Legume Aquafaba: Finding the Optimal Plant-Based Substitute for Egg White Foam

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Abstract

As increasing carbon emissions induce rising global temperatures, plant-based food products are being investigated as sustainable alternatives with less environmental detriment compared to animal products. For example, aquafaba, the cooking wastewater from legumes, functions as a substitute for egg whites due to its foaming properties. Therefore, the intent of this study was to determine the most effective legume source of aquafaba to maximize foaming potential as an alternative to egg whites. It was hypothesized that, if the legume source of aquafaba is related to foaming capacity, then egg white foam will display the greatest foaming capacity, followed by lima bean, green lentil, and chickpea aquafaba, because water-soluble proteins found in egg whites have superior film formation properties. Additionally, legumes contain chemical compounds called saponins which aid in lowering surface tension; lima beans contain the highest concentration, followed by lentils and chickpeas. The effect of the legume source of aquafaba on foaming capacity was determined by obtaining cooking water from chickpeas, lentils, and lima beans, and the foaming capacity of each experimental group was compared to that of egg whites as a control for desired foaming properties. The results collected demonstrated that egg whites had a mean foaming capacity of 401% , followed by lima bean aquafaba with a mean of 279%, lentil aquafaba with a mean of 271%, and chickpea aquafaba with a mean of 200%. The results showed that the hypothesis was supported as a result of varying protein compositions and saponin contents of the experimental groups tested.

Keywords: aquafaba, legume, egg whites, saponins, foaming capacity, water-soluble proteins

Introduction

Aquafaba, or the wastewater from cooked beans and legumes, has recently been discovered to function as an effective plant-based substitute for egg whites due to its foaming capabilities. As carbon emissions and global temperatures rise, the use of sustainable, plant-based alternatives for animal products is becoming increasingly relevant. By lessening the quantity of animal-based products in the diet of the global population, carbon emissions from the livestock cultivation sector would decrease, in addition to reducing fresh water usage from this specific industry (Hunnes, 2020). Compared to the cultivation of livestock for human consumption, plant-derived food sources provide a more sustainable alternative for the future.

This study aimed to investigate the foaming capabilities of different legume sources of aquafaba in an attempt to maximize foaming potential and determine an effective substitute for egg whites while still preserving foaming capacity. Due to the fact that the use of aquafaba as an egg white substitute is relatively recent development, a limited amount of research has been conducted investigating its substitory function. Moreover, the majority of published research regarding the functions of aquafaba utilizes wastewater from canned legumes rather than pressure cooked beans and solely investigates chickpea aquafaba. Therefore, the purpose of this study was, in addition to maximizing the foam yield of aquafaba, to determine alternative sources of aquafaba with potentially less detriment to the environment than chickpea production, and to expand the use of aquafaba as a more sustainable substitute to areas where chickpeas may be less prevalent.

The chemical compositions of legumes influence the foaming capabilities of legume aquafaba. Legumes contain different concentrations of chemical compounds which seep into the cooking water during the soaking and cooking process, including water-soluble proteins, polysaccharides, and surfactant agents called saponins which contribute to the foaming capabilities of aquafaba. (Alsaman et al., 2020). Saponins are amphipathic molecules composed of a lipid-soluble, hydrophobic aglycone molecule (a specific functional group covalently bonded to a glucose molecule) with water-soluble, hydrophilic polysaccharide chains attached. Their properties as surface active agents allow them to orient themselves such that the hydrophobic aglycones align towards air phase and hydrophilic polysaccharides towards water phase, disrupting hydrogen bonds between water molecules and causing the solution to foam. Additionally, saponins interact with denatured water-soluble proteins which concentrate at the air-water interface of the foam, further lowering surface tension as polar and nonpolar polypeptide chains align with the aqueous and nonaqueous phases respectively, and finally increasing foam stability and yield.

