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Restrictions to use oven in determining water content for Brazil nuts

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ABSTRACT: The Brazil nut plant (*Bertholletia excelsa* Bonpl.) is native from Amazonia region. The nuts are extensively used as food and in cosmetic industries. During its processing, one of the most important parameters to be determined is its water content, which is critical to drying and storage steps. Thus, the objective on this study was to verify the accuracy of the method of oven at 105 °C to 24 h on water content measurement for Brazil nuts, in comparison to water content levels determined by Karl Fisher titration. It was verified that applying oven at 105 °C to 24 h to determine water content of Brazil nuts is not adequate, once the results were higher than those obtained from Karl Fisher titration. The samples submitted to oven showed high peroxide value, indicating its oxidation and consequent formation of volatile secondary compounds, which could be the reason to overestimation of water content determined.

Keywords: Karl Fischer, oil, peroxide, quality.

Restrições ao uso da estufa para determinação de umidade de castanha do Brasil

RESUMO: A castanheira-do-brasil (*Bertholletia excelsa* Bonpl.) é uma planta nativa da região da Amazônia. As nozes são amplamente utilizadas como alimento e nas indústrias de cosméticos. Durante o seu processamento, um dos parâmetros mais importantes para ser determinado é o teor de umidade, o qual é crítico para as etapas de secagem e armazenagem. Assim, o objetivo deste estudo foi verificar a exatidão do método de estufa a 105 °C por 24 h sobre a determinação de umidade para a castanha do Brasil, em comparação com os níveis de umidade determinados por titulação de Karl Fisher. Verificou-se que a aplicação da estufa a 105 °C por 24 h para determinar o teor de umidade da castanha não é eficiente, uma vez que os resultados foram mais elevados do que os obtidos a partir de titulação de Karl Fisher. As amostras submetidas à estufa apresentou alto índice de peróxidos, indicando a sua oxidação e consequente formação de compostos voláteis, que poderia ser a razão para a superestimação do teor de umidade determinado.

Palavras-chave: Karl Fischer, óleo, peróxido, qualidade.

1. INTRODUCTION

Bertholletia excelsa Bonpl. (Lecythidaceae) or Brazil nut is nutritionally important (SILVA et al., 2010), the seeds are composed by 60 to 70% of lipids which the major portion of fatty acid is unsaturated (85% of total) (MONTEIRO et al., 2016).

During the harvest, the Brazil nut has approximately 26% of water content on wet basis (% w.b.) (NOGUEIRA et al., 2014). During processing in industry, this value ranges from 3.50 to 11.25% w. b. (ARRUS et al., 2005), reaching 2.00% w.b. which is an appropriated water content for the market (ALVARES et al., 2012). Regardless of which stage the product is in, a suitable methodology for analyzing the water content, safely, is required. Since the processes applied to determine the water content, the oven method is one of the most used. On these technique, the samples lose water by drying, which the direct heating of the samples, until the samples reach constant weigh, remove the free water from the product. However, some compounds may decompose when exposed to high temperatures (INSTITUTO ADOLFO LUTZ, 2008). The lipids are one of the chemical compounds that suffer most decomposition when exposed to high temperatures, being the oxidation, the manly reason for its deterioration (ALLEN; HAMILTON, 1994; TOMAINO et al., 2005). During the oxidation of lipids, a lot of reactions occur simultaneously, leading the synthesis of secondary products such as peroxides, aldehydes, ketones, alcohols and hydrocarbons, which, in most cases, are volatile (ALLEN; HAMILTON, 1994; BOBBIO; BOBBIO, 1992).

Thus, applying oven to determine the water content of products with high oil level, mainly those which the major portion of fatty acids is unsaturated, may produce wrong results, considering the oil oxidation and volatilization of the compounds.

As an alternative for the method of direct evaporation of water in oven there are some techniques: as vacuum oven, Karl Fisher titration and others (INSTITUTO ADOLFO LUTZ, 2008). Karl Fisher titration is considered a safe method to water content determination in products with high oil content (LUZ et al. 1993). In this method, the iodine present in the reagent is oxidized by sulfur dioxide, in the presence of water. Using a Karl Fisher titrator, a solution containing the samples is titrated and the reagent amount is related to water content in the product (INSTITUTO ADOLFO LUTZ, 2008). Considering that it is a chemical method based on the amount of water in the samples, it may be used as a reference method to calibrate or verify the accuracy of other methods (TILLMAN; CICERO, 1996; HART et al. 1959).

