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RESEARCH PAPER

Freezing effect on Brazil nut shelf life

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Abstract

The Brazil nut (*Bertholletia excelsa* HBK) and its derivatives hold substantial significance in the global commercial market. This seed's edible part boasts high nutritional value despite being subject to rapid deterioration, a notable limiting factor. Recognizing the seed's economic importance beyond its dehydrated form, this study subjected frozen Brazil nuts to two distinct freezing techniques. The physicochemical properties and stability of these nuts were evaluated at 30-day intervals for a period of six months, and the samples underwent both rapid freezing (-25 °C) and slow freezing (-18 °C) techniques before storage. Visually, the frozen Brazil nut seeds closely resembled their fresh counterparts. In terms of acidity index and peroxide index results, both methods demonstrated high efficiency, maintaining acceptable levels of conservation within the limits established by Brazilian legislation throughout the storage period. Notably, the physicochemical analyses revealed a lack of significant difference between the two freezing methods ($p > 0.05$), which underscores the viability of employing either technique. These findings are instructive for the food industry as they provide a potential approach for producing frozen Brazil nuts without necessitating dehydration and, as a result, they cater to consumers desiring to enjoy the distinctive characteristics of this seed.

Keywords

Bertholletia excelsa: protein, lipid, tree nut

Introduction

The pursuit of healthier foods represents one of the prominent lifestyle needs of contemporary society. In an effort to mitigate the detrimental impacts of highly processed foods on health, the food industry has sought alternatives that reduce the quantity of preservatives and additives in food. The aim is to produce food items that closely resemble the sensory characteristics of natural foods. Given this context, the use of cold storage in food preservation is a longstanding technique, with freezing being widely employed in the food industry due to its ability to ensure more extended food preservation with minimal impacts on quality (Mulot et al. 2019).

Freezing is applied to a variety of food items, including vegetables, fruits, meat products, fish, and others, as a conservation or pre-treatment method. Additionally, the technique tends to maintain and preserve the properties of food, including nutritional composition, texture, and taste, which are criteria that consumers highly value (Li et al. 2018). Nevertheless, freezing as a treatment method is not commonly applied to heat-treated foods such as tree nuts, which are regarded as nutritious due to their high protein, fat content, and micronutrients such as potassium, copper, calcium, fiber, and vitamins (Gama et al. 2018).

Among tree nuts, the Brazil nut (*Bertholletia excelsa*)—a tropical seed indigenous to the Amazon region, also found in neighboring regions like Bolivia and Peru (Brito et al. 2019)—is noteworthy. It has a high protein (15%) and lipid content (71%) and on average, an exceptionally high selenium content (290.5 µg g^{-1}) (Stockler-Pintoet et al. 2015). Due to its remarkable nutritional composition, the consumption of Brazil nuts has been the subject of numerous studies, many of which have transcended the realm of food science, indicating broad applications in the health sector, including links to decreasing the risk of cardiovascular diseases.

Brazil nut production primarily relies on traditional plant extraction methods in the Amazon region, with little investment in technological development (Cardosoet al. 2017). The seed is collected by native or indigenous people of the region, and the commercialization of this food forms a critical source of income for many Amazon residents. However, being an activity performed by inhabitants of regions distant from major urban centers, these collectors face significant challenges, particularly in storing Brazil nut hedgehogs, leading to substantial product losses even at the harvest stage.

In many developed communities, cooperatives play a central role in the processing and sale of Brazil nuts, forming an essential part of the production chain due to their significant contribution to the economy and regional development (Krag et al. 2017). In fact, the Brazil nut is highly valued in the commodities market (IBGE 2019); its production is a lucrative activity, and the Brazilian state of Amazonas is particularly notable for being one of the largest producers. In 2019, Brazil nut production stood out among non-timber products, totaling approximately 4.5 thousand tons.

The climate has a significant role in Brazil nut production. The flowering of the Brazil nut tree is annual, long-lasting, and synchronous, typically spanning an average of six months during the season of lower rainfall (Tonini 2011). The trade and consumption of Brazil nuts are largely focused on the dried seed (the edible part), which can be consumed as a snack, used as raw material for other products, or included as a meal ingredient. Such products include oil, ingredients for cereal bars, biscuits, bread, and candies, among others (Brito et al. 2019). Despite this, there is a lack of data on the use of freezing to preserve Brazil nuts.

