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# Ozonation of Brazil nuts (*Bertholletia excelsa* H.B.K.): effect of column height on the saturation process and changes in quality

# Ozonização de castanha-do-Brasil (*Bertholletia excelsa* H.B.K.): efeito da altura da coluna no processo de saturação e alterações na qualidade

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## Highlights

Column height influences the ozone saturation process.
Increasing ozone concentrations reduced saturation time at different column heights.
Ozone did not change product quality to the point of rendering it unmarketable.

## Abstract

The objective of this study is to examine the saturation process in a column containing Brazil nuts and possible changes in the quality of the product. Brazil nut samples were initially placed in a cylindrical PVC column 15 cm in diameter and 110 cm in height. The ozone gas concentrations of 2.5, 4.5, 9.0, and 14.0 mg $L^{-1}$ and a flow rate of 3.0 L $min^{-1}$ were applied at a temperature of 25 ºC. Ozone gas was injected at the base of the cylindrical column, and the seed column height values adopted were 0.25, 0.50, and 0.75 m. Saturation concentration and time were determined. To measure possible changes in the quality of ozonized Brazil nuts, moisture and color, as well as qualitative variables of the crude oil were evaluated at the exposure times of 0, 3, 6, 9, and 12 h. To evaluate the quality of the crude oil extracted from ozonized nuts, the free fatty acid content, peroxide value, and iodine value were analyzed. Increasing ozonation times increased ozone concentration at all inlet gas concentrations. Saturation time decreased as the inlet gas concentration was increased, at the different product column heights. There was no change in product moisture in response to ozonation. Ozonation did not induce significant changes in color or in the crude oil, due to the triple interaction between column height, ozone concentration, and exposure time. In conclusion, the height of the product's column influences saturation time and concentration during the ozonation process. Considering the color of the product and characteristics of its crude oil, the use of

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ozone under the conditions adopted in the present study does not affect the quality of Brazil nuts to the point of rendering them unmarketable.
**Key words:** Ozone. Saturation time. Saturation concentration. Color. Crude oil.

## Resumo

O objetivo do presente trabalho é estudar o processo de saturação em coluna contendo castanha-do-Brasil e possíveis alterações na qualidade do produto. Inicialmente as amostras de castanha-do-Brasil foram acondicionadas em coluna cilíndrica de PVC de 15 cm de diâmetro e 110 cm de altura. Foram adotadas as concentrações do gás ozônio de 2,5, 4,5, 9,0 e 14,0 mg $L^{-1}$ e vazão de 3,0 L $min^{-1}$, na temperatura de 25 ºC. O gás ozônio foi injetado na base da coluna cilíndrica e os valores adotados de altura da coluna de grãos foram de 0,25, 0,50, e 0,75 m. Determinaram-se o tempo e a concentração de saturação. Na avaliação de possíveis alterações na qualidade de castanhas-do-Brasil ozonizadas foram determinados a umidade, coloração e variáveis qualitativas do óleo bruto, com tempos de exposição de 0, 3, 6, 9 e 12 h. Para avaliação da qualidade do óleo bruto extraído de castanhas ozonizadas foram analisadas o teor de ácidos graxos livres, o índice de peróxido e o índice de iodo. A elevação do período de ozonização promoveu aumento da concentração do ozônio para todas as concentrações de entrada do gás. No que se refere aos valores de tempo de saturação, à medida que se elevou a concentração de entrada do gás, houve redução do tempo de saturação, nas diferentes alturas de coluna do produto. Não houve variação da umidade do produto em decorrência da ozonização. A ozonização não provocou alterações significativas na cor e no óleo bruto, em decorrência da interação tripla entre altura da coluna do produto, concentração do ozônio e tempo de exposição. É possível concluir que a altura da coluna do produto influencia o tempo e a concentração de saturação, durante o processo de ozonização. O uso do ozônio nas condições adotadas no presente estudo não afeta a qualidade da castanhado-Brasil, considerando-se a cor do produto e características do óleo bruto, de tal forma a inviabilizar a comercialização.
**Palavras-chave:** Ozônio. Tempo de saturação. Concentração de saturação. Coloração. Óleo bruto.

## Introduction

The Brazil nut is a monotypic tree of the genus *Bertholletia,* family Lecythidaceae, that is native to the Amazon region. Its only species, *B. excelsa,* produces large, oil-rich seeds of biological and nutritional interest due to their high concentrations of selenium and other minerals (Lima et al., 2019).

Brazil nuts are the second most profitable extractive product from the Amazon region, only after açaí, with a production of 33,118 t (Instituto Brasileiro De Geografia e Estatística [IBGE], 2020). This product possesses high nutritional value and health benefits, making it a valuable export commodity (Cardoso et al., 2017). However, one of the main concerns related to the safety of Brazil nuts is contamination with aflatoxigenic fungi (Gallo et al., 2016). Aflatoxins induce high toxicity, both acute and chronic, and can cause cancer, with the liver being the most affected organ (Afsah-Hejri et al., 2020).

As the food industry grows and becomes increasingly competitive, it is important to develop top-quality products that are ready to be consumed with safety. One of the current focuses of research is the application of ozone, a non-thermal, residue-free technology capable of degrading mycotoxins in food (Sujayasree et al., 2022).

Thanks to its high oxidative potential, and mainly when used at high concentrations, ozone is able to generate radicals, affecting the structure of mycotoxin molecules. This can lead to changes in these substances, forming products of lower molecular weight and reduced toxicity, which implies detoxification of contaminated foods (Pandiselvam et al., 2019).