Thus, the objective of this study was to verify the accuracy of the method of oven at 105°C for 24 hours in determining water content of Brazil nuts, using Karl Fisher titration as reference.

2. MATERIAL AND METHODS

Four samples of shelled Brazil nuts, provided by a processing industry from municipality of Sinop, Mato Grosso State, Brazil, containing 2 kg each, with different water content were used. All of the samples was grinded in an industrial blender and sieved through a 3.5 mm sieve to homogenize the particles. Each sample were divided into oven and Karl Fisher's procedure.

2.1. Water content determination

2.1.1. By Karl Fisher titration

In this procedure, 1 g of grinded samples of Brazil nut was submitted to the test, in three repetitions. Each sample was solubilized in 40 mL of anhydrous methanol; this solution was titrated in the presence of Karl Fisher's reagent - SO2, I2 and an organic base (INSTITUTO ADOLFO LUTZ, 2008).

2.1.2. By oven method

In the oven procedure, 20 replications were used (each one with 15 g of grinded Brazil nut) for each sample send to Karl Fisher titrator. The samples were weighed and sent to oven with forced air circulation at a temperature of 105 ± 3°C and reweighed after cooled in desiccator (AOAC, 1995) for the intervals of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours. The samples were remained in the desiccator for an average of 5 minutes, until the samples reached about room temperature. Based on differences between the initial and final weigh, the water content was calculated (AOAC, 1995).

2.2. Peroxide value determination

The peroxide value (PV) was determined in the oil extracted from samples, before and after oven procedure at 105 ± 3°C for 24 hours, in three repetitions. The Bligh & Dier method was used to oil extraction from Brazil nut samples (BLIGH; DYER, 1959).

To determine peroxide value in the Brazil nut samples it was added 5 g of extracted oil to 30 mL of a solution of acetic acid-chloroform 3:2 into an Erlenmeyer with 250 mL of capacity. The mixture was stirred for one minute to its dissolution, being added 0.5 mL of saturated KI and then kept standing and in darkness for one minute more. After that, it was added 30 mL of distilled water and the solution was titrated with sodium thiosulfate 0.1 N, using starch solution at 1% as indicator. This test was carried out in two replications, and also to benchmark the procedure, a blank test was performed under the same conditions.

After titration, the peroxide value was calculated based on Equation 1:

$$PV = \frac{(A - B) \times f \times N \times 1000}{M} \quad (1)$$

where:

PV - peroxide value, meq.kg^{-1};

A - volume of sodium thiosulfate 0.1 (or 0.01 N) used in sample titration, mL;

B - volume of sodium thiosulfate 0.1 (or 0.01 N) used in blank titration, mL;

f - factor of sodium thiosulfate solution (from standardization), dimensionless;

N - normality of sodium thiosulfate solution, dimensionless;

M - mass of sample, g.

2.3. Data analysis

Data from water content of samples obtained by oven method (105°C for 24 hours) was compared to those obtained by Karl Fisher titration using the confidence interval for means.

In addition, the water contents was evaluated based on the adjustment of a linear regression for the values observed in the Karl Fisher with those predicted by oven (105ºC to 24 h) (PROC REG - SAS 9.0). The regression parameters estimated was tested by the hypothesis of joint nullity according to Mayer et al. (1994), and by exclusive null hypothesis for each parameter as described:

H0: $\beta 0 = 0$ and $\beta 1 = 1$ Ha: no H0

H0: $\beta 0 = 0$ and Ha: $\beta 0 \neq 0$

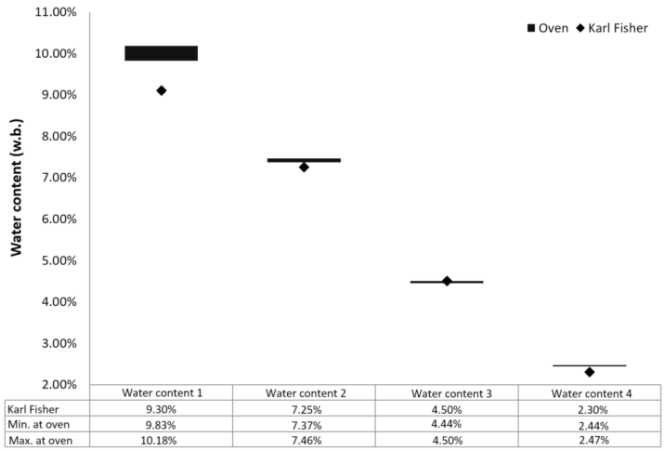
H0: $\beta 1 = 1$ and Ha: $\beta 1 \neq 1$

For the non-rejection of the null hypothesis (H0), it may be conclude that the values observed in the Karl Fisher method and those predicted by oven are equivalent; otherwise, there is no equivalence between the methods.

