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Aquafaba obtained from chickpea cooking: chemical characterization, standardization of use and viability in a recipe

Aquafaba proveniente da cocção do grão-de-bico (Cicer arietinum L.): características químicas, padronização do uso e aplicação culinária

Abstract

Introduction: Aquafaba is a by-product of chickpeas (*Cicer arietinum* L.) that may represent an interesting alternative for providing foam formation, at the same time as being a vegetal ingredient that has pleasant physicochemical and sensory characteristics in culinary vegan recipes. ***Objective***: Standardize the homemade process to obtain aquafaba, with the aim of using it in vegan recipes. ***Material and Methods***: After soaking chickpeas overnight, different proportions of chickpea and water (v/v) were used during the cooking process, in six experiments. The homemade aquafaba samples were analyzed with regard to foam formation and stability, and the results were compared to those of egg white, in order to determine the best proportion of grain:water for developing a vegan chocolate mousse. The samples of standard (egg white) and vegan (aquafaba) mousse went through an acceptability test using a 9-point structured hedonic scale (n= 95 untrained tasters). ***Results***: The foam obtained from egg white presented lower stability (p<0.05), while the aquafaba obtained from a 2:3 grain:water proportion formed foam more quickly, when compared to that obtained from other experiments. This same sample did not present any significant difference in foam formation and stability, compared to the other samples and was selected for the vegan mousse preparation, which did not differ in terms of flavor (p>0.05) and presented better means in the other features evaluated, when compared to standard mousse (p<0.05). ***Conclusion***: The homemade chickpea (*Cicer arietinum* L.) aquafaba obtained from a 2:3 proportion of grain:water (v/v) presented viability for culinary application in vegan mousse.

Keywords: Cicer. Food Technology Coadjuvants. Diet, Vegan.

Resumo

Introdução*:* A aquafaba, proveniente da cocção do grão-de-bico (*Cicer arietinum* L.), pode ser uma alternativa interessante para formação de espuma, sendo um ingrediente vegetal capaz de conferir características físico-químicas e sensoriais agradáveis a preparações culinárias veganas. ***Objetivo****:* Padronizar o processo de obtenção da aquafaba caseira visando aplicação em preparação culinária vegana. ***Material e Métodos***: Após remolho dos grãos, foram utilizadas diferentes proporções de grão e água (v:v) durante o cozimento, totalizando seis experimentos. As amostras de aquafaba caseira foram analisadas quanto à capacidade de formação e estabilidade de espuma e os resultados foram comparados à clara de ovo, visando escolher a melhor proporção grão:água para desenvolvimento de uma mousse de chocolate vegana. As amostras de mousse padrão (clara de ovo) e vegana (aquafaba) foram submetidas ao teste de aceitação utilizando escala hedônica de nove pontos (n = 95 provadores não treinados). ***Resultados:*** A espuma da clara de ovo apresentou menor estabilidade (p<0,05), sendo que a aquafaba obtida a partir da proporção 2:3 formou espuma em menor tempo comparado aos demais experimentos. Essa mesma amostra não apresentou diferença significativa na formação e estabilidade de espuma em relação às demais, sendo então utilizada para elaborar a mousse vegana, a qual não diferiu quanto ao sabor (p>0,05) e apresentou melhores médias dos demais atributos avaliados, comparada à mousse padrão (p<0,05). ***Conclusão:*** A aquafaba caseira de grão-de-bico (*Cicer arietinum L.)*, 2:3 (grão:água v/v) mostrou boa viabilidade para aplicação culinária em mousse vegana.

Palavras-chave: Grão-de-bico. Tecnologia de alimentos. Dieta vegana.