Furthermore, the chemical properties and composition of egg whites impart a significant influence on their foaming capacity. The combination of proteins which makes up egg whites is collectively called albumen, primarily composed of water-soluble ovalbumin protein. Albumen, the composition of proteins found in egg whites, acts as hydrophilic and hydrophobic groups during the foaming process due to polar and nonpolar nature of specific proteins. Hydrophilic protein groups orient towards the water phase, while hydrophobic proteins align towards the air phase, lowering surface tension at the air-water interface (M et al., 2018). During the beating process, air is introduced to the solution, and hydrophobic proteins facilitate adsorption, or rapidly encasing pockets of air within a thin, viscoelastic film of the aqueous phase at the interface. The protein molecules then partially unfold, a process known as surface denaturation, due to the force introduced by beating (Lomakina & Míková, 2006). Consequently, some of these partially denatured proteins lose their solubility due to disrupted hydrogen bonds and the exposure of nonpolar amino acids. The newly-insoluble proteins collect at the air-water interface to avoid interactions with water molecules, reducing surface tension. Additionally, partially-denatured protein molecules associate to form a film around air pockets as new interfaces are created, holding air in place, and forming a stable foam.

Prior to experimentation, it was hypothesized that, if the legume source of aquafaba affects its change in volume before and after foaming, and aquafaba has properties similar to that of egg whites in its protein, polysaccharide, and chemical composition and function that allow its use as a substitutory foaming agent, then egg whites will undergo the greatest change in volume after beating, followed by aquafaba sourced from lima beans, green lentils, and chickpeas. This will occur because, although legumes have similar compositions of water-soluble proteins which are vital to the foaming process, the collection of proteins in albumen, with superior foaming capabilities, are found exclusively in egg whites. The ability of albumen protein to form a cohesive film through intermolecular interactions and adsorb at the air-water interface confers significant foaming properties to egg whites (Lomakina & Míková, 2006). Contrastingly, legumes contain saponins, amphipathic chemical compounds with surfactant properties that allow them to reduce the surface tension at the air-water interface, increasing foaming capabilities, with lima beans containing the highest concentrations of these chemical compounds, followed by lentils and chickpeas respectively (Kregiel et al., 2017). If saponin content correlates with foaming capabilities, the hypothesis will be supported.

Methods

Experimental Design

The study was conducted by testing the foaming capacity of three experimental groups against the foam yield of the control group. The independent variable tested was the legume source of aquafaba, with different varieties of legumes serving as the levels of the independent variable. These experimental groups were chickpeas, lima beans, and green lentils. Egg whites served as a baseline control for optimal foaming capacity to which data acquired for the experimental groups was tested against. The dependent variable tested was the foaming capacity, measured as a percentage. Each level of the independent variable was tested five times, constituting five total trials in the experiment. Variables which remained constant throughout experimentation were the legume to water ratio (1:3.5), the amount of aquafaba in each sample tested, the duration of whisking each sample, the duration of cooling the aquafaba, and the device used to cook the legumes (addressed in the procedure section).

Materials

The materials used to conduct the experiment were as follows:

- 100 grams dried chickpeas (*Cicer arietinum*)
- 100 grams dried lima beans (*Phaseolus lunatus*)
- 100 grams dried green lentils (*Lens culinaris*)
- 5 grade AA large eggs
- 900 milliliters tap water (for soaking the legumes)
- 1,050 milliliters tap water (for cooking the legumes)
- Graduated cylinder
- Digital metric scale
- Stopwatch/timer
- Instant Pot® electric pressure cooker
- 4 small bowls for measuring beans and eggs
- 3 large bowls for storing aquafaba
- 4 medium bowls for eggs and strained aquafaba
- Wire sieve
- Fridge
- 3 600 mL capacity plastic containers with lids
- 20 250 mL capacity graduated beakers
- Small electric whisk with adjustable speed
- Heat resistant oven gloves

Procedure

1. 5 eggs were cracked into a medium bowl and the yolks were carefully separated from the whites using the hands. The yolks and shells were discarded.
2. 30 mLs of egg whites were measured using the graduated cylinder and the measured egg whites were placed into one of the beakers. This process was repeated until five total beakers were filled with 30 mLs of egg white each. Any additional egg white leftover was discarded.
3. The electric foaming whisk was plugged into an outlet. The end of the whisk was inserted into one of the filled beakers to submerge the whisk in the egg whites, and the whisk was turned on to the lowest power setting, immediately starting the stopwatch as the whisk was turned on.
4. After two minutes of whisking had passed, the power setting of the whisk was increased to medium speed. Whisking was continued until four minutes total had passed, or two additional minutes of whisking on medium speed. The whisk was turned off.
5. The volume of the egg white foam was observed as signified by markings on the side of the graduated beaker. The volume of the foam was recorded in mLs.
6. The volume change of the egg white foam was calculated by utilizing the formula $\frac{Vf-Vi}{Vi} \times 100$, where V_f equals the final volume of the foam in mLs, and V_i equals the initial volume of the egg white sample, 30 mLs (Buhl, Christensen, & Hammershøj). The value calculated was recorded as a percentage.
7. Steps three through six were repeated four more times with the four remaining samples of egg white for five total trials of the egg white foam group.
8. 100 grams of dried chickpeas were measured in a small bowl using the digital metric scale. The dried chickpeas were placed into a plastic container.
9. 300 mLs of tap water were measured with the graduated cylinder and poured into the plastic container over the dried chickpeas, ensuring all of the chickpeas were covered with water. The container was covered with a lid.