Aside from drying to extend the shelf life of food, freezing aims to decrease enzymatic activity and the amount of water within food items. This reduction can slow food deterioration reactions, thereby enhancing the shelf life of frozen foods (Zhu et al. 2020). Some research has been conducted on freezing tree nuts, particularly pecans (*Carya illinoinensis*), wherein samples were subjected to different freezing temperatures to reduce microorganisms (Brar et al. 2015). Nonetheless, the freezing of such nuts also presents potential drawbacks, as demonstrated in a study on frozen Portuguese chestnuts (*Castanea sativa*), as there were higher costs associated with energy requirements for frozen storage and processing losses compared to the use of fresh ones (Rosa et al. 2017). Given this context, there is still a considerable lack of studies on the freezing of Brazil nuts. Most food products require preservation techniques to delay degradation or maintain their natural characteristics over extended periods; therefore, cold-based strategies (e.g., freezing) are often utilized for this purpose (Palacz et al. 2021).

The food industry constantly changes to deliver products that closely mimic fresh food, and this is driven by both consumer demand and market needs. One of the industry's biggest challenges is ensuring food freshness throughout the production chain. Consequently, cooling and freezing processes are applied post-harvest to prolong food shelf life (Stebel et al. 2020). In light of this, the study aimed to validate Brazil nut seeds' stability and physiochemical characteristics using two freezing techniques over six months.

Materials and methods

Raw materials and processing

This study utilized commercially sourced medium-sized fresh Brazil nuts harvested in 2019/2020 from a local market in Manaus (3°08'27.5"S, 60°01'19.0"W), Manaus State, northern Brazil. A total of 4 kg of Brazil nuts in the shells was purchased. The samples were manually peeled and sanitized with a sodium hypochlorite solution (Nuclear, Brazil, P.A.®) with an active chlorine content of 2.0–2.5% w/w. After a soaking period of 15 minutes, they were rinsed with potable water to remove excess solution. These shelled seeds were vacuum packed in transparent, slotted low-density polyethylene bags, each weighing 300 g. Each sample was specifically numbered before undergoing different freezing methods (slow freezing and quick freezing).

Processing

Slow freezing

Post-sanitization and weighing, the vacuum-packed Brazil nuts underwent slow freezing in a vertical freezer (CVU18, Consul, Brazil). Each 300-g package was subjected to freezing temperatures of approximately -18 °C, protected from sunlight, and stored in the same freezer until analysis. The required freezing time was around 6 hours. In order to monitor the freezing process, temperature checks were performed every 30 minutes using an infrared thermometer (Scan Temp ST-600, Incoterm, Brazil). The stored frozen seeds were then subjected to physicochemical analysis every 30 days for 6 months.

Quick freezing

The same steps were repeated for quick freezing, with the application of the air freezing method in a quick-freezing tunnel. The nuts were frozen in a cold chamber (08C-DCP, Gallant, Brazil), achieving a freezing temperature of nearly -25 °C. The freezing process required approximately 2 hours. Temperature oversight was undertaken at 30-minute intervals using the same infrared thermometer. Each 300-g package, identified similarly to the slow-freezing samples, was then stored in a vertical freezer under -18 °C and protected from sunlight. These frozen nuts also underwent physicochemical analysis every 30 days for 6 months.

Shelled Brazil nut samples (frozen and *in natura* seeds) were ground in a food processor (Chef FPE11, Electrolux, Brazil) for 30 seconds. A pilot test was performed to determine the ideal drying time in a circulating air oven to attain maximum yield from cold pressing of the Brazil nut kernels. The test considered 24-, 48-, and 72-hour drying periods in a circulating air oven (Tecnal, Brazil), maintaining a steady temperature of 45 °C. Following each period, oil extractions from the Brazil nut samples were conducted utilizing the cold pressing method. After evaluating the test results, it was determined that the most effective drying parameters for achieving the highest extraction yield involved a drying temperature of 45 °C for 48 hours. Thus, this process was implemented for all frozen samples.