INTRODUCTION

During the last decade, different lifestyles have emerged, and the vegan diet has gained more popularity, especially amongst younger people, due to health and sustainability.[1] The main reasons for this decision are animal welfare, cultural beliefs, and environment and health concerns.[2]

A research study conducted by the Brazilian Public Opinion and Statistics Institute (IBOPE) in 2018,[3] cited by Hargreaves et al.,[4] reported that 14% of the Brazilian population declared themselves to be vegetarian, representing a total of 30 million Brazilians. There are still no studies that evaluate the exact number of vegans in Brazil; however, most of those interviewed in the study referenced reported that they would consume more vegan products if this information was present in the label (55%), or if they were similarly priced to other products (60%).[3] Thus, along with the increasing number of people with this lifestyle, the search for products that can substitute foods and animal ingredients has also increased. Replacing eggs in food preparations, for example, is a challenge, since this ingredient presents properties such as foam formation and an emulsifying capacity.[5] The foam produced by egg white can be used in meringues, cakes, sponge cakes and mousses.[6] According to Mustafa et al.,[5] some studies[7-9] have found replacers for eggs, such as emulsifiers and starch, but none of these presented foam formation capacity and stability.

Given the increasing adoption of the vegan lifestyle and the search for food replacers, plant-based proteins have aroused interest from big industries since the beginning of the XXI century.[10] These proteins are low-cost options with a nutritional equivalence comparable to those of animal-origin proteins and contain interesting technological functional properties[10] and characteristics, as observed for chickpea (*Cicer arietinum* L.).

Chickpea (*Cicer arietinum* L.) is the third most produced legume in the world, making up 20% of world farming.[11] This grain contains mostly carbohydrates (41-47%) and proteins (15-29%),[11] where these proteins promote water and oil soaking capacity, and can be used as an emulsifier agent and foamer.[12] The consumption of chickpea (*Cicer arietinum* L.) depends on it being cooked; as such, the cooking water derived from chickpea preparation results in a by-product known as aquafaba. Aquafaba was discovered in 2014 and represents a good egg replacer for cookery and has been utilized in food formulations, especially in vegan baking,[13] where it can provide foam formation and can be used in meringue and cake preparation, for instance.[14] Foam formation and foam stability are afforded by the amphiphilic behavior of proteins solubilized in water.[15] However, some variables can interfere in aquafaba foam formation, such as sugar addition and temperature.[16]

Despite its excellent application in cooking, there have only been a few studies about aquafaba, such as that by Buhl, Christensen & Hammershoj,[17] who reported on the aquafaba derived from canned chickpea (*Cicer arietinum L.*). In the study led by these authors, chickpea water presented 13g of proteins per liter. Recently, Meurer, Souza and Marczak[13] studied the effects of high intensity ultrasound on the functional and technological properties of chickpea (*Cicer arietinum* L.) cooking water and observed a dry basis of 17.79% protein.

Thus, the present study aimed to standardize the homemade process for obtaining aquafaba, aiming to apply this ingredient in a vegan chocolate mousse recipe to replace egg white. This recipe was then subjected to an acceptance purchase intention test.