Fig. 1. Venting control knob turned to "sealing"

Fig. 2. Venting control knob turned to "venting"

Fig. 3. Depressurized inner pot, indicated by the dropping of the button next to the venting control

Fig. 4. Pressurized inner pot, indicated by the raised button next to the venting control

10. The chickpeas were left to soak for 12 hours.
11. After 12 hours had passed, the lid of the container was removed. The water was drained from the chickpeas using the wire sieve, ensuring all water was drained. The drained chickpeas were placed into the inner pot of the instant pot.
12. 350 mLs of tap water were measured using the graduated cylinder and poured into the cooking chamber of the instant pot over the chickpeas.
13. The instant pot was plugged into an outlet. It was ensured that the rubber ring had been inserted into the lid of the instant pot before properly closing it, and that the venting control knob had been turned to 'sealing' (see *Figure 1*).
14. The manual setting was selected and a cooking time of 25 minutes was entered. The beans were left to cook.
15. Once the instant pot timer ended, signalling the cooking had ended, the chickpeas were left inside the instant pot. The instant pot was allowed to depressurize completely, signaled by the dropping of the metal button next to the venting control (see *Figure 3*). Once this had happened, the control knob was moved to 'venting' (see *Figure 2*), while wearing the heat resistant glove. The lid of the instant pot was opened, being wary of steam.
16. The inner pot was removed from the instant pot, still wearing heat resistant gloves, and the chickpeas were left to cool for 30 minutes, uncovered.
17. After 30 minutes had passed, the chickpeas and water were poured from the instant pot inner pot into a large bowl.
18. The bowl of chickpeas and cooking water were placed into the fridge for five hours to cool completely. Once five hours had passed, the chickpeas and water were removed from the fridge.
19. The wire sieve was placed on top of a medium-sized bowl and the chickpeas and cooking water were poured through the sieve into the bowl. All water was allowed to drain from the chickpeas. The chickpeas were set aside.
20. 30 mLs of the cooled chickpea water were measured using the graduated cylinder and poured into one of the graduated beakers. This process was repeated until five total beakers were filled with 30 mLs of chickpea water each. Any additional chickpea water left over was discarded.
21. The electric foaming whisk was plugged into an outlet. The end of the whisk was inserted into one of the filled beakers to submerge the whisk in the egg whites, and the whisk was turned on to the lowest power setting, immediately starting the stopwatch as the whisk was turned on.
22. After two minutes of whisking had passed, the power setting of the whisk was increased to medium speed. Whisking was continued until four minutes total had passed, or two additional minutes of whisking on medium speed. The whisk was turned off.
23. The volume of the chickpea aquafaba foam was observed as signified by markings on the side of the graduated beaker. The volume of the foam was recorded in mLs.
24. The volume change of the chickpea aquafaba foam was calculated by utilizing the formula $\frac{Vf-Vi}{Vi} \times 100$, where V_f equals the final volume of the foam in mLs, and V_i equals the initial volume of the chickpea aquafaba sample, 30 mLs (Buhl, Christensen, & Hammershøj, 2019). The value calculated was recorded as a percentage.
25. Steps 21 through 24 were repeated four more times with the four remaining samples of chickpea water for five total trials of the chickpea aquafaba group.
26. Steps 8 through 25 were repeated two more times, using dried lima beans in place of the chickpeas the first time and dried green lentils the second time. When using lima beans, all steps were followed as detailed, with the exception that the lima beans were cooked in the instant pot for 15 minutes instead of 25. When using dried green lentils all steps were followed as detailed, with the exception that the lentils were not soaked, and instead, were cooked unsoaked. Additionally, the lentils were cooked in the instant pot for 20 minutes instead of 25.[1] This added to two additional experimental groups.