Because ozone is highly reactive when in contact with organic materials, a kinetic evaluation of the decomposition of the gas in a medium containing different plant products is required to determine parameters such as saturation concentration and time, as well as possible changes in product quality. Several studies can be found in the literature evaluating the ozone saturation process, but only one report addresses the saturation process in Brazil nuts (Oliveira et al., 2020). In said study, the authors used samples of 1.0 kg, thus not considering the product's column height, an essential variable for the application of the ozonation process.

In view of the above descriptions, this study was undertaken to evaluate the process of ozonation of Brazil nuts considering different combinations of inlet gas concentrations and product column heights, as well as to investigate possible changes in product color and in the quality of the crude oil.

## Material and Methods

The study was carried out at the Agricultural Product Pre-Processing and Storage and Food Analysis laboratories at the Faculty of Agriculture and Veterinary Medicine (FAV), University of Brasília (UnB).

### *Generation of ozone gas*

Ozone gas was obtained using an ozone generator based on the Dielectric Barrier Discharge method. In the ozone generation process, oxygen ($O_2$) was used as inlet gas, with a purity of approximately 90% and free of moisture, which was obtained from an oxygen concentrator attached to the ozone generator.

### *Ozone saturation process in a column containing Brazil nuts*

Initially, the Brazil nut samples were placed in a cylindrical PVC column 15 cm in diameter and 110 cm in height (Figure 1). To support the samples, a 1.4-mm-thick metal plate with 22.6% perforated area and 5.0-mm-diameter holes was installed at the base of the cylindrical column. The ozone gas concentrations of 2.5, 4.5, 9.0, and 14.0 mg $L^{-1}$ and flow rate of 3.0 L $min^{-1}$ were used at a temperature of 25 ºC. These ozone concentrations were applied considering the study developed by Oliveira et al. (2020), who analyzed the effect of ozone on *Aspergillus flavus* and on the quality of Brazil nut. Ozone gas was injected at the base of the cylindrical column and the seed column heights adopted were 0.25, 0.50, and 0.75 m (Figure 1). The residual concentration of ozone was determined after the passage of the gas through the cylindrical column containing Brazil nuts, adopting different seed column heights. Initially, the ozone saturation process was evaluated by quantifying the residual concentration, by the iodometric method, until it became constant. A thermal catalyst was used to degrade the residual ozone (Figure 1).

The ozone concentration was determined before and after the gas was passed through the product column by the iodometric method, described by Clescerl et al. (2000), by bubbling the gas containing ozone in 50 mL of 1N potassium iodide (KI) solution, producing iodine ($I_2$). To ensure that the reaction shifted towards the production of $I_2$, the solution was acidified with 2.5 mL of 1 N sulfuric acid ($H_2SO_4$), followed by titration with 0.01 N sodium thiosulphate ($Na_2S_2O_3$). A 1% starch solution was used as an indicator.

To relate the residual concentration of ozone gas with time, a sigmoidal equation was fitted to the obtained data (Equation 1):

$$C = \left[ \frac{a}{1 + e^{-(t-b)/c}} \right] \quad \text{Equation 1}$$

in which C = ozone gas concentration (mg $L^{-1}$); t = time (min); a, b, and c = constants of the equation.

Constants b and c were used to determine the saturation time for each inlet gas concentration (Equation 2) and, subsequently, to obtain the saturation concentration:

$$t_{Sat} = b + 2c \quad \text{Equation 2}$$

in which tSat = saturation time (min).