MATERIAL AND METHODS

Raw material

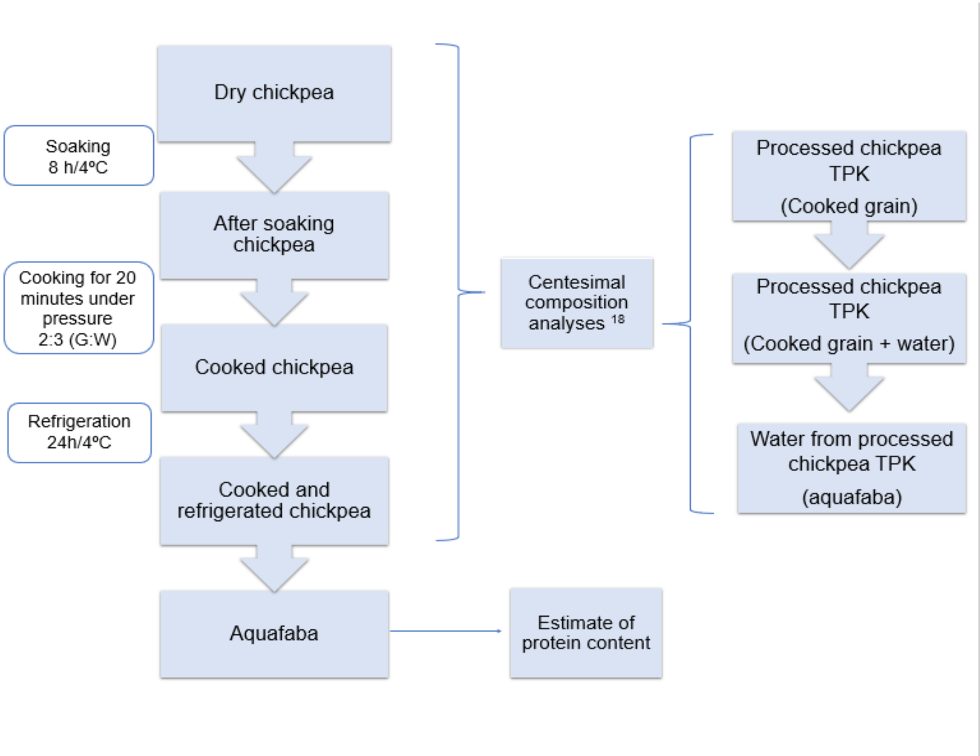
Processed chickpeas (Tetrapak®) and dry grains (*Cicer arietinum L.*) were kindly donated by Camil Alimentos S.A. (São Paulo, SP, Brazil). The remaining foodstuffs used in the production of vegan and standard chocolate mousses (containing pasteurized egg white - Fleisheggs®, Ohio, USA) were acquired in a wholesale supermarket chain, in the city of Limeira, SP, Brazil.

Centesimal Composition

Chemical composition analyses were performed to determine humidity, proteins, lipids, and ash concentrations, according to AOAC[18] methods, in the following samples: dry chickpeas (*Cicer arietinum L.*), chickpeas after soaking for eight hours, after cooking for 20 minutes; after refrigeration for 24 hours, and the processed chickpeas (Tetrapak® - grain and water, only grains and only water), as shown in Figure 1.

The analyses were performed in triplicate at the Biochemistry Laboratory of the School of Applied Sciences, Unicamp, Limeira, Brazil, and the results are expressed as means (± standard deviation). The protein content was determined using the micro-Kjeldahl method, multiplying the total nitrogen content by a factor of 5.75.[19] Results are presented as a dry basis as means ± SD (standard deviation).

Figure 1. Centesimal Composition flowchart



Standardization of the obtention process and evaluation of foam formation

To obtain homemade chickpea aquafaba (*Cicer arietinum L.*), the grains were subjected to a soaking process for eight hours, under refrigeration (4±1ºC), with deionized water, which was then discarded replaced with fresh water to cook the grains for 20 minutes. During the cooking stage, different proportions (in cups) of chickpea and water (v/v) were utilized (table 2) and the cooked grains were stored along with the cooking water for 24 hours (4±1ºC). The best proportion was selected based on its foaming capacity and stability results.

For the foaming test, an initial chickpea aquafaba volume of 200 mL (using the samples cooked in different water proportions) was beaten in a planetary mixer (Arno, modelo Deluxe SX80, Cajamar-SP, Brazil) at maximum speed for 30 minutes. Afterwards, the foam obtained was transferred to a graduated beaker to measure the final volume. After five hours, a visual appearance analysis of the foam was performed, along with the measurement of the foam volume and drained liquid using a graduated beaker. The percentage of liquid drainage was determined by the formula described below. The same procedure was conducted with 200 mL of pasteurized egg white (Fleisheggs®, Ohio, USA), which was the standard sample used for comparison. The foam formation and stability parameters were also evaluated when refined sugar was used in the process (table 3).

$$\%LD = Vdr/Vf \times 100$$

Where %LD represents the percentage of liquid drainage; Vdr the volume of drained liquid after 5 hours and Vf the foam volume at the end of 30 minutes of beating in a planetary mixer (initial time).

All the tests were performed in triplicate. Experiments were performed using different grain:water proportions, the addition of refined sugar, absence of soaking and absence of refrigeration of the cooked grain.

