}	23.36 ± 0.19^{b}
F	15.6	94.28 ± 0.02^{bc}	34.66 ± 0.06^{c}	24.61 ± 0.15^{cd}
G	12.7	94.70 ± 0.03^{bc}	33.78 ± 0.13^{b}	22.75 ± 0.19^{ab}
H	15.1	92.98 ± 0.00^{a}	37.30 ± 0.10^{f}	24.49 ± 0.26^{c}
I	16.3	93.63 ± 0.00^{ab}	35.85 ± 0.00^{e}	23.20 ± 0.02^{ab}
J	13.1	95.12 ± 0.00^{c}	34.77 ± 0.05^{c}	25.22 ± 0.44^{d}
Each value is presented as the mean ± SD (n = 3) except freeze dried mass. Values followed by different letters are significantly different ($p < 0.05$). NA = not available. ND =not determined				

Table 5: Composition of aquafaba.

Brand D had the highest *L* value which is associated to seed lightness followed by chickpeas of brand H, on the other hand, brand J had the lowest value which indicates that the seed is darker (**Table 6**). This could be related to increased values of cooking time and/or seed quality. Additionally, this might be associated with the physical quality of the resulting foam. It is evident that a lighter product is more desirable for use as a thickener.

Brand code	*L*	*a*	*b*
A	58.02 ± 0.96^{cd}	8.64 ± 0.95^{abc}	27.23 ± 1.14^{ab}
B	57.66 ± 1.92^{bcd}	8.66 ± 0.97^{abc}	24.84 ± 1.64^{a}
C	58.45 ± 0.93^{cde}	10.31 ± 0.70^{d}	29.62 ± 2.18^{b}
D	60.49 ± 0.55^{e}	7.95 ± 0.69^{a}	27.74 ± 0.87^{b}
E	55.65 ± 1.16^{ab}	9.92 ± 0.28^{cd}	27.39 ± 0.87^{ab}
F	57.25 ± 0.52^{bcd}	8.51 ± 0.58^{ab}	28.09 ± 1.16^{b}
G	59.02 ± 1.20^{de}	7.58 ± 0.34^{a}	28.80 ± 1.58^{b}
H	56.63 ± 1.78^{abc}	10.44 ± 0.57^{d}	26.77 ± 0.74^{ab}
I	56.25 ± 1.34^{abc}	9.71 ± 1.02^{bcd}	28.87 ± 2.51^{b}
J	54.94 ± 0.90^{a}	9.34 ± 0.09^{bcd}	28.29 ± 0.64^{b}
Each value is presented as the mean ± SD (n = 3). Values followed by different letters are significantly different ($p < 0.05$).			

Table 6: Color parameters of chickpea seed.

The protein contents in the supernatant increased when MWCO increased (**Table 7**). When a 3 kDa MWCO membrane was used for aquafaba filtration, no protein was found in the supernatant confirming that proteins present in the chickpea juice had MWs greater than 3 kDa. As membrane MWCO increased, some proteins were observed in the supernatant. The retentate after diafiltration was a gel-like pellet, therefore, it was dissolved in 0.02 M Tris HCl at pH 7.4 or PBS at pH 7.4 buffer.

Fraction	MWCO (kDa)		
	3	10	50
Supernatant	0.05 ± 0.00 g/L	0.17 ± 0.01 g/L	0.89 ± 0.01 g/L
Retentate[1]	0.88 ± 0.01 g/L	1.36 ± 0.00 g/L	0.82 ± 0.02 g/L
[1]The retentate was dissolved in 0.02 M Tris HCl at pH 7.4. Similar trend and results were obtained when PBS at pH 7.4 was used as solvent.			

Table 7: Protein content (g/L) of supernatant from filtering brand H juice using different MWCO.

Aquafaba from 10 commercially available chickpea products was analyzed using DPFSE-^{1}H-NMR. A typical annotated aquafaba ^{1}H-NMR spectrum is depicted in **Figure 2**. The resonances of constituents were assigned according to the previous publications[21]. A total of 20 compounds were identified including alcohols (isopropanol, ethanol, methanol), organic acids (lactic acid, acetic acid, succinic acid, citrate, formate, malate), sugars (glucose, sucrose), amino acids (alanine), and nucleosides (inosine, adenosine).