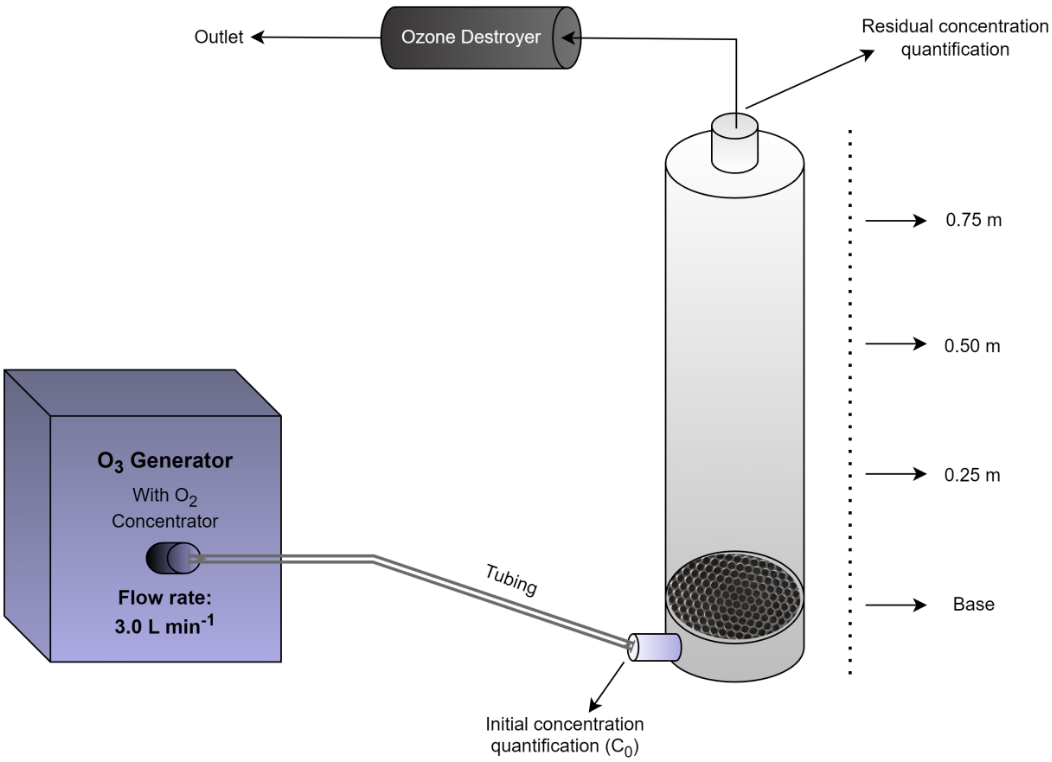


**Figure 1.** Experimental scheme for the ozonation of Brazil nuts.

### *Qualitative analyses of ozonized Brazil nuts*

This step was performed using the inlet concentrations of 2.5, 4.5, 9.0, and 14.0 mg $L^{-1}$ and flow rate of 3.0 L $min^{-1}$, at a temperature of 25 °C. The ozonation periods were 0 (non-ozonized), 3, 6, 9, and 12 h, and the maximum product column height was 0.75 m. Samples of 80 g of Brazil nuts were previously packed in organza bags and placed at 0, 0.25, 0.50, and 0.75 m in the cylindrical column (Figure 1). Three replications were used for each combination between ozone concentration, product column height, and exposure period. To assess possible alterations in the quality of ozonized Brazil nuts, moisture and color as well as qualitative variables of the crude oil were evaluated. To evaluate the quality of the crude oil extracted from ozonized nuts, the free fatty acid content and peroxide value were analyzed.

The moisture of the Brazil nuts was determined by drying in a forced-air oven at a temperature of 103±2 ºC to constant weight, according to ISO 665-2000 (United Nations Economic Commission for Europe [UNECE], 2000).

The color of the nuts was evaluated using the Colorquest XE spectrophotometer (HunterLab, Reston, United States), which defined the values of coordinates L* (lightness, measurement of intensity from black to white), a* (measurement of intensity from green to red), and b* (measurement of intensity from blue to yellow) of the Hunter system. Based on the a* and b* coordinates, it was possible to obtain the hue angle (h*) (Equation 1) and the saturation color or chroma C* (Equation 2) (Maskan, 2001).

$$h^* = \arctan (b^*/a^*) \quad (1)$$

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (2)$$

Lipid extraction for analysis of free fatty acid (FFA) content and peroxide value (PV) was performed by the method of Bligh and Dyer (1959). An aliquot of 2.5 g of ground samples was weighed and transferred to a 50-mL Falcon tube, to which 5.0 mL of chloroform, 10.0 mL of methanol, and 4.0 mL of distilled water were added. The mixture was homogenized on a rotary shaker for 30 min. An additional 5 mL of chloroform and 5 mL of 1.5% sodium sulfate solution were added. A new agitation was performed for 2 min, followed by centrifugation at 1,000 rpm for 2 min. Then, the upper methanol layer was pipetted and discarded. Next, the lower layer was collected and placed in a 50-mL tube. The sample collected in the lower layer was filtered through a funnel with qualitative filter paper. The filtrate was then collected in a 50-mL beaker, which was subsequently taken to an oven at 40 ºC for the evaporation of residual chloroform. This procedure was repeated eight times per replication, so it was possible to obtain sufficient oil mass for the analyses of quality.

The FFA content was determined according to American Oil Chemists Society [AOCS] (1993) Method Ca 5a-40, and expressed as a percentage of oleic acid. The peroxide value was determined as per AOCS (1993) Method Cd 8-53, with results expressed in mEq $kg^{-1}$.

### *Experimental design*

The experiment was laid out in a completely randomized design with three replications. For the saturation step, the experiment was carried out in a 4 × 4 factorial arrangement with four gas concentrations (2.5, 4.5, 9.0, and 13.5 mg $L^{-1}$) and four heights (0, 0.25, 0.50, and 0.75 m). To evaluate the effect on quality, a 4 × 4 × 5 factorial arrangement was adopted, which corresponded to four concentrations (2.5, 4.5, 9.0, and 13.5 mg $L^{-1}$), four heights (0, 0.25, 0.50, and 0.75 m), and five ozonation periods (0, 3, 6, 9, and 12 h). Analysis of variance was performed at 5% probability, followed by Tukey's test or regression analysis. StatPlus v.5 software (AnalystSoft Inc, Canada) was used for analysis of variance and Tukey's test, whereas SigmaPlot v.10 software (Systat Software Inc, Germany) was employed to obtain the regression equations and plot the graphs.

## Results and Discussion

### *Ozone saturation process in a column containing Brazil nuts*

Figure 2 illustrates the residual ozone concentration in Brazil nuts in different combinations of column heights (0, 0.25, 0.50, and 0.75 m) and inlet gas concentrations (2.5, 4.5, 9.0, and 13.5 mg $L^{-1}$). Based on the results observed during the saturation process, increasing ozonation times increased ozone concentration for all inlet gas concentrations. These results corroborate other studies (Roberto et al., 2016; Souza et al., 2018; Silva et al., 2019; Oliveira et al., 2020). The ozone transported through the seed mass is very reactive and acts in two steps. In principle, the gas reacts with active sites on the surface of the product, accelerating its degradation. Then, as the ozone moves through the seed mass, more active sites react with the gas, and the rate of degradation slows.