For all statistical procedures it was adopted a significance level of 0.05 for the error type I.

3. RESULTS

The means and standard deviation for water contents of Brazil nut samples obtained by Karl Fisher titration were 2.30 ± 0.05; 4.50 ± 0.10; 7.25 ± 0.20 and 9.10 ± 0.10% w.b., being value of 2.30% w.b (Figure 1). getting from shelled nuts from industry; the sample with 4.50% w.b. of water content from in-shell nuts received directly from industry; and other two values (7.25 and 9.10% w.b.) getting from samples rewetted after conditioning into climatic chamber. These same samples, submitted to oven at 105°C for 24 hours, showed higher results for its water content when compared to those obtained by Karl Fisher, considering the confidence interval test at 5% of probability. The differences between methods were significantly, especially for water content



	Water content 1	Water content 2	Water content 3	Water content 4
Karl Fisher	9.30%	7.25%	4.50%	2.30%
Min. at oven	9.83%	7.37%	4.44%	2.44%
Max. at oven	10.18%	7.46%	4.50%	2.47%

Figure 1. Comparison between water contents determined by Karl Fisher titration and by oven, analyzed by confidence interval at 5% of probability.
Figura 1. Comparação entre os teores de água determinados pela titulação de Karl Fischer e por forno, analisados por intervalo de confiança a 5% de probabilidade.

1 (9.10% w.b.), where the observed difference was higher than 1% in absolute terms and more than 10% in relative terms for two methods. Only for water content 3 (4.50% w.b.) the means were similar, comparing the two methods for water content determination (Table 1).

The differences between methods were confirmed by linear regression for the values observed in the Karl Fisher with those predicted by oven (105ºC to 24 h). As showed in Table 2, by the parameters of linear regression, the null hypotheses (H0) are rejected, considering the exclusive and joint nullity. For all of those null hypotheses, the significance of the data were less than 0.0001.

Table 1. Water content in samples of Brazil nuts by Karl Fisher titration an oven at 105°C for 24 hours.
Tabela 1. Teor de água em amostras de castanha do Brasil por titulação de Karl Fischer em estufa a 105 ° C por 24 horas.

Water content (% w.b.)	
Karl Fisher	**Oven**
2.30 ± 0.05 a	2.46 ± 0.04 b
4.50 ± 0.10 a	4.47 ± 0.07 a
7.25 ± 0.20 a	7.42 ± 0.10 b
9.10 ± 0.10 a	10.00 ± 0.40 b

Different letters in the same line indicate statistical significant difference by test T at $p < 0.05$.

Table 2. Parameters of regression model for the values of moisture content obtained by Karl Fisher titration and by oven method (105°C to 24 h).
Tabela 2. Parâmetros do modelo de regressão para os valores de teor de umidade obtidos por titulação de Karl Fisher e por método de forno (105 ° C a 24 h).

Parameters	R^2		0.9873
	Slope	P^1	<.0001
		SE	0.0116
		β_0	0.8942
	v-intercept	P^1	<.0001
		SE	0.0786
		β_0	0.3508
	Item		KF - ESTF

[1] H_0: $\beta_0 = 0$ and H_a: $\beta_0 \neq 0$; [2] H_0: $\beta_1 = 1$ and H_a: $\beta_1 \neq 1$; [3] H_0: $\beta_0 = 0$ and $\beta_1 = 1$ H_a: no H_0

In Figure 2 is showed the variation of water content obtained in the samples submitted to oven throughout 24 hours considering the steps of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours. For water content 1 (9.10% w.b. obtained by titration), the values obtained by the oven ranged between 10.00 and 10.09% w.b. The results for the water content 2, represented by the sample with 7.25% w.b., were between 7.42 and 7.51% wb. Applying the technique of oven in samples with a water content of 4.50% w.b., the results were consistent with that found by Karl Fischer titration, ranging from 4.48 to 4.59% w.b.