Physicochemical analyses

The nutrients, including moisture content (mc%), proteins, and lipids in the Brazil nut seeds were analyzed *in natura*. The characterization was carried out using water activity tests (Aw), acidity, and peroxide index tests every 30 days for 6 months, as outlined by AOAC (2016). In the frozen Brazil nut kernel samples, both freezing techniques were employed, and the same analyses were administered following the same period.

Statistical analyses

The Student t-test was utilized to assess the results of laboratory tests on samples tested in triplicate to determine whether the mean value found follows a normal distribution. Analysis of variance (ANOVA) was conducted to identify any significant differences between the means obtained at various stages of product storage. The statistical analysis was conducted using the Bioestat software (version 5.3).

Results and discussion

Sample description

After freezing and subsequent preservation at -18 °C, the samples remained stable during the 6 months of storage, as indicated by the analyses. Parts of the seeds directly in contact with the packaging appeared slightly more yellow than those not, and this discoloration may be attributed to the permeability of the packaging to the entry of small molecules, such as gases or water vapor, due to the variation in permeability across different packaging materials. Treatments, both thermal and mechanical, can influence this permeability. Additionally, the food's characteristics, such as pH or the high fat content present in Brazil nuts, may impact the barrier the packaging provides. This could result in a minor color changes in the Brazil nuts that were directly in contact with the packaging (Siracusa 2012). The statistical analysis did not indicate any significant difference ($p > 0.05$) for mc% and Aw, lipid content, acidity index, and peroxide index in the samples for both methods employed.

Sample freezing rate

Initially, the freezing method and temperature parameters were established in order to develop a profile of the freezing process, informed by prior research. These profiles began from the time and temperature at which the samples were placed in the freezer and ended when they achieved the predetermined freezing temperature. Temperature-time profiles were recorded commencing the freezing process, starting at 26 °C for the quick-freezing samples and 25.6 °C for the slow-freezing samples, with an end temperature of -25 and -18 °C, respectively. Figure 1 demonstrates the temperature behavior during the freezing process of the Brazil nuts.

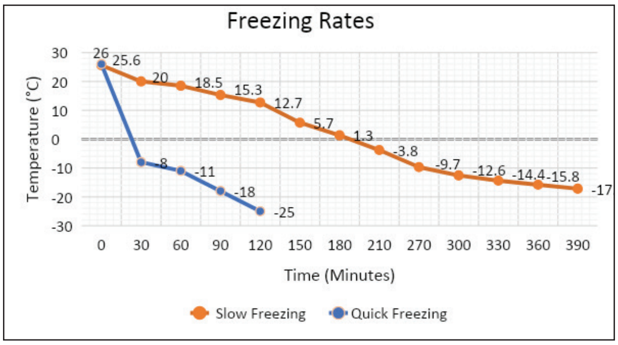

Figure 1. Brazil nut freezing rates in slow and quick methods.

It seems that the freezing method significantly influenced the freezing rate, given all samples had the same weight, a factor that could have otherwise caused variations in freezing time. For the quick-freezing process, the average cooling rate was faster, taking approximately 2 hours to reach a temperature of -25 °C. Conversely, the slow freezing process had a more gradual average cooling rate, taking roughly 6 hours to reach a temperature near -18 °C. One noteworthy observation in the final measurements of the slow freezing temperature is the decreasing speed of the freezer temperature; it appears to decline slowly in the last readings. One plausible explanation could relate to the point where the freezing threshold is being reached or the number of times equipment doors were opened for temperature readings. This approach offers a helpful method to evaluate the impact of the freezing rate on the stability and properties of Brazil nuts, laying a foundation for future research on freezing this food item. This information is beneficial for industries in their constant pursuit of new consumer markets, as frozen Brazil nuts represent a promising product and end users seeking effective food preservation methods aiming for products akin to fresh foods.