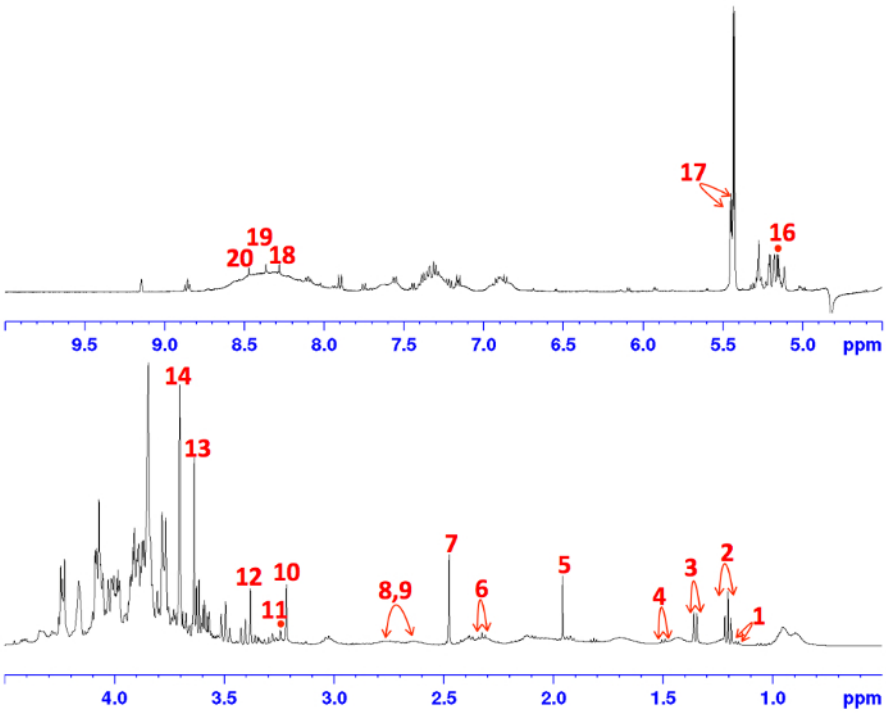


Figure 2: Representative ^{1}H-NMR Spectrum of the total region of aquafaba. 1, Isopropanol; 2, ethanol; 3, lactic acid; 4, alanine; 5, acetic acid; 6, glutamine; 7, succinic acid; 8, citrate; 9, malate; 10, choline; 11, phosphocholine; 12, methanol; 13, sucrose; 14, glucose; 15, β-glucose; 16, α-glucose; 17, sucrose; 18, inosine; 19, adenosine; 20, formate. Please click here to view a larger version of this figure.

Most of the ^{1}H-NMR spectra of aquafaba from commercial samples (except brands E, G and J) showed a triplet at 1.2 ppm from ethanol methyl group (**Figure 3**). Acetic acid fermentation in aquafaba can also be verified through acetate signal at 1.95 ppm. Aquafaba from brand F show high level of lactic acid (1.35 ppm), while aquafaba from A show high level of lactic acid and succinic acid (2.48 ppm). The singlet at 3.2 ppm in the ^{1}H-NMR spectra of all aquafaba investigated indicates the presence of choline. It is probable that ethanol, acetic acid and lactic acid are produced during steeping of the chickpeas prior to canning. Sucrose, choline and other molecules might arise from the chickpeas as normal metabolites.

