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Antioxidant concentration effect on stability of Brazil nut (*Bertholletia excelsa*) crude oil

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SUMMARY. Shelled and broken Brazil nuts easily lose quality, if not properly stored. Pressing is an alternative use of these nuts and the crude oil stability was studied. Our previous studies demonstrated that TBHQ (200 mg kg^{-1}) was very efficient to prevent rancidity development in oils bottled in brown and clear glass. As TBHQ has higher price than other phenolic antioxidants like BHT and BHA, an oven test (at 63°C) was conducted to determine the economical and best concentration of TBHQ for Brazil nut crude oil. An assay at ambient temperature was conducted in brown and clear glass flasks with and without the economical concentration of TBHQ calculated (83 mg kg^{-1}) for 90 days. Acid, peroxide, and iodine indices and the absorptivity at 232 nm were determined. TBHQ, even at this low dosage, was very efficient in both brown and clear glass flasks. Peroxide value increased from 11.5 meq O_2 kg^{-1} to average 15 and 35, in TBHQ and control samples after 90 days. The absorptivity at 232 nm remained at 1.3 in samples with TBHQ while the control increased to 1.6.
Key words: Antioxidant concentration, Brazil nut, crude oil, oxidative stability.

RESUMO. Efeito da concentração de antioxidante na estabilidade do óleo bruto de Castanha do Pará (*Bertholletia excelsa*). Castanhas do Pará quebradas e descascadas facilmente se deterioram, se não forem armazenadas adequadamente. A prensagem é uma forma alternativa de encontrar um uso para o óleo e por isso sua estabilidade durante o armazenamento foi estudada. Estudos nossos anteriores já haviam demonstrado a eficiência do TBHQ (200 mg kg^{-1}) como antioxidante de óleos embalados em frascos de vidro transparente e âmbar. Contudo o TBHQ tem um preço consideravelmente mais alto do que o de antioxidantes fenólicos BHA e BHT. Por isso foi conduzido um teste de estufa (a 63°C) para determinar a dose econômica e a melhor dose de TBHQ para o óleo bruto de castanha do Pará. Um ensaio de armazenamento à temperatura ambiente foi conduzido com o óleo adicionado da dose econômica calculada de TBHQ (83 mg kg^{-1}) em frascos de vidro transparente e âmbar por um período de 90 dias. Foram determinados índices de acidez, de peróxido, de iodo e absorptividade em 232 nm. O TBHQ foi muito eficiente em ambos tipos de vidros. O índice de peróxido aumentou de 11,5 meq O_2 kg^{-1} para um valor médio de 15 e 35, nas amostras com TBHQ e da testemunha, respectivamente, ao final do armazenamento. A absorptividade em 232 nm permaneceu em 1,3 nas amostras com TBHQ e aumentou para 1,6 na testemunha.
Palavras-chave: Concentração de antioxidante, castanha do Pará, óleo bruto, estabilidade oxidativa.

INTRODUCTION

Brazil nut (*Bertholletia excelsa*), native to the Amazonian region, has a well known nutritional value due to its high contents of lipids (70%) and proteins (20%) (1). Brazilian production is extractive, although some rational cultivation can be found in the rain forest region, with higher yields and earlier harvests. Almost all of the 20 thousand tons collected annually are exported to the United States (in shell) and to Europe (shelled). Domestic consumption amounts to 1% of the total harvesting. Nuts broken during shelling are not accepted for exportation and if not properly stored, they spoil rapidly and become moldy or rancid. An alternative use for these nuts is pressing for crude oil production. If nuts are of good quality, oil is flavorful and highly accepted. Oil quality is directly dependent on nuts conservation; when not properly stored or spoiled, there is an increase in oil acidity, however up to a maximum 20% of spoilage did not affect seriously the oil quality (2). Shelled Brazil nuts stored on polypropylene trays covered with PVC film for six months at ambient temperature showed an increase in oil peroxide value from 11.39 to 31.36, after 90 days, and to 50.49, after 180 days. Acid values increased from 0.34 mg g^{-1} to 2.74 (3).

Brazil nut oil is reported to be very unstable due to its over 75% polyunsaturated fatty acids (a balance between oleic and linoleic acid) (4-6). Refined Brazil nut oil was the least stable in frying tests (7). Refining may have consumed all the natural antioxidants detected in crude oil methanolic extract which showed phenolic character and synergistic effect with BHT (8). Broken nuts, in general, do not keep very long and have

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very little protection from natural antioxidants (9). Expeller pressed crude Brazil nut oil in brown glass bottles was stored for 184 days and protective effect of the addition of BHT and citric acid was not observed (10). Oven test for 120 hours at 63°C had already indicated 200 mg kg^{-1} TBHQ to be the best antioxidant for Brazil nut crude oil protection when compared to BHA, BHT and BHA/BHT mixtures (11).