There was a significant effect of inlet gas concentration on saturation time, which was obtained from the equations described in Table 1. Overall, as the inlet gas concentration ($C_0$) was increased, saturation time decreased, at the different product column heights. An exception to this trend was observed at the column height of 0.25 m, in which the ozone concentration of 2.5 mg $L^{-1}$ resulted in a saturation time of 59.5 min. On the other hand, when the gas concentration of 4.5 mg $L^{-1}$ was used, saturation time was 65.1 min. The greatest difference between saturation times was found when comparing the values obtained for the concentrations of 2.5 mg $L^{-1}$ (65.5 min) and 13.5 mg $L^{-1}$ (26.5 min), when the product column height of 0.50 m was adopted. Saturation concentration ($C_{Sat}$) increased as the inlet gas concentration increased, and this trend was more pronounced at the product height of 0.50 m.

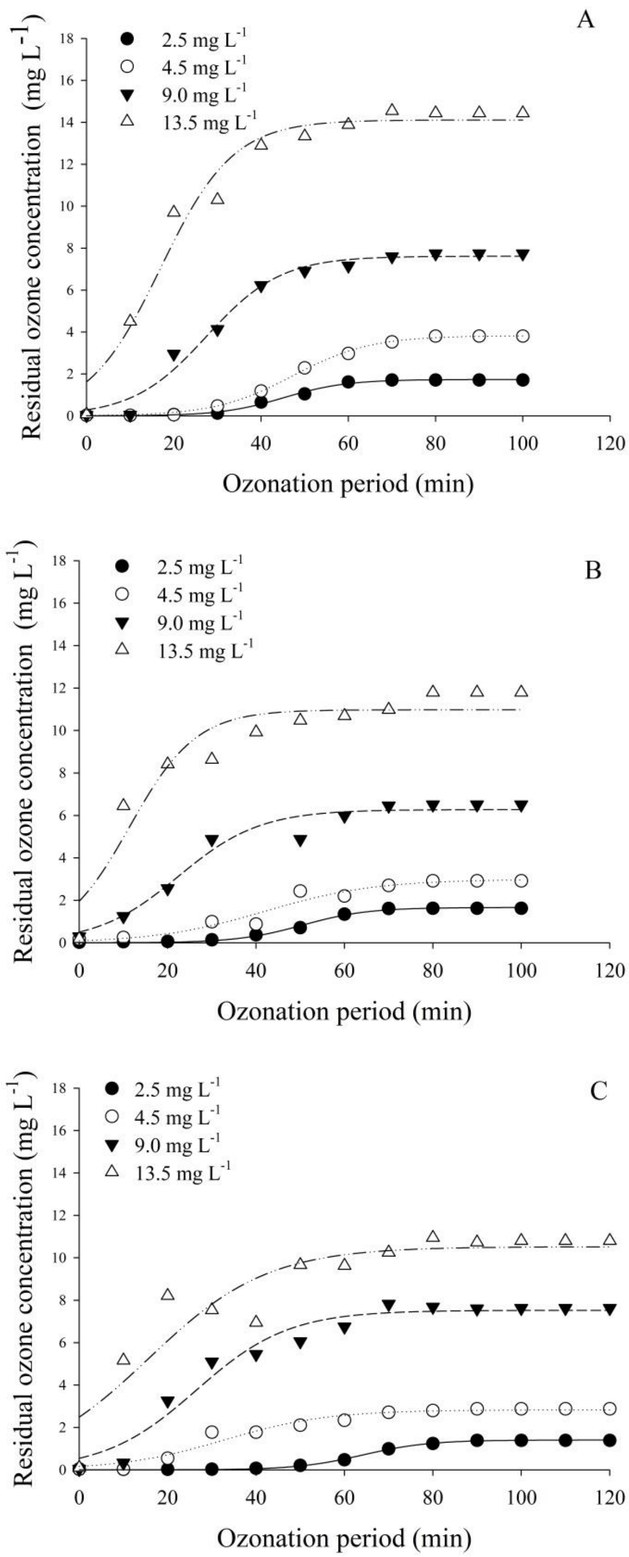


**Figure 2.** Residual ozone concentration in Brazil nuts at different combinations of column height (0.25 m – A; 0.50 m – B; 0.75 m – C) and inlet gas concentrations (2.5, 4.5, 9.0, and 13.5 mg $L^{-1}$) as a function of time.

**Table 1**
**Adjusted regression equations and respective coefficients of determination ($R^2$) for residual ozone concentration (mg $L^{-1}$) in different combinations of column height and inlet gas concentrations as a function of time**