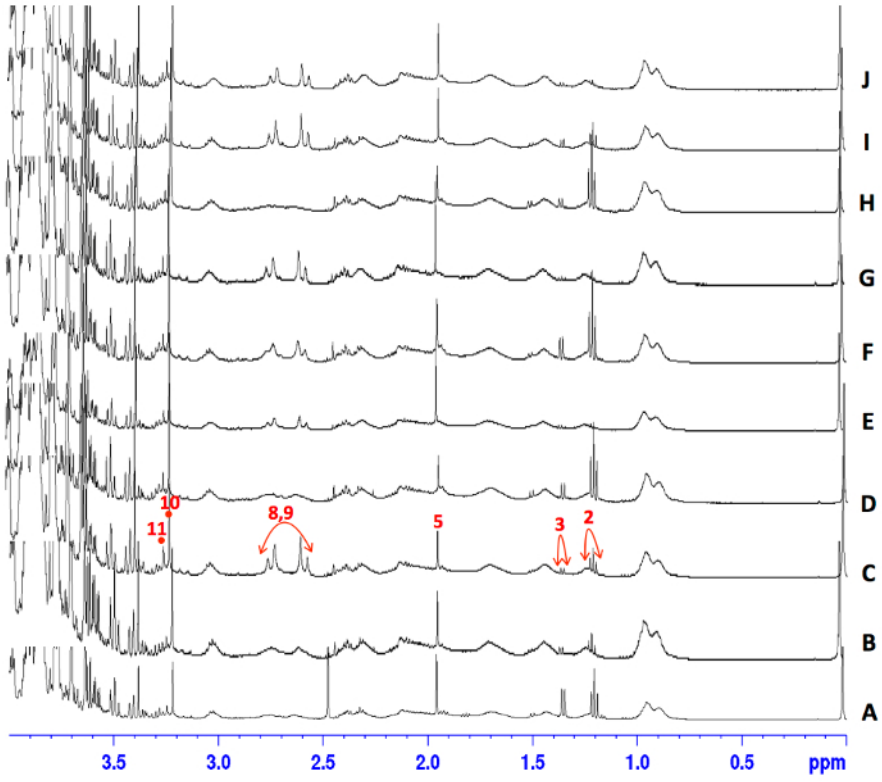


Figure 3: ^{1}H-NMR Spectrum of aquafaba from different commercial product. 2, Ethanol; 3, lactic acid; 5, acetic acid; 8, citrate; 9, malate; 10, choline; and 11, phosphocholine. Please click here to view a larger version of this figure.

To understand the foam composition, the ^{1}H-NMR spectrum of the liquid separated from the foam after 12 h from brand A was compared with the spectrum of the foam (**Figure 4**). Proton signals in the region (5.0-5.4 ppm) were highly enriched in the foam spectrum. The sucrose concentration (3.63 ppm) was greater in the foam than in the liquid. Volatile components such as methanol (3.40 ppm), ethanol (1.20 ppm), lactic acid (1.35 ppm), acetic acid (1.95 ppm) and succinic acid (2.48 ppm) decreased in the foam layer, likely due to evaporation. Proton signals of proteins (0.5-3.0 ppm, the protons of aliphatic amino acid side chain) are present in the foam.