The objective of this work was to determine the lowest efficient TBHQ concentration to be added to Brazil nut crude oil. This economical dose of TBHQ was calculated from oven test oxidative data and applied to an ambient storage essay in brown and clear flasks for three months.

MATERIAL AND METHODS

Broken nuts were hydraulically pressed at 5lb in^{-2} in a laboratory hydraulic Carver press, model B. Crude oil was filtered and stored under nitrogen atmosphere, in a freezer, until the beginning of the assays. Two experiments were conducted: for economical antioxidant concentration determination (under accelerated condition) and for best packaging (glass color) at ambient temperature, for 90 days.

Assay 1: Accelerated oven test was conducted at 63°C for 120 hours to determine the best and the economical dosage of TBHQ. 0, 25, 50, 75 and 100 mg kg^{-1} were added to Brazil nut crude oil bottled in 30 mL clear glass flasks. Each treatment was conducted in triplicate. Bottles were withdrawn and analyzed after 120 hours for peroxide value and polynomial regression analysis was done (14) to determine the best and the economical dosage of TBHQ.

Assay 2: Brazil nut crude oil was poured in 30 ml clear and brown glass bottles (surface : volume ratio = 1.48:1), sealed with a plastic cap, and stored with the addition of the economical antioxidant concentration calculated from Assay 1, at room temperature (25-27°C) for 90 days. Every 30 days samples flasks were withdrawn from shelf and analyzed for acid, peroxide and iodine values and for UV spectrophotometry. Control samples (without any antioxidant or sinergistic agent addition) were analyzed as well, for comparison.

Analytical procedures: Absorptivity at 232 nm was determined following the analytical methods described by IUPAC (1979) method II.D.23 (12). Oil samples were dissolved in isooctane. A Shimadzu UV 1203 spectrophotometer was used. Peroxide, acid, and iodine values were determined according to AOCS (1983) (13), methods Cd 8-53 e Ca 5a-40 and Cd 1-25, respectively.

Statistical analysis was conducted, analyzing data as a factorial model in a randomly distributed experiment, in triplicate. Tukey's test ($P<0.05$) was performed for time, antioxidants, color of glass bottles and for their interactions (14).

RESULTS AND DISCUSSION

Brazil nut freshly defrozen crude oil presented 3.00 mg KOH g^{-1} acid value and 10.03 meq O2 kg^{-1} peroxide value. According to the Brazilian food law (CNNPA 22/77) (15), maximum peroxide value accepted for crude oils is 20 meq O_2 kg^{-1}.

TBHQ showed an increasing effect in controlling Brazil nut crude oil stability during oven test with increasing concentration (Figure 1). A polynomial regression of data resulted in a quadratic equation, valid for 0-100 mg kg^{-1}, yielding the best and economical concentration values:

$$y = 0.003x^2 - 0.45x + 23.79 \qquad \text{(Eq. 1)}$$

where: y = peroxide value (meq kg^{-1})
x = TBHQ concentration (mg kg^{-1})

Application of dy / dx furnished the best TBHQ concentration (85 mg kg^{-1}) to achieve the same performance of 100 mg kg^{-1}, the maximum concentration of the studied period.

The economical dose calculated from the following equation (Eq.2) presented a similar value (83 mg kg^{-1}). In this case, prices of the oil and the antioxidant were taken into consideration besides the results of dy / dx. The calculated value of 83 mg kg^{-1} offers the minimum cost to achieve a good performance.

$$[(\text{oil price}) / \text{TBHQ price}) - 0.45] / 2 \times 0.003 \qquad \text{(Eq.2)}$$

where: oil price = US$ 600.00/ton
TBHQ price = US$ 47.00/kg

FIGURE 1
Peroxide value of the Brazil nut crude oil submitted to oven test (63°C, 120 hours) - polynomial regression curve drawn originated Equation 1

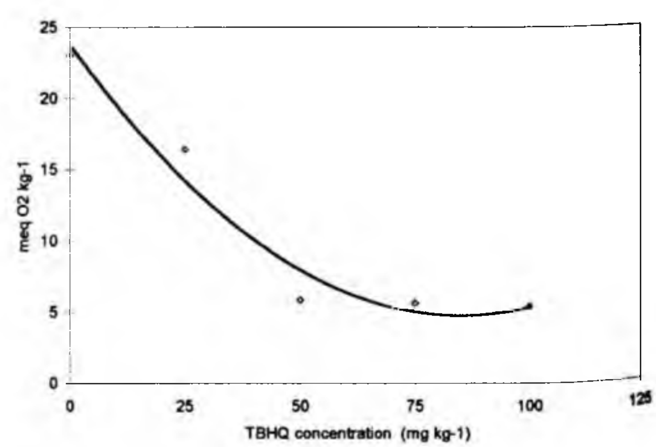


Figure 2 confirms that TBHQ economical dose, 83 mg kg^{-1}, was efficient on the preservation of Brazilian crude oil quality. Peroxide values reached 14.2 and 15.01 in brown and transparent glasses, respectively, while the control reached much higher values (< 30.0 mg kg^{-1}). Tukey's test for storage time of TBHQ factor demonstrated no variation in peroxide index or absorptivity at 232 nm during the three months at 1% significance level (Figure 3), which indicates the efficiency of the low dosage. The low stability of control oil samples could be attributed to its high initial peroxide value, as well as to its fatty acid composition as mentioned earlier.