| Column height (m) | Inlet concentration ($C_0$; mg $L^{-1}$) | Adjusted regression | $R^2$ | SEE | Saturation time ($t_{Sat}$, min) | Saturation concentration ($C_{Sat}$, mg $L^{-1}$) | $C_{Sat}/C_0$ |
|---|---|---|---|---|---|---|---|
| 0.25 | 2.5 | $\hat{y} = \frac{1.732}{1+e^{-\left(\frac{x-45.167}{7.180}\right)}}$ | 0.99 | 0.0615 | 59.5 | 1.52 | 0.61 |
| | 4.5 | $\hat{y} = \frac{3.824}{1+e^{-\left(\frac{x-47.265}{8.893}\right)}}$ | 0.99 | 0.0705 | 65.1 | 3.37 | 0.75 |
| | 9.0 | $\hat{y} = \frac{7.615}{1+e^{-\left(\frac{x-27.561}{8.485}\right)}}$ | 0.98 | 0.4319 | 44.5 | 6.70 | 0.74 |
| | 13.5 | $\hat{y} = \frac{14.111}{1+e^{-\left(\frac{x-16.982}{8.322}\right)}}$ | 0.97 | 0.9477 | 33.6 | 12.42 | 0.92 |
| 0.50 | 2.5 | $\hat{y} = \frac{1.660}{1+e^{-\left(\frac{x-50.454}{7.379}\right)}}$ | 0.99 | 0.0539 | 65.2 | 1.46 | 0.58 |
| | 4.5 | $\hat{y} = \frac{2.987}{1+e^{-\left(\frac{x-42.133}{12.396}\right)}}$ | 0.95 | 0.2828 | 66.9 | 2.58 | 0.57 |
| | 9.0 | $\hat{y} = \frac{6.273}{1+e^{-\left(\frac{x-22.462}{9.226}\right)}}$ | 0.96 | 0.5076 | 40.9 | 5.52 | 0.61 |
| | 13.5 | $\hat{y} = \frac{10.976}{1+e^{-\left(\frac{x-11.460}{7.507}\right)}}$ | 0.91 | 1.1468 | 26.5 | 9.67 | 0.72 |
| 0.75 | 2.5 | $\hat{y} = \frac{1.404}{1+e^{-\left(\frac{x-64.127}{7.476}\right)}}$ | 0.99 | 0.0284 | 79.1 | 1.23 | 0.49 |
| | 4.5 | $\hat{y} = \frac{2.828}{1+e^{-\left(\frac{x-32.850}{11.709}\right)}}$ | 0.96 | 0.2320 | 56.3 | 2.49 | 0.55 |
| | 9.0 | $\hat{y} = \frac{7.521}{1+e^{-\left(\frac{x-26.504}{10.408}\right)}}$ | 0.96 | 0.5776 | 47.3 | 6.63 | 0.74 |
| | 13.5 | $\hat{y} = \frac{10.520}{1+e^{-\left(\frac{x-15.746}{13.440}\right)}}$ | 0.86 | 1.2858 | 42.6 | 9.26 | 0.69 |

SEE – Standard Error of Estimate.

The highest $C_{Sat}/C_0$ ratio obtained was 0.92, when an inlet concentration of 13.5 mg $L^{-1}$ was used, at a product column height of 0.25 m (Table 1). On the other hand, the lowest value was 0.49, for the inlet concentration of 2.5 mg $L^{-1}$ and seed column height of 0.75 m. It is important to point out that the saturation time of 65.1 min, at an ozone concentration of 4.5 mg $L^{-1}$ and for a column height of 0.25 m, is associated with a $C_{Sat}/C_0$ ratio of 0.75. However, when the ozone concentration of 2.5 mg $L^{-1}$ was adopted, at the same product column height, the $C_{Sat}/C_0$ ratio was 0.61. We can therefore state that in studies on the ozone saturation process, variables such as saturation time and saturation concentration should not be analyzed separately, and the $C_{Sat}/C_0$ ratio should be considered.

Souza et al. (2018) indicated the possibility that in addition to the chemical composition of the seed, its physical properties may influence the saturation process of the porous medium. Oliveira et al. (2020) observed that as ozone concentration is increased, there is an exponential reduction in the saturation time of Brazil nuts.

### *Quality of ozonized Brazil nuts*

Despite a slight downward trend in the moisture of the ozonized product, this variation was not significant ($p>0.05$) according to analysis of variance when considering the triple or double interaction between the *inlet ozone concentration, product column height,* and exposure time factors, or the factors alone. Product moisture remained in the range between 2.30±0.10 and 3.70±0.20%.

Other studies have shown that ozonation does not significantly influence the moisture content of the ozonized product (Roberto et al., 2016; Afsah-Hejri et al., 2020; Silva et al., 2022). It is important to stress that the relative humidity during ozonation is crucial for maintaining the product's moisture and also for inactivating microorganisms. According to Luo et al. (2014), an increase in relative humidity implies an increase in the ozone oxidation potential.

The FFA content of the crude oil varied significantly ($p<0.05$) when only the inlet ozone concentration was considered alone. Despite this, no significant difference was found between the means ($p>0.05$) according to Tukey's test. The mean FFA values varied between 0.23±0.01 and 0.39±0.04%.

Peroxide value exhibited a significant variation ($p<0.05$) as a result of inlet ozone concentration and exposure time separately. However, there was no significant difference between the mean peroxide values at the different inlet gas concentrations, according to Tukey's test ($p>0.05$). Regarding the effect of time of exposure to ozone on PV, the variation was not significant, according to regression analysis (Table 2 and Figure 3A). It is noteworthy that the peroxide value of crude oil, whose limit as established by the Codex Alimentarius is 15 mEq $kg^{-1}$ (Food and Agriculture Organization of the United Nations [FAO], 1999), remained in the range between 6.87±0.76 and 9.15±1.00 mEq $kg^{-1}$. For Oliveira et al. (2020), this range of variation can be attributed to the initial quality of the raw material and should therefore not be attributed to the ozonation process.

The color of Brazil nuts was evaluated by determining the lightness (L*), color saturation or chroma (C*), and hue angle (h*) parameters. According to analysis of variance, lightness varied significantly ($p<0.05$) only according to inlet gas concentration and exposure time, when analyzed separately. On the other hand, color saturation varied significantly ($p<0.05$) in response to exposure time alone. Hue angle showed a significant variation due to the double interaction between inlet gas concentration and exposure time ($p<0.05$).