Culinary applications

Procedure for making the chocolate mousses

Following the tests performed to determine the best conditions for foam formation, two versions of chocolate mousse were developed, one containing aquafaba in the composition (vegan mousse) and another containing pasteurized egg white (standard mousse). The mousses were prepared using three cups of aquafaba or egg white foam, 40g of sugar and 125g of non-dairy chocolate.

Mousses were made by beating the aquafaba or pasteurized egg white in a planetary mixer (Arno, model Deluxe SX80, Cajamar-SP, Brazil), using maximum speed for 30 minutes, while the sugar was gradually added during the beating process. At the end of this step, the dark chocolate was melted and added to the foam obtained. The preparation was kept under refrigeration at 4±1°C to acquire an adequate texture.

Sensory analysis: Acceptance test

The sensory analysis of standard and vegan chocolate mousses was conducted after the approval of the Ethics Committee, Unicamp (UNICAMP protocol number: 3.181.067). The acceptance test was performed by 95 untrained volunteers tasters, who were men and women of over 18 years old that showed an interest in taking part in the study; all were students or employees of the School of Applied Sciences (FCA – UNICAMP), Limeira, Sao Paulo, Brazil. The tests occurred during the day, in individual cabins, at room temperature (24 ± 1°C) and in the Dietetic Technique Laboratory of the School of Applied Sciences. After signing a consent form agreeing to participate in the study, the volunteers received samples containing ± 25g of standard and vegan mousses in a monadic form, coded with three random digits, without knowing which ingredients were part of the sample composition. Water was provided during the time between each tasting. The tasters evaluated the products using a 9-point structured hedonic scale,[20] ranging from 1 (dislike extremely) to 9 (like extremely), for five different attributes (aroma, flavor, appearance, texture, and overall impression). On the same form, the tasters responded regarding their purchase intention for each product. Before starting each tasting, the volunteers received instructions from the researchers about how to perform the sensory evaluation of the samples and how to complete the analysis forms. The results for each attribute for each sample were expressed as means ± SD (standard deviation).

Statistical Analysis

The results obtained were tabulated using Microsoft Excel software and analyzed using Graph-Pad Prism (version 8.4) software. After testing data for normality (Shapiro-Wilk test), variance analyses (one-way ANOVA) and mean comparison test (Tukey) were performed for comparing foaming capacity and stability. A variance test (one-way ANOVA) and multiple comparisons (Dunnett) were used for the centesimal results, using the dry chickpea as standard reference. The Wilcoxon signed-rank test was used to analyze the results of the sensory analysis attributes. The significance level adopted was 5%.

RESULTS AND DISCUSSION

Centesimal composition analyses

The composition analyses of the different samples of chickpea (*Cicer arietinum* L.) are presented in Table 1. Statistically significant difference ($p<0.05$) could be seen between all the nutrients compared with dry chickpea, signifying that cooking reduces the macronutrients of the sample. Data found in this study are in accordance with those presented at the International Food Composition Table of the Department of Agriculture (US).[21]

Table 1. Centesimal composition of chickpea samples (CP), before and after cooking and refrigeration. Results calculated on a dry matter basis, as mean ± standard deviation (g/100g). Limeira, Brazil, 2020.

Samples (%)	Dry Chickpeas$	Chickpeas after 8 hours of soaking	Cooked Chickpeas	Cooked and Refrigerated Chickpeas	Canned Chickpea TPK# (grain)	Canned Chickpea TPK (grain + water)	Chickpea Water TPK
Moisture	10.99 a±0.64	50.49 b±0.30	74.32 b±0.89	70.17 b±0.68	67.20 b±1.3	72.71 b±0.08	89.76 b±0.04
Ashes	3.12 a±0.05	1.62 b±0.03	0.85 b±0.12	0.67 b±0.03	1.11 b±0.06	0.98 b±0.03	1.06 b±0.11
Proteins	18.40 a±0.22	10.31 b±0.76	8.71 b±0.11	7.01 b±0.16	5.67 b±0.19	4.52 b±0.18	1.69 b±0.04
Lipids	3.29 a±0.10	4.56 b±0.03	6.16 b±0.22	6.44 b±0.04	6.84 b±0.14	6.32 b±0.20	----
Carbohydrates	64.2	33.02	9.96	15.71	19.18	15.47	

a,bDifferent letters on the same lines show significant difference ($p<0.05$) in comparison to dry chickpea (Dry chickpea) by Dunett test; $CP: chickpea; #TPK: canned/ Tetrapak®.