Risk and Safety

The use of the instant pot electric pressure cooker introduces a risk into the conduction of the experiment. In normal operational conditions, the likelihood of harm is small. Possible risks include burns from steam released from the pressure cooker or hot water. To minimize these risks, heat resistant gloves were worn and direct

1 Each legume was prepared in a manner to obtain aquafaba with optimal foaming capacity, based on previous research and trial and error testing prior to the conduction of the experiment. Due to the different chemical composition of each legume, different cooking and soaking durations are necessary to maximize foam yield accordingly.

contact with the venting control with bare skin was avoided. The cooling of the water within the instant pot for 30 minutes after cooking also minimized risk of burns. By following safety precautions and ensuring the conduction of the experiment was supervised, risks were minimized as much as possible. These precautions appropriately contained the risk. Operating a pressure cooker introduced the risk of contents of the pressure cooker exploding if opened too early. Likelihood of this risk is small; allowing pressure to depressurize naturally as detailed in the procedure contained the risk appropriately. Procedures regarding the operation of the instant pot were followed carefully in order to avoid risk of malfunction.

Caution was exercised when plugging the instant pot into the power outlet. The rubber ring was ensured to be inserted into the lid of the instant pot before closing it and beginning cooking to maintain a pressurized seal while cooking. The venting control was ensured to be moved to the 'sealing' setting before starting the cooking process. The instant pot was not opened until all pressure had been released, indicated by the dropping of the metal button next to the venting control, and the venting control knob was moved to 'venting' once this occurred. Caution was exercised when opening the instant pot and changing the venting control to 'venting'; a heat-resistant glove was worn to minimize risk of steam burns. Discretion was used when transferring and disposing of hot water; a heat-resistant glove was worn. The conduction of the experiment was supervised by the qualified designated supervisor to minimize the risk when operating the electric pressure cooker and ensuring its correct use. The supervisor ensured that all safety measures, precautions, and procedures were followed accordingly.

Data Analysis

The foaming capacity of each sample of aquafaba or egg whites was calculated using an equation from the study conducted by Buhl, Christensen, & Hammershøj (2019): $\frac{Vf-Vi}{Vi} \times 100$, where V_f is the final volume of the foam, in milliliters, and V_i is the initial volume of the foam. The values calculated were recorded as a percentage. The results were analyzed by determining the mean foaming capacity across all levels of the experiment and observing which experimental group displayed the greatest mean foaming capacity. The data for the foaming capacities of the three experimental groups (chickpeas, lima beans, and green lentils) was compared to that of the baseline control group (egg whites). This data was validated through three statistical t-tests, each comparing the data of one of the experimental groups to that of the control group. The P-values were analyzed to determine whether the null hypothesis was rejected or failed to be rejected.

Results

The results of the study illustrate the effect of legume source of aquafaba on foaming capacity. Egg whites, which served as a baseline control group, underwent the greatest change in volume after beating, with an mean of 401% foaming capacity, followed by lima bean aquafaba with a mean of 279%, then green lentil aquafaba with a mean of 271%, and finally chickpea aquafaba with a mean of 200% foaming capacity (see *Table 1*). There was little variation between the lima bean and lentil aquafaba groups across all five trials; additionally, they demonstrated the greatest mean foaming capacity out of all experimental groups tested. The chickpea aquafaba group demonstrated the lowest foaming capacity out of all experimental groups. During experimentation, the lima bean and green lentil aquafaba groups exhibited heightened foam stability in comparison with the chickpea aquafaba; these experimental groups remained in the foam state for a longer duration and did not revert to the aqueous state as rapidly.

The Effect of the Legume Source of Aquafaba on Foaming Capacity

Legume Source of Aquafaba (Type)	Foaming Capacity (%)					
	Trials					
	1	2	3	4	5	Mean
Chickpea	190	203	193	203	210	200
Lima Bean	283	277	290	273	273	279
Green Lentil	277	273	270	270	267	271
Egg White (Control)	397	403	407	393	403	401