**Table 2**

**Regression equations for the peroxide value of crude oil (mEq kg$^{-1}$, A), lightness (L*, B), and color saturation (C*, C) in Brazil nuts as a function of ozonization period, regardless of inlet gas concentration and product column height, and respective coefficients of determination ($R^2$)**

| Variables | Adjusted regression | $R^2$ | SEE |
|---|---|---|---|
| Peroxide value of crude oil | $\hat{y} = 7.812$ | - | - |
| Lightness (L*) | $\hat{y} = \frac{77.967}{1+e^{-\left(\frac{x+2.401}{1.116}\right)}}$ | 0.99 | 0.121 |
| Color saturation (C*) | $\hat{y} = \frac{12.800}{1+e^{-\left(\frac{x+5.248}{2.411}\right)}}$ | 0.99 | 0.479 |

SEE – Standard Error of Estimate.

In terms of lightness, although analysis of variance indicated a significant variation due to inlet gas concentration, there was no significant difference between the mean values according to Tukey's test ($p>0.05$). Lightness displayed an upward trend as the ozonation period increased, with the most significant increase occurring in the first three hours of ozonation (Figure 3B and Table 2), with values remaining between 70 and 80. Color saturation exhibited a similar behavior, increasing with the time of exposure to ozone (Figure 3C and Table 2). Finally, hue angle (Figure 4 and Table 3) showed an increasing trend as the time of exposure to ozone increased, which was more pronounced at the inlet gas concentrations of 9.0 and 13 .5 mg L$^{-1}$.

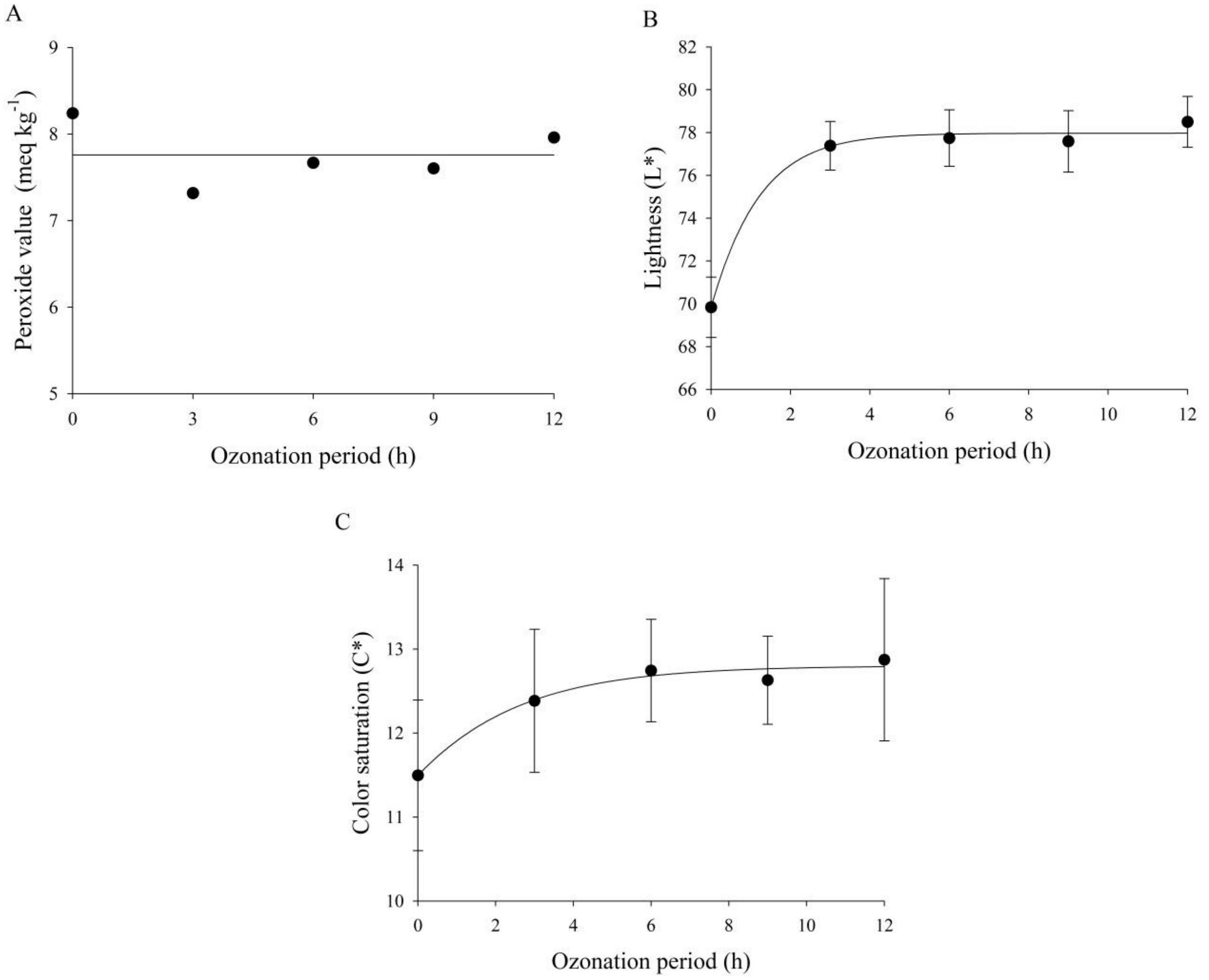


**Figure 3.** Regression curves for the peroxide value of crude oil (mEq $kg^{-1}$, A), lightness (L*, B) and color saturation (C*, C) in Brazil nuts as a function of ozonation period, regardless of inlet gas concentration and product column height.