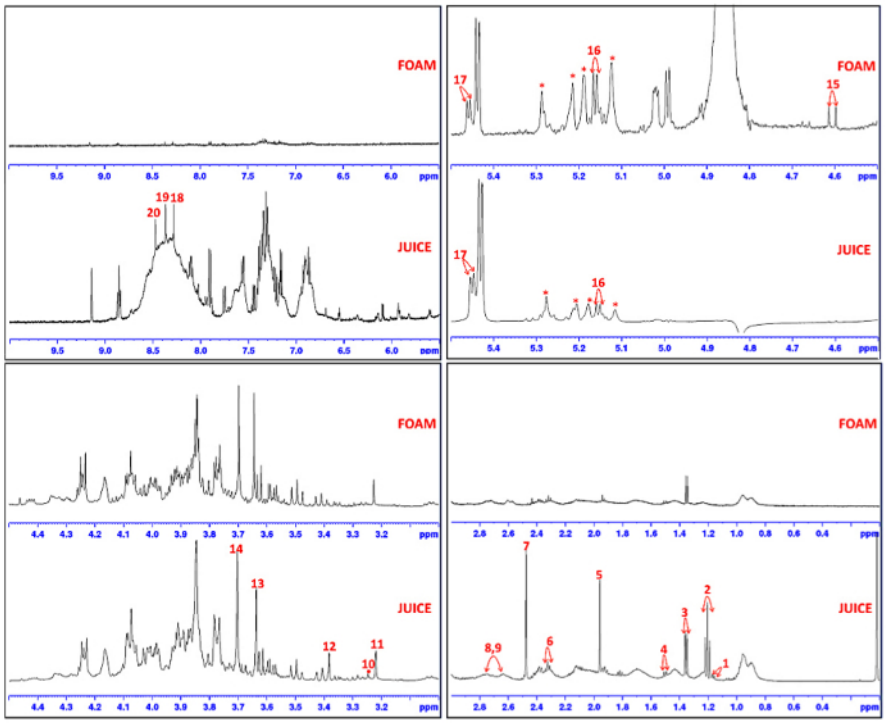


Figure 4: ^{1}H-NMR Spectrum of aquafaba foam and juice from brand A. 1, Isopropanol; 2, ethanol; 3, lactic acid; 4, alanine; 5, acetic acid; 6, glutamine; 7, succinic acid; 8, citrate; 9, malate; 10, choline; 11, phosphocholine; 12, methanol; 13, sucrose; 14, glucose; 15, β-glucose; 16, α-glucose; 17, sucrose; 18, inosine; 19, adenosine; 20, formate; and *, polysaccharide. Please click here to view a larger version of this figure.

The aquafaba juice from brand H was subjected to ultrafiltration using three different MWCO membranes and the ^{1}H spectra were compared (**Figure 5**). The 10 kDa membrane separated an apparent polynucleotide (6.5-8.5 ppm), while polysaccharides, contributing peaks of 5.0-5.2 ppm, were passed by the 50 kDa membrane. Peptide signals (0.5-2.5 ppm) were detected in the 3 kDa filtrate.

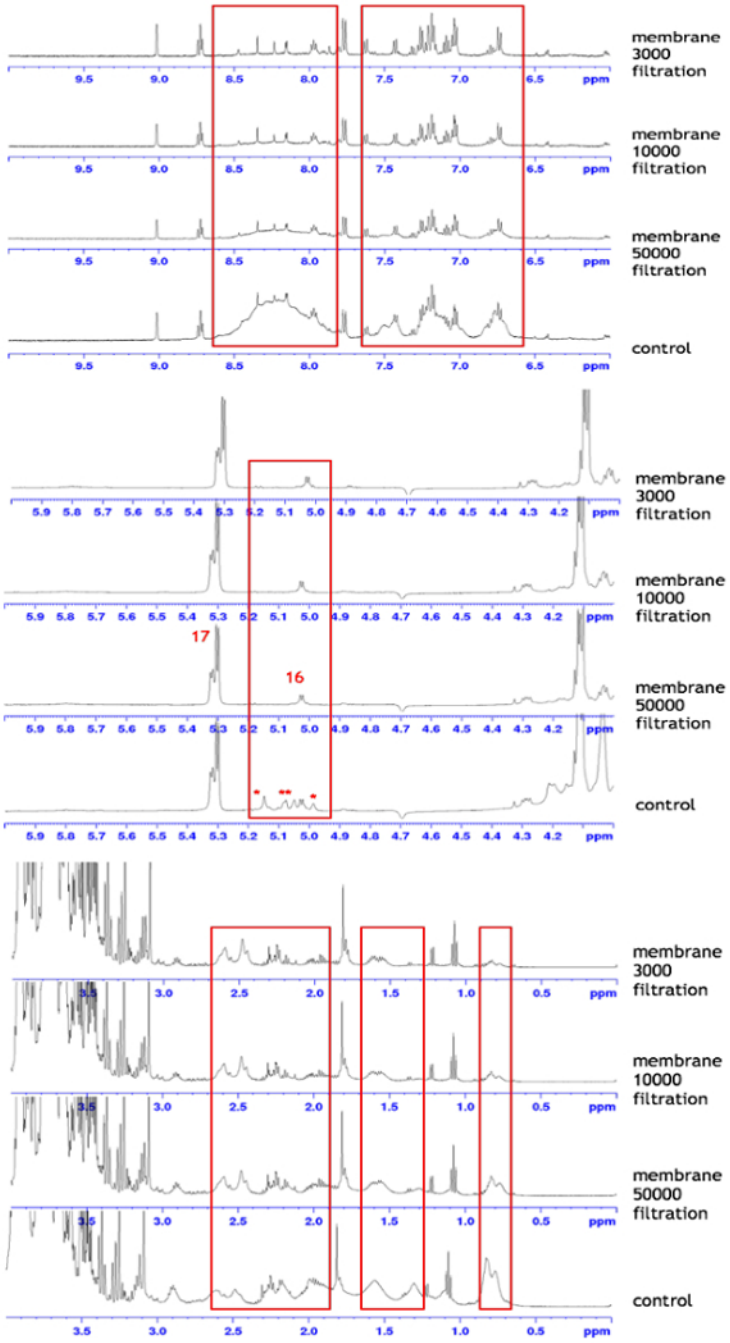
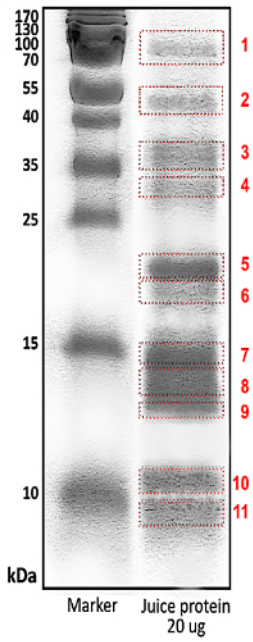