Nutrients

Protein content

The mean protein content for the *in natura* samples was 10.95% ± 0.13, and after 6 months of storage, 12.40% ± 0.28 for the final quick-freeze sample and 11.35% ± 0.10 for the final slow-freeze sample. Interestingly, the Brazil nut, from a nutritional point of view, contains 15–20% of protein content. However, these results differ from those found by Cardoso et al. (2017) in dried Brazil nuts for consumption, as the authors found protein content values of >15%, and Yang (2009), who reported 17% protein content in Brazil nuts. When comparing our data with other frozen products, the samples should ideally have protein content similar to Brazil nuts subjected to comparable processing; thus, animal meat is the most comparable product. Beef, for instance, comprises a mean of 16–22% protein (Soares et al. 2017), and variations may occur depending on the type of meat cut and geographical region. Another study compared freezing methods for pork leg muscle to obtain data on quality and oxidative stability (Truel et al. 2020). In the present study, air freezing was deployed in Brazil nuts freezing, and samples were stored in a conventional freezer at -30 °C, which did not significantly influence protein content. As for the Brazil nuts, the variation can be attributed to the inherent fluctuations of the analyzed samples.

Moisture content

The moisture content (mc%) in the *in natura* samples and after applying both methods displayed, in all instances, a mean of mc% >19%. The sample stored for 60 days had the highest values, at 28.77% for quick freezing and 25.53% for slow freezing. Most studies on tree nuts involve drying processes, including utilizing greenhouses, ovens, or even sun-drying methods. These procedures aim to decrease the mc% and water activity, creating a safer food storage environment. In a study conducted with freshly harvested macadamias, the samples were dried until reaching 1.5% mc%, deactivating any potential deterioration reactions (Buthelezi et al. 2021). Unlike this approach, our study chose to vacuum pack the fresh Brazil nuts to retain their freshness; thus, no drying techniques were employed. Hence, the mc% levels are expected to remain similar to those of the fresh sample. While the high mc% values of the frozen Brazil nuts could facilitate a favorable environment for microbial growth, their preservation at low temperatures (-18 °C) and the utilization of vacuum packing ensure safe and stable preservation throughout the entire storage period.

Water activity

The Aw values in the *in natura* samples and after applying two types of freezing are depicted in Fig. 2. The data indicates that the Aw levels in the untreated samples are above 0.7, exceeding the maximum prescribed level for Brazil nuts according to the Codex Alimentarius Commission (2010) to prevent fungal contamination that could potentially produce the carcinogenic contaminant aflatoxin. The lowest Aw values observed for both frozen samples were recorded at 30 days of storage, with values of 0.9839 for the quick freezing and 0.9801 for the slow freezing. Typically, drying operations are implemented to decrease the Aw in Brazil nuts to <0.7, promoting better preservation and more efficient control of aflatoxin-producing fungi.

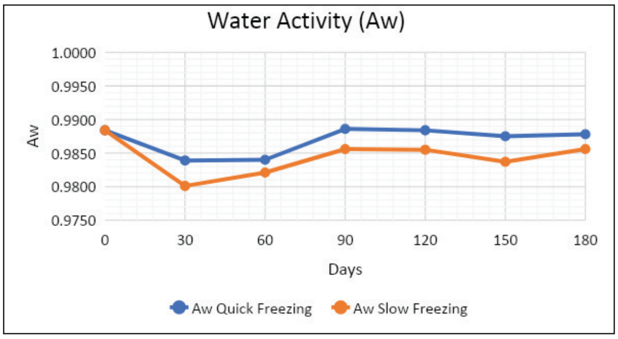

Figure 2. Brazil nuts Aw values: *in natura* and after freezing after 180 days of storage.

Evidence has shown that *Aspergillus* sp. growth is hindered at low temperatures, requiring an environment with temperatures above 20 °C for optimal growth (Ribeiro et al. 2021). Despite the Aw results indicating a suitable medium for microbial, chiefly fungal, growth, these microorganisms also necessitate ideal temperature conditions (25–37 °C) for development. In our study, the lower temperatures positively affected Brazil nut preservation by significantly inhibiting the growth of microorganisms involved in the degradation process.