Table 1. The Effect of the Legume Source of Aquafaba on Foaming Capacity

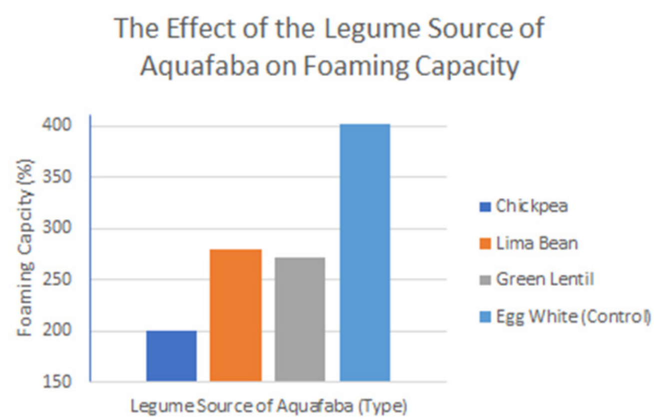

Fig. 5. The Effect of the Legume Source of Aquafaba on Foaming Capacity

Table 1 depicts the relationship between the legume source of aquafaba and foaming capacity, as a percent. The control group, egg whites, demonstrated the highest foaming capacity. Lima bean and green lentil aquafaba displayed very similar foaming capacities, although the average of lima bean aquafaba was slightly greater, and chickpea aquafaba demonstrated the lowest foaming capacity. *Figure 5* visually illustrates the average foaming capacities of the four levels of the independent variable. Furthermore, across all five trials of the experiment, the chickpea aquafaba group displayed the greatest variability in foaming capacity with a range of 20% between the minimum and maximum foam yields. The green lentil aquafaba group exhibited the least variation in foaming capacity out of all experimental groups and the control group tested, with a range of 10%, followed by egg whites with a range of 14%, and lima bean aquafaba, with a range of 17%.

Experimental Group	T-Test P-Value
Chickpea	5.83E-10
Lima Bean	5.11E-09
Green Lentil	8.40E-10

Table 2: T-Test Comparing the Control Group and Experimental Groups

Table 2 depicts the P-values obtained by performing three t-tests comparing the data from each experimental group against the control. The P-value represents the probability that the two data sets are statistically equivalent. The null hypothesis in this study states that the two means are equal. If the P-value is greater than the level of significance (0.01), the null hypothesis is failed to be rejected and the two data sets are concluded to be equivalent. If the P-value is less than the level of significance, the null hypothesis is rejected and the two data sets are concluded to be statistically unequal. The P-value comparing the lima bean group and control group was greatest, followed by that which compared the control to the green lentil group and finally to the chickpea group. Although the P-values obtained were less than the level of significance, the P-values of each t-test correspond with the data in *Table 1*, which compares the mean foaming capacities of each level of the independent variable.

Discussion

The results of the study demonstrated that egg whites, the control group underwent the greatest change in volume after beating, followed by lima bean, green lentil, and chickpea aquafaba. Data for lima bean aquafaba groups demonstrated little variation across all five trials and the greatest mean foaming capacity out of all experimental groups tested, an indication that lima bean aquafaba is the optimal substitute for egg whites in terms of foaming capacity. This conclusion was validated through three independent statistical t-tests, each comparing one of the three experimental groups with the control group. Because the P-value obtained from the t-test comparing the foaming capacities of the lima bean and egg white groups was the greatest, the data for the lima bean group has the highest probability of being similar to the control group out of the experimental groups tested. In other words, *Table 2* displays convincing evidence that, because the P-value comparing the lima bean and egg white groups is greatest, their means are the most likely to be significantly similar; lima bean aquafaba is the optimal substitute for egg whites amongst the legumes studied. This data corresponds with the conclusions drawn from *Table 1*.

However, because the P-values calculated in each of the three t-tests were less than the level of significance, 0.01, the null hypothesis, which states that the two data sets are statistically equivalent, was rejected. Therefore, the data for the foaming capacity of each of the experimental groups was concluded to be unequal to the data for the control group. For this reason, future experimentation could include research into the effect of different legume preparations on the foaming capacity of aquafaba, in order to optimize the foam yield of legume aquafaba so that its foaming capacity further approaches or reaches that of egg white foam. This research would allow for a one to one substitution of egg whites to aquafaba foam. Additional future research could also include investigating other legumes or plants with high saponin concentrations to be used in food foams, or continuing to research plant-based substitutes for animal products based on the chemical compositions of each.