Figure 5: ^{1}H-NMR Spectrum of aquafaba subjected to membrane filtration. 16, α-Glucose; 17, sucrose; *, polysaccharide. Please click here to view a larger version of this figure.

Enrichment of proteins from product H, the one yielding the most stable foam by membrane filtration followed by SDS-PAGE of the retentate revealed clear bands of heat soluble proteins with MWs greater than 3 kDa (**Figure 6A**). Curiously, five identified protein bands appeared to contain proteins from the chickpea pathogenic fungus *Didymella rabiei*. Most of the other proteins belonged to known heat soluble proteins such as late embryogenesis abundant proteins and dehydrins (**Figure 6B**). Identified proteins also included heat shock protein, defensin, histone, non-specific lipid transfer protein and superoxide dismutase. Major storage proteins provicillin and leguminin were also present.

A



B

Gel	Protein name	MW(kDa)	Accession No.
1a	tRNA (Cytosine-5-)-methyltransferase	92.9	A0A163M953
1b	O-acyltransferase	92.1	A0A163IUM5
2a	Provicilin	51.5	Q304D4
2b	Legumin	56.7	Q9SMJ4
3a	Uncharacterized protein	36.0	A0A163HIE9
3b	Oxidoreductase	36.3	A0A163HIE9
4	Dehydrin 1	20.4	Q8GUS4
5	LEAP 2	16.7	O49817
6	LEAP 2	16.7	O49817
7a	LEAP 2	16.7	O49817
7b	LEAP 4	15.7	E7BSD7
7c	Superoxide dismutase [Cu-Zn]	15.3	Q9ZNQ4
7d	LEAP	13.2	A0A076KXB6
8a	LEAP	13.2	A0A076KXB6
8b	Superoxide dismutase [Cu-Zn]	15.3	Q9ZNQ4
8c	LEAP 4	15.7	E7BSD7
8d	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
8e	Histone H3	15.4	A0A163J7K4
8f	LEAP 2	16.7	O49817
8g	Histone H2B	14.9	A0A163DU27
8h	Histone H2AX	14.6	O65759
9a	LEAP	13.2	A0A076KXB6
9b	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
9c	Putative Pi starvation-induced protein	13.9	O65757
10a	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
10b	Non-specific lipid-transfer protein	13.8	A0A076KXC0
11	Defensin	8.8	Q2I2W0

Figure 6: (A) SDS-PAGE Separation of chickpea juice protein; (B) Potential protein identification per band. Five identified protein bands are highlighted. LEAP = Late embryogenesis abundant protein, HSP = Heat shock protein. Please click here to view a larger version of this figure.