Buhl, Christensen and Hammershoj[17] and Mustafa, Shim and Reaney[5] studied aquafaba obtained from canned chickpeas (*Cicer arietinum L.*) and found 1.3% and 1.5% protein contents, respectively. The results obtained in the present study were similar, where aquafaba presented a protein content of 1.7%. Pasteurized egg white, used as standard, presented a 12.56% protein content.

The percentage of protein solubilized in aquafaba was estimated as 3%, from the analysis of the centesimal composition data for chickpeas (*Cicer arietinum* L.), before and after cooking. Cooking legumes can modify the concentrations of nutrients such as soluble fiber,[22] carbohydrates[23] and proteins.[24] Therefore, we suggest that protein solubilization occurred, thereby contributing to the capacity of aquafaba to form foam. However, it was not possible to investigate other nutrients such as carbohydrates and/or fibers, which could also contribute to this process.

Standardization of the culinary process to obtain aquafaba

When performing tests with different proportions of grain:water (G:W) (table 2), the proportion of 2:2 + ½ (G:W v/v) + 24 hours of refrigeration (±4ºC) (experiment 7) resulted in an extremely diluted aquafaba that could not form foam in a food mixer. The same happened when chickpeas were not soaked for 8 hours (±4ºC) (experiment 8), since the chickpeas were burned in the cooking process and, as a consequence, the cooking water did not form foam. Additionally, refrigerating the grains, along with their water after cooking, for 24 hours (4±1ºC), was also found to be necessary for foam formation. Thus, experiment 3 was also excluded from analyses, since the lack of refrigeration did not provide foam formation by the aquafaba.

Table 2. Foam formation capacities of different test samples. Limeira, Brazil, 2020.

Experiment	1	2	3	4	5	6	7	8
Chickpea and water proportion (v/v)	2:3	2:4	2:3 SRef[$]	2:3 Aç[#]	-	-	2:2 1/2	2:3 SRem[£]
Experiment specification (quantity-g)	308g:612g	310g:810g	310g:612g	310g:612g	200g TPK[¶]	200g Clara de ovo	308g:562g	314g:612g

Foam formation and stability results were used as references to standardize the culinary process to obtain aquafaba (Table 3). With regard to the foam formation volume, a larger volume was observed ($p<0.05$) for the foam from canned chickpeas (TPK), when compared to egg white and the remaining samples. In addition, samples with different water proportions and with sugar addition did not present any differences between each other ($p>0.05$). The percentage of drained liquid did not present significant difference in any of the experiments ($p>0.05$), when samples were compared with each other, with the exception of egg white, which is less stable ($p<0.05$) and presented increased drained liquid after 5 hours. Thus, the proportion of 2:3 (Grain:Water v/v – experiment 1) was chosen as ideal, since it formed a consistent foam more rapidly during the beating process and did not present any difference from TPK, when the percentage of drained liquid was compared.

Table 3. Formation capacity (formed volume) and foam stability (volume and drained liquid percentage) of homemade aquafaba samples; aquafaba from canned/ Tetrapak® and egg white (initial volume=200mL). Limeira, Brazil, 2020.

Experiments	1	2	4	5	6
Volume of foam formed (mL)	$1136.70\pm1.18\text{x}10^{2}$ [a]	$1000.00\pm2\text{x}10^{2}$ [a]	$1333.30\pm4\text{x}10^{2}$ [a]	2000.00 ± 0.00 [b]	$967\pm1.52\text{x}10^{2}$ [a]
Volume of drained liquid (mL)	102.00±7.20 [b]	74.70±12.7 [b]	70.70±62.00 [b]	125.30±6.10 [a]	128.70±4.20 [a]
Drained Liquid (%)	8.97 [b]	7.47 [b]	5.30 [b]	6.27 [b]	13.20[a]