The results of the experiment support the hypothesis; egg whites demonstrated the greatest mean foaming capacity, followed by lima bean aquafaba, green lentil aquafaba, and chickpea aquafaba. Significantly, the data collected indicates that the saponin content of legumes correlates positively with foaming capacity of legume aquafaba due to the significance of saponins in the foaming process. Mohan et al. (2016) report that dried chickpeas have a saponin content of 0.36 grams per kilogram and dried lima beans have a saponin content of 1.2 grams per kilogram. According to Caballero, Finglas, & Toldrá (2016), dried lentils have a saponin content of 1.1

Figures 6 - 9. Samples of each level of the independent variable, before and after beating.

Before *After*

Fig. 6. Chickpea aquafaba

Before *After*

Fig. 7. Lima bean aquafaba

Before *After*

Fig. 8. Green lentil aquafaba

Before *After*

Fig. 9. Egg White aquafaba

grams per kilogram of dried lentils, varying between different lentil varieties. This data correlates with the foaming capacity of aquafaba sourced from each legume type researched in this study; chickpeas have a significantly lower saponin content and demonstrated the lowest foaming capacity, while lima beans and lentils have similar saponin contents and displayed similar foaming capacities. To review, saponin content correlates with foaming capacity because saponins, as amphipathic, surfactant molecules, are able to orient themselves such that surface tension at the air-water interface is lowered, creating a foam. Soluble proteins also play a role in the foaming process by lowering surface tension, and egg albumen may contain proteins with superior adsorption and film-formation properties as an explanation for the significantly greater foaming capacity of the control group. Overall, the results of this study suggest that the saponin content of different legumes and the protein composition of egg whites influences foaming capacity.

The restricted scope of the research introduced limitations into the conduction of the experiment. Time limitations prevented further trial and error testing with different preparations of legumes prior to experimentation and additional trials conducted, which may have increased the accuracy of the data. Additionally, because the experiment was not conducted in a scientific environment, access to equipment was limited, and the specific chemical composition of each legume, or the exact saponin content of each could not be determined. Possible sources of error during experimentation include human error in calculation or measurement, in addition to the fact that further trial and error testing prior to the experiment may have exposed different optimal legume cooking methods. The data collected in this study contrasts with the results of a study conducted by Alsaman, et al. (2020), which reported an optimal foaming capacity of chickpea aquafaba to be 120%, with a chickpea to water ratio of 2:3. Discrepancies in findings may have arisen due to different chickpea sources and therefore chemical compositions, legume to water ratios (2:3 vs. 1:3.5) ,pressure cookers used (standard vs. electric), soaking durations (2 hours vs. 12 hours), cooking durations (30 minutes vs. 25 minutes), and beating durations (2 minutes vs. 4 minutes).

Based on the results of the experiment, it was concluded that, although the foam yield of the aquafaba tested was less than that of the control group, with further research, legume aquafaba can be used as a functional substitute for egg white foam and can be applied as a more sustainable plant based alternative. As carbon emissions and global temperatures rise, the use of plant-based alternatives, which generally have a lesser carbon footprint, for animal products has become increasingly relevant. Reduction in the consumption of animal products on a global scale would reduce the significant level of carbon emissions from this sector, as well as fresh water usage, lessening the carbon footprint of livestock cultivation food industries and their contribution to global warming. According to the USGS (2016), approximately 50 gallons of water are required to produce one chicken egg, with most of the water utilized for feeding the chickens. In contrast, approximately 40 gallons of water are required to produce 30 grams of lima beans, the amount utilized to produce aquafaba equivalent to one egg in this experiment. However, chickpea and lentil cultivation have a greater water footprint than chicken cultivation due to application of fertilizer, indicating the lima bean aquafaba is the optimal egg white substitute in terms of limiting fresh water usage amongst the legumes studied. Egg production has a relatively high carbon footprint of approximately 4.8 CO2e[2] per kilogram of eggs, while lima bean production has a carbon footprint of 1.14 CO2e per kilogram of lima beans, lentil production has a carbon footprint of 0.9 CO2e per kilogram of dried lentils, and chickpea production has an estimated carbon footprint of 0.64 CO2e per kilogram of dried chickpeas (Healabel, 2021). In terms of limiting carbon emissions, chickpeas are the most sustainable sources of aquafaba in place of egg whites. Finally, the use of aquafaba and other byproducts of agro-industrial food processing provides a means to reduce and reuse agro-industrial wastes in a resource overexploitation crisis, lessening human detriment to the environment.

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2 According to the Environmental Protection Agency (n.d.), CO2e is "the number of metric tons of CO2 emissions with the same global warming potential as one metric ton of another greenhouse gas," such as methane and nitrous oxide.

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