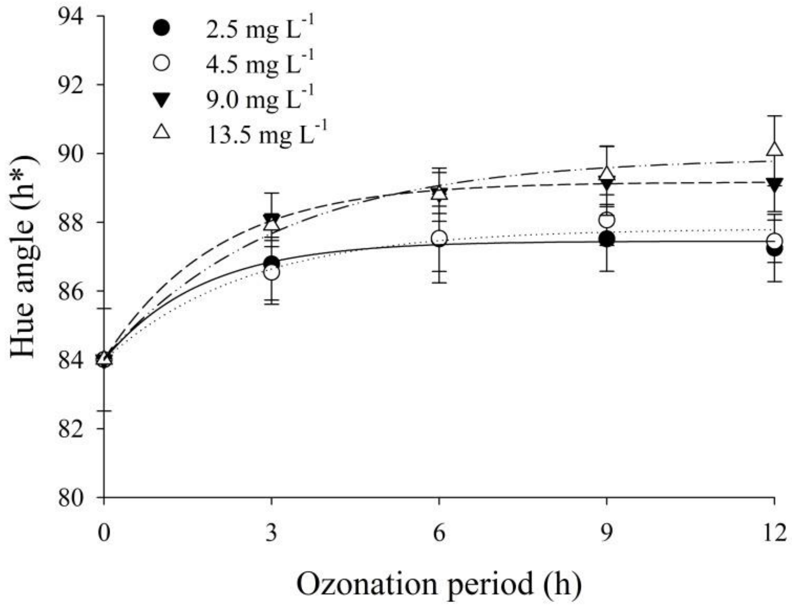


**Figure 4.** Regression curves for hue angle (h*) in Brazil nuts as a function of the ozonation period, at different inlet gas concentrations, regardless of product column height.

**Table 3**
**Regression equations for hue angle (h*) in Brazil nuts as a function of ozonation period and inlet gas concentrations, regardless of product column height, and respective coefficients of determination ($R^2$)**

| Inlet concentration ($C_0$; mg $L^{-1}$) | Adjusted regression | $R^2$ | SEE |
|---|---|---|---|
| 2.5 | $\hat{y} = \frac{87.450}{1+e^{-\left(\frac{x+5.383}{1.686}\right)}}$ | 0.99 | 0.198 |
| 4.5 | $\hat{y} = \frac{87.815}{1+e^{-\left(\frac{x+7.618}{2.467}\right)}}$ | 0.98 | 0.356 |
| 9.0 | $\hat{y} = \frac{89.168}{1+e^{-\left(\frac{x+5.319}{1.907}\right)}}$ | 0.99 | 0.084 |
| 13.5 | $\hat{y} = \frac{89.883}{1+e^{-\left(\frac{x+7.903}{2.962}\right)}}$ | 0.99 | 0.364 |

SEE – Standard Error of Estimate.

Other authors observed a variation in the color of Brazil nuts, considering L*, C*, and h*, in response to the ozonation process (Freitas-Silva et al., 2013; Oliveira et al., 2020; Ferreira et al., 2021; Silva et al., 2022). These alterations may be related to the oxidation of compounds in the dark brown skin that characteristically surrounds the nut. Alencar et al. (2011) and Sanchez et al. (2016) also described these alterations in peanut seeds. It should be noted, however, that these color changes possibly do not compromise the acceptance or consumption of the product. Future studies are warranted to investigate the sensory characteristics of ozonized Brazil nuts.

## Conclusions

The obtained results allow us to conclude that the height of the product's column influences saturation time and concentration during the ozonation process. The increase in inlet ozone concentration implies a reduction in saturation time, at the different heights of the Brazil nut column. The application of ozone under the adopted conditions does not affect the quality of the product to the point of rendering it unmarketable, considering its color parameter and free fatty acid content as well as the peroxide value of Brazil nut crude oil.

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## References

Afsah-Hejri, L., Hajeb, P., & Ehsani, R. J. (2020). Application of ozone for degradation of mycotoxins in food: a review. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 1777-1808. doi: 10.1111/1541-4337.12594

Alencar, E. R. D., Faroni, L. R., Martins, M. A., Costa, A. R. D., & Cecon, P. R. (2011). Decomposition kinetics of gaseous ozone in peanuts. *Engenharia Agrícola, 31*(5), 930-939. doi: 10.1590/S0100-69162011000500011

American Oil Chemists Society (1993). *Official methods and recommended practices* (4nd ed.). AOCS2v.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911-917. doi: 10.1139/o59-099

Cardoso, B. R., Duarte, G. B. S., Reis, B. Z., & Cozzolino, S. M. (2017. Brazil nuts: Nutritional composition, health benefits and safety aspects. *Food Research International, 100*(2), 9-18, doi: 10.1016/j.foodres.2017.08.036

Clescerl, L. S., Greenberg, A. E., & Eaton, A. D. (2000). *Standard methods for the examination of water and wastewater.* Denver.