[a,b]Different letters on the same line indicate significant difference ($p<0,05$)

Egg proteins, especially albumin, demonstrate foam formation capacity;[25] due to their amphiphilic behavior, proteins can retain and encapsulate air, which can promote the formation and stability of whipped egg white.[15] As such, the use of pasteurized egg white, which contains 9.8% protein, presents adequate foam formation for the preparation of a mousse. In contrast, the foam obtained from canned chickpeas presented a larger foam volume formation, differing significantly from those of the other samples ($p<0.05$). Since it was not possible to observe any difference ($p>0.05$) in foam formation for the 2:3 (G:W v/v) proportion, when compared to egg white, we conclude that this whipped aquafaba preparation presents viability for replacing whipped egg in cooking recipes. According to Foegeding, Luck & Davis,[16] foam formation capacity in products that contain protein is improved by adding sugar. Accordingly, better foam stability was observed in experiment 4 (2G:3W) that evaluated sugar addition.

These results imply that the best procedure to obtain homemade aquafaba is by soaking the grains in water (8hs ± 4ºC), followed by cooking with 2 parts of chickpea and three parts water under pressure for 20 minutes, followed by refrigeration (± 4ºC) for 24 hours.

Although some studies[5,17] have suggested aquafaba to be an interesting substitute for egg white, due to its emulsifying and foaming properties,[17] and similar physicochemical properties in sponge cakes[5] for example, there are still no studies that have used standardization parameters to obtain aquafaba in order to analyze foam formation.

Sensory Analysis

The results from the mousse sensory analysis (n=95) are presented in Table 4. All samples demonstrated a good acceptance, since the mean of each attribute was above 7.0.[26] When comparing the sensory analysis results from vegan and standard mousse, it is possible to observe significant differences ($p<0.05$) between the characteristics of appearance, aroma, texture, and overall impression, where the aquafaba mousse received better evaluations than the standard mousse. No difference ($p>0.05$) was found between samples with regard to their flavor, indicating that aquafaba can act as an egg replacer and did not interfere in the flavor of the chocolate mousse.

With regard to the intention to buy, 85% of tasters would certainly or probably buy aquafaba mousse, while 84% would certainly or probably buy egg white mousse, reaffirming the viability of egg white replacement by aquafaba.

Table 4. Sensory analysis for the acceptance of chocolate mousses made with aquafaba or pasteurized egg white. Results are means ± standard deviation (n=95). Limeira, Brazil, 2020.

	Aquafaba mousse	Standard mousse	*p* Value
Appearance	8.19±0.89	7.72±1.23	*0.0005*
Aroma	8.07±1.12	7.46±1.44	*0.0023*
Flavor	7.99±1.13	8.08±1.00	0.6734
Texture	7.97±1.23	7.19±1.48	*<0.0001*
Overall impressions	8.06±0.93	7.76±0.93	*0.0080*

Consistent with the results of this study, Damian, Huo & Serventi,[27] found that the use of aquafaba from legumes (chickpea and dry yellow pea) in mousses did not change the results of the qualities; aroma, color, glow, consistency and sweetness. As such, the different types of aquafaba were suggested as interesting substitutes for egg white in mousses.[27] It is important to note that the methodology used to obtain aquafaba (homemade or canned) varies between studies, explaining the challenge in comparing results.

CONCLUSION

The standardization of the aquafaba preparation and obtention process demonstrated its viability for culinary application as a substitution for egg white to form foam. The development of a vegan chocolate mousse proved to be a good alternative for the application of this vegetal culinary ingredient, and the use of aquafaba may, therefore, contribute to diet diversity and sustainability, since the water from cooking of the grains is reused. Finally, further exploration of the homemade aquafaba composition is suggested, including the determination of its bioactive compounds for the discovery of new application and health possibilities, as well as expanding its applicability as a culinary ingredient.

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Contributors

Capitani CD participated in study conception, design, review and final approval.
Zaminelli CX and Landert MD participated equally in study conception and design, analysis, and interpretation.
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