Lipid content

The values from lipid analysis are presented in Fig. 3. A key characteristic of tree nuts, including Brazil nuts, is their abundant lipid content, offering numerous benefits to consumers. For instance, pecans (*Carya illinoinensis*) share a similar high lipid content (50–75%) with the Brazil nut and are also a source of monounsaturated and polyunsaturated unsaturated fatty acids (Ribeiro et al. 2020). The lipid content of the Brazil nut is typically 60–70% (Yang 2009), which is comparable with the 66.4% reported by Kluczkovski (2019). However, in this study, the highest lipid values recorded for both freezing methods occurred at 120 days of storage time: 30.37% for quick freezing and 34.29% for slow freezing. These findings are lower than

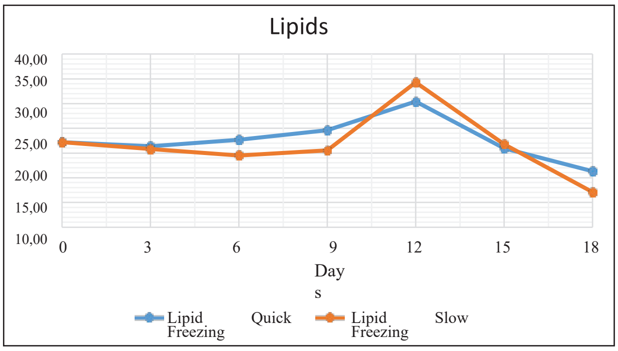

Figure 3. Lipids (%) in the samples of Brazil nuts in two types of freezing, for 180 days.

the levels previously reported for Brazil nuts, although it is unclear in earlier work whether the sampled nuts had undergone dehydration.

The process of drying seeds in a circulating air oven at 45 °C for 48 hours is critical for Brazil nut oil extraction. Drying is essential because fresh or frozen Brazil nuts with high moisture content do not yield sufficient oil through cold pressing due to poor adherence in the press and subsequent leakage. The resulting oil should ideally possess a light-yellow color and characteristic odor and flavor.

Another point that deserves attention is the need for the vacuum packaging of seeds to be opened and the seeds placed on trays for efficient drying; otherwise, a process known as "dripping," "drip loss," or simply "drip" may occur. This happens when water leaches out during defrosting due to the damage in the cellular structure that occurs during freezing. This phenomenon is undesirable to consumers and food facilities as it can result in the loss of nutrients and economic value. Furthermore, products that display dripping (Fig. 4) are generally inferior in sensory quality.

Figure 4. Defrosting process and appearance of the drip phenomenon.

Previous research indicates that defrosting methods reduce drip losses and lead to softer products. The conventional defrosting method tends to result in greater losses due to decreased capacity to reabsorb water from ice crystals formed during freezing (Bedane et al. 2018). Effective defrosting processes preserve fresh food attributes (Rahbari et al. 2018). Therefore, seeds were thawed in a circulating air oven at 45 °C for 48 hours outside the vacuum packaging to minimize drip. Arranging the kernels on trays allowed for a more uniform drying (Fig. 5), subsequently improving Brazil nut oil extraction by cold pressing. This study, however, did not evaluate the defrosting process.

Acidity index

The acidity index values of the frozen samples are illustrated in Figure 6. After 6 months of storage, the frozen Brazil nut samples that exhibited the highest acidity index values were those measured at 120 days for quick freezing with a recorded value of 0.9026 KOH/g and at 90 days

Figure 5. Arrangement of Brazil nuts on trays for the drying process.

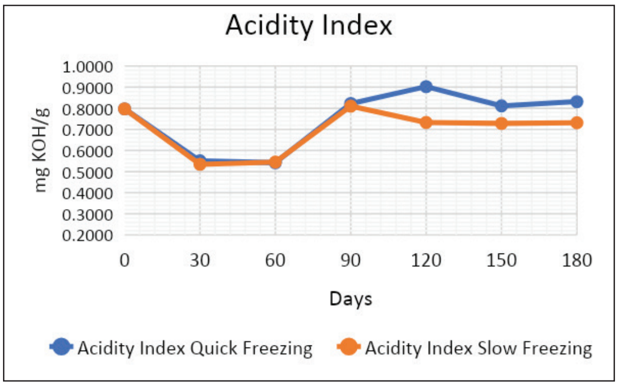

Figure 6. Acidity index (mg KOH/g) *in* Brazil nut samples of two types of freezing, for 180 days.

for slow freezing with a value of 0.8102 KOH/g. As per Brazilian legislation (Brasil 2021b), the maximum limit for the acidity index is 4.0 mg KOH/g for cold-pressed and unrefined oils. All samples fell within the specifications for the acidity index, with all observed results below 1.0 mg KOH/g.