FIGURE 2

Peroxide value of the Brazil nut crude oil stored in glass bottles at ambient conditions
(Variance coefficient: 11.953%)

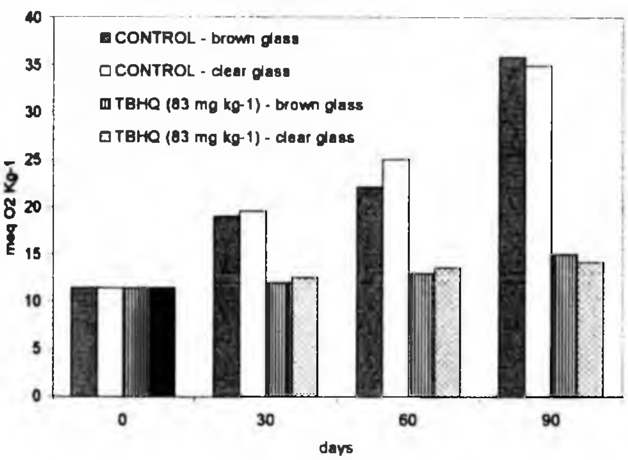
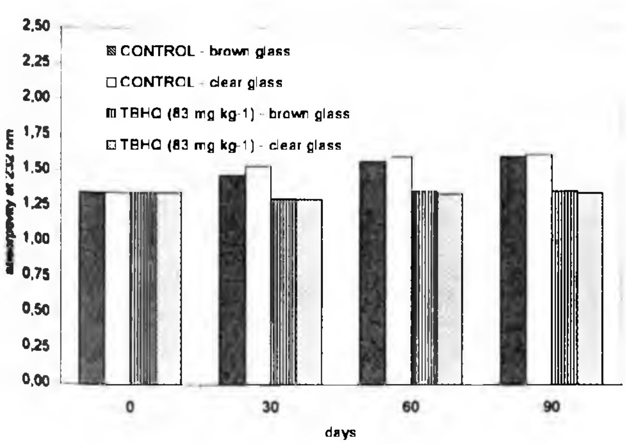


FIGURE 3

Absorptivity at 232 nm of the Brazil nut crude oil stored in glass bottles at ambient conditions
(Variance coefficient: 7.214%)



When 200 mg kg^{-1} of TBHQ was used in an previous study (11) peroxide value and absorptivity at 232 nm of Brazil nut crude oil were kept without alterations during 90 days at ambient storage. The protection of 83 mg kg^{-1} against oil rancidity was similar to 200 mg kg^{-1} of TBHQ. Our practice has indicated the most economical and best antioxidant concentrations can be determined for each oil, and varies with initial oil quality and type of accelerated oxidative tests like microwave or photoxidation chamber (16-18).

Iodine values decreased slightly with time of storage under ambient conditions, although average data did not differ statistically. Initial value was 101 cg g^{-1} and after 90 days it reached 98.98. Table 1 indicates hydrolysis is not a significant alteration in stored oils and is not related to stability. Neither antioxidants nor the glass color had effect on the acidity development.

TABLE 1

Acid value of the Brazil nut crude oil stored at ambient conditions

Storage days	TBHQ 83 mg kg^{-1} brown glass	TBHQ 83 mg kg^{-1} clear glass	Control brown glass	Control clear glass
0	3.01	3.01	3.01	3.01
30	2.96	2.95	2.98	2.97
60	3.03	3.06	3.05	3.08
90	3.03	3.10	3.15	3.10

Variance coefficient: 1.811%

Glass bottles provide protection for the oil, they are recyclable and can be filled at the retail shops and returned for refilling in small communities. Glass has better performance than PET bottles (19). Light protection is mandatory for prolonged oil shelf life and peroxide formation prevention (20). Research with nuts oils also indicated brown and clear glass bottles, in this order, to be the most protective (21), although our data do not confirm that. No statistical difference was detected between brown and transparent glasses.

CONCLUSIONS

TBHQ can be an efficient antioxidant for Brazil nut crude oil, even at 83 mg kg^{-1}, a dose much lower than the maximum allowed (200 mg kg^{-1}). In this study the glass color of the bottles had no influence on the oil stability preservation. The proposed method for TBHQ economical dose calculation could be adopted by industries.

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