Discussion

In this research, we have found that chickpea aquafaba from different commercial sources produces foams that vary in both properties (volume and stability of foam) and chemical composition. There was a positive correlation between aquafaba viscosity and moisture content. Foam volume increase (V_{f100}) was not related to these parameters. Additives such as salt and disodium EDTA might suppress viscosity and foam stability as aquafaba from chickpea canned with these additives had lower viscosity and produced foams with lower foam stability. This result differs from that of Behera *et al*. (2014)[22] where it was reported that micellar foam volume was reduced in the presence of salt and that foam collapse rate was slowed by salt. Chickpea aquafaba obtained from samples cooked with added salt also had higher moisture and lower carbon contents compared to aquafaba from chickpea canned with salt. Adding salt prior to cooking chickpea may have limited starch and protein swelling[23].

Protein separations by membrane filtration followed by SDS-PAGE and peptide mass fingerprinting showed that aquafaba proteins were largely known heat soluble hydrophilic species. Curiously, there was also evidence of tryptic fragments that suggested this material was contaminated with ascochyta blight. The NMR analysis revealed up to 20 small organic solutes including alcohols, acetate, lactic acid and succinic acid. These results appear to indicate that fermentation products have accumulated in the aquafaba. These compounds may have been produced by microorganisms during steeping of the seed prior to canning. Proton NMR spectra of the foam and juice showed that isoflavones and volatile components were present in the juice while the foam contained mainly polysaccharides, sucrose and protein. Also of interest the ^{1}H-NMR indicates the presence of nucleic acids (possibly DNA).

Clearly processes used in canning affected aquafaba properties and quality. It is possible that a consumer could identify better sources of aquafaba based on solution concentration. Primarily a source could be selected that was canned without added salt or EDTA. After a can is opened the consumer could measure the ratio of the mass of chickpeas and aquafaba to determine the concentration. However, a manufacturer could standardize processing conditions and produce standardized aquafaba as a commercial product.

In this research peptide mass fingerprinting and ^{1}H-NMR were used to analyze the composition of aquafaba. Peptide mass fingerprinting identified aquafaba proteins contributing to foaming properties. The thermostability of the proteins that were identified is well known. The technique was sufficiently discerning to identify the presence of proteins associated with seed fungal organisms. However, various factors might influence the results[24]. The critical steps are sample preparation, protein purification, separation prior to gel electrophoresis, trypsin digestion and mass search that leads to identification. Software database searches of tryptic fragments consider the presence of many peptide modifications including carbamylated lysine, oxidized methionine, pyroglutamic acid, deamidated asparagine, phosphorylated serine, threonine, and tyrosine as variable modifications. The validated hits from the two stage analysis are then searched using a semi-trypsin non-specific C-terminus, and semi-trypsin non-specific N-terminus. After the iterative analysis the validations are provided for peptide and proteins with a 1% false discovery rate.

Proton NMR was used to determine the presence of organic small molecules in aquafaba. Several compounds were identified by their spectra. Filtration and centrifugation of aquafaba samples was conducted to eliminate insoluble particles which can interfere with NMR peak shapes and lower sensitivity. In addition, solvent suppression was employed to improve the NMR detector sensitivity for molecules that overlap with the broad baseline caused by the strong water resonance.

Proton NMR is an established tool in quality control of food products. Compared to chromatographic methods such as GC/MS, LC/UV, and LC/MS, ^{1}H-NMR method is typically less sensitive. However, this method requires very little sample preparations and achieves rapid results and broad information in one measurement[25]. Where sufficient material is present ^{1}H-NMR is also a very reliable method for quantitative analysis and elucidation of chemical structure of unknown compounds.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This research was supported by the Institute of International Education's Scholar Rescue Fund (IIE-SRF).

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