Research conducted on pecans revealed that the lowest acidity indexes after six months of storage were seen in samples kept at 1.5 °C in 20 kPa pO_2, suggesting optimal conservation at lower temperatures (Ribeiro et al. 2020). The practice of freezing followed by preservation of the seeds at a temperature of -18 °C emerged as an effective procedure for maintaining the quality of the Brazil nuts, as the acidity index values remained below the maximum limit stipulated by international standards. Until 90 days of storage in this study, samples from both types of freezing exhibited similar characteristics, with minimal differences in later periods. This variation might potentially be linked to the inherent properties of the Brazil nut samples.

Peroxide index

The results for the peroxide index are illustrated in Fig. 7. Notably, unsaturated fatty acids present in tree nuts (e.g., Brazil nuts) are highly susceptible to deterioration when exposed to light, oxygen, heat, moisture, and post-harvest processing

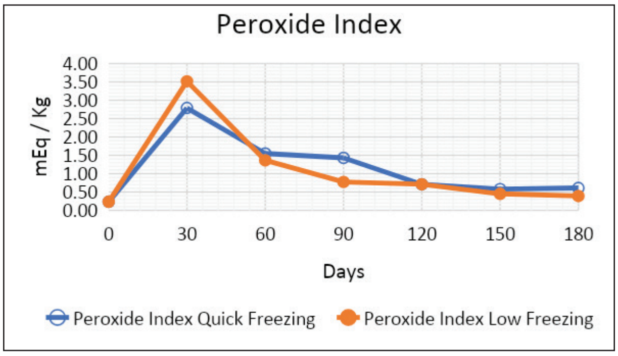

Figure 7. Peroxide index (mEq/Kg) in Brazil nut samples of two types of freezing, for 180 days.

(Bai et al. 2019). The oxidation of tree nut oil significantly accelerates the onset of rancidity, thus reducing the product's shelf life (Tahmasbian et al. 2021). Rancidity, a key indicator of poor nut quality, occurs in Brazil nuts in the same way it does in macadamia seeds. There are two main pathways to rancidity: oxidation and hydrolysis (Buthelezi et al. 2021).

When unsaturated oil interacts with air, it reacts with oxygen (enzymatically or non-enzymatically) during oxidation. This interaction alternately produces hydroperoxides, which react with proteins and amino acids, creating unpleasant odors and flavors (Bai et al. 2017). Conversely, hydrolysis results from the enzymatic breakdown of triacylglycerols due to hydrothermal activity and heat coupled with the release of free fatty acids. These free fatty acids act as catalysts for self-oxidation reactions, causing hydrolytic rancidity (Buthelezi et al. 2021).

After 30 days of storage, both frozen samples demonstrated the highest peroxide index of the entire storage period (2.79 mEq/Kg for quick freezing and 3.52 mEq/Kg for slow freezing), which is nearly 75% below the maximum limit established for the peroxide index as per Brazilian legislation (Brasil 2021a, 2021b), which sets the maximum index for cold-pressed and unrefined oils at 15 mEq/Kg. Intriguingly, the peroxide contents in all samples decreased after 30 days of storage. Therefore, the freezing process and subsequent oven heating at 45 °C for 48 hours did not negatively influence the oxidation levels of the Brazil nut samples.

Conclusion

Different freezing methods were applied to Brazil nuts and showed potential for Brazil nut preservation, achieving good stability, and yielding results within the required limits for key parameters such as acidity and peroxide indices. Hence, our findings lead us to conclude that freezing and subsequent low-temperature storage enhanced product stability over a 6-month storage period. Furthermore, vacuum packaging significantly extended the product's shelf life, corroborating previous studies that evaluated this packaging method. Additionally, there were no significant differences between the results for fast and slow freezing methods in the Brazil nut samples. This work provides valuable insights into the food industry and paves the way for innovative product development, such as frozen Brazil nuts.

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