Food and Agriculture Organization of the United Nations (1999). *Codex standard for edible fats and oils not covered by individual standards.* FAO. http://www.fao.org/docrep/004/y2774e/y2774e03.htm

Ferreira, W. F. D. S., Alencar, E. R. de, Blum, L. E. B., Ferreira, M. D. A., Mendonca,

M. A., Racanicci, A. M. C., & Urruchi, W. M. I. (2021). Ozonation of Brazil nuts in aqueous media at different ph levels: ozone decomposition, *Aspergillus flavus* inactivation, and effects on nut color and crude oil lipid profile. *Ozone: Science & Engineering, 43*(4), 351-362. doi: 10.1080/01919512.2020.1799189

Freitas-Silva, O., Morales-Valle, H., & Venâncio, A. (2013). Potential of aqueous ozone to control aflatoxigenic fungi in Brazil nuts. *International Scholarly Research Notices Biotechnology, 2013*(1), 859830. doi: 10.5402/2013/859830

Gallo, A., Solfrizzo, M., Epifani, F., Panzarini, G., & Perrone, G. (2016). Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Aspergillus flavus* on almond medium. *International Journal of Food Microbiology, 217*(1), 162-169. doi: 10.1016/j.ijfoodmicro.2015.10.026

Instituto Brasileiro de Geografia e Estatística (2020). *Produção extrativa vegetal - auantidade produzida na extração vegetal por tipo de produto extrativo.* https://www.ibge.gov.br/explica/producao-agropecuaria/castanha-do-para/br

Lima, L. W., Stonehouse, G. C., Walters, C., Mehdawi, A. F. E., Fakra, S. C., & Pilon-Smits, E. A. (2019). Selenium accumulation, speciation and localization in Brazil nuts (*Bertholletia excelsa* HBK). *Plants, 8*(8), 1-17. doi: 10.3390/plants8080289

Luo, X., Wang, R., Wang, L., Li, Y., Wang, Y., & Chen, Z. (2014). Detoxification of aflatoxin in corn flour by ozone. *Journal of the Science of Food and Agriculture, 94*(11), 2253-2258. doi: 10.1002/jsfa.6550

Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave drying. *Journal of Food Engineering, 48*(2), 169-175. doi: 10.1016/S0260-8774(00)00154-0

Oliveira, J. M., Alencar, E. R. de, Blum, L. E. B., Souza Ferreira, W. F. de, Botelho, S. D. C. C., Racanicci, A. M. C., & Silva, C. R. da. (2020). Ozonation of Brazil nuts: decomposition kinetics, control of *Aspergillus flavus* and the effect on color and on raw oil quality. *LWT LWT-Food Science and Technology, 123*, 109106. doi: 10.1016/j.lwt.2020.109106.

Pandiselvam, R., Subhashini, S., Banuu Priya, E. P., Kothakota, A., Ramesh, S. V., & Shahir, S. (2019). Ozone based food preservation: a promising green technology for enhanced food safety. *Ozone: Science & Engineering, 41*(1), 17-34. doi: 10.1080/01919512.2018.1490636

Roberto, M. D. A., Alencar, E. R. D., Ferreira, W. F. D. S., Mendonça, M. A., & Alves, H. (2016). Saturação do ozônio em coluna contendo grãos de amendoim e efeito na qualidade. *Brazilian Journal of Food Technology, 19*(1), 1-8. doi: 10.1590/1981-6723.5115

Sanchez, B. A. O., Alencar, E. R. de, Oliveira Pineli, L. D. L. de, Souza Ferreira, W. F. de, & Almeida Roberto, M. de. (2016). Tracing interactions among column height, exposure time and gas concentration to dimension peanut antifungal ozonation. *LWT-Food Science and Technology, 65*(1), 668-675. doi: 10.1016/j.lwt.2015.08.051

Silva, M. V. A., Faroni, L. R. A., Sousa, A. H., Prates, L. H. F., & Abreu, A. O. (2019). Kinetics of the ozone gas reaction in popcorn kernels. *Journal of Stored Products Research, 83*(1), 168-175. doi: 10.1016/j.jspr.2019.06.014

Silva, M. V. D. A., Faroni, L. R. D. A., Alencar, E. R. de, Sousa, A. H. de, Cecon, P. R., Nogueira, J. V. F., & Mason, V., Fº. (2022). Ozone injection at low pressure: decomposition kinetics, control of *Sitophilus zeamais,* and popcorn kernel quality. *Ozone: Science & Engineering, 44*(1), 66-78. doi: 10.1080/01919512.2021.1937043

Souza, J. V. D. S., Alencar, E. R. D., Junqueira, A. M., & Oliveira, G. P. D. (2018). Ozone saturation and decomposition kinetics in porous medium containing different hybrids of maize. *Revista Brasileira de Engenharia Agrícola e Ambiental, 22*(4), 286-291. doi: 10.1590/1807-1929/agriambi.v22n4p286-291

Sujayasree, O. J., Chaitanya, A. K., Bhoite, R., Pandiselvam, R., Kothakota, A., Gavahian, M., & Mousavi Khaneghah, A. (2022). Ozone: an advanced oxidation technology to enhance sustainable food consumption through mycotoxin degradation. *Ozone: Science & Engineering, 44*(1), 17-37. doi: 10.1080/01919512.2021.1948388

United Nations Economic Commission for Europe (2000). *Oilseeds - determination of moisture and volatile matter content, standard. UNECE.* http://www.unece.org/fileadmin/DAM/trade/agr/standard/standard/dry/dry_e/02WalnutKernels_e.pdf