Finally, for the sample of shelled nuts, whose water content of 2.30% w.b. was determined by titration, the oven produced results ranging from 2.36 to 2.47% w.b.

Peroxide values (PV) for Brazil nut samples before and after submitting to oven condition (105°C for 24 hours) allowed indentifying the oxidation level of lipid portion from product. For samples that were not submitted to oven, the mean and standard deviation of PV was 1.90 ± 0.01 meq kg^{-1}. After 24 hours into oven at 105°C, the samples showed a significant increase for this parameter, being quantified 183.53 ± 9.16 meq kg^{-1} (Table 3).

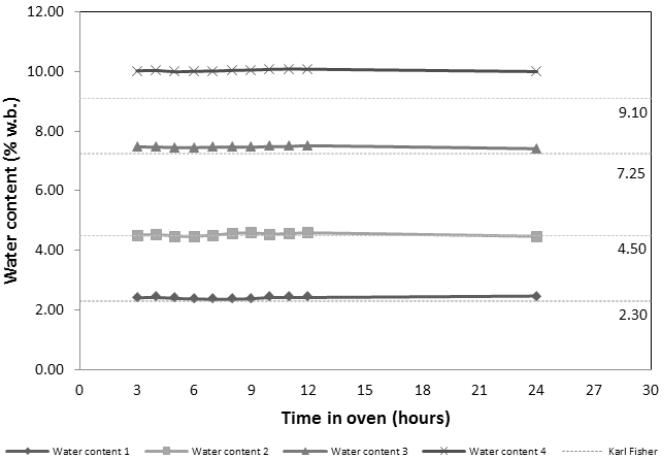


Figure 2. Water content for Brazil nut samples determined by oven for intervals between 3 and 24 hours.
Figura 2. Teor de água para amostras de castanha do Brasil, determinado por forno para intervalos entre 3 e 24 horas.

Table 3. Peroxide values in samples of Brazil nuts in nature and submitted to oven at 105°C for 24 hours.
Tabela 3. Valores de peróxido em amostras de castanha do Brasil em natureza e submetidos a forno a 105 ° C por 24 horas.

	Peroxide value (meq kg^{-1})
In natura	1.90 ± 0.01
Submitted to oven	183.53 ± 9.16

4. DISCUSSION

Water contents in the studied samples indicate typical waters for Brazil nuts during processing and marketing steps. Álvares et al. (2012) studying the quality of processed Brazil nuts marketed in Rio Branco city, Acre State, Brazil, found water contents varying from 2.00 to 3.12% w.b., being this interval compatible with value for shelled nuts from industry, that showed a water content of 2.30% w.b. Arrus et al. (2005) analyzing in-shell nuts from industry and storing the samples for 60 days at 30°C and 97% of relative humidity, found water contents from 3.50% to 11.26 % w.b. These values comprise

those three remained contents for the studied samples, which are 4.50; 7.25 and 9.10% w.b. So, tested contents for nuts water are characteristic to processing industries and to market, that needs an efficient and safe method to determine the water content on its product. It is noteworthy also that the range of water contents tested comprises typical values for researches involving Brazil nuts post-harvest.

Based on the results for water content in samples submitted to oven at 105°C for 24 hours, in comparison to those obtained submitting the samples to Karl Fisher titration, there is statistical differences between the methods, being the contents obtained by oven higher ($p< 0,05$) than those obtained by titration, that was the reference method (Table 1). Tillman; Cicero, (1996) comparing water contents for maize and soybeans using oven at 105°C with the contents determined by Karl Fisher titration, cites that to water contents higher than 16%, for both products, the oven method overestimates its parameter. Hart et al. (1959) mentions that products with high oil content may present oxidation on its lipid portion during the heating of product into an oven, and it may be a source of error to this method.

It may be verified in Figure 1 that the differences between the methods have direct relationship, that is, the differences increase as the water content are higher. This is especially important to processing industry, because the product with high water content is more susceptible to become rotten, to mould growth and to mycotoxins synthesis. This information is corroborated by several authors as Freire et al. (2000); Arrus et al. (2005); Baquião et al. (2012) and Reis et al. (2012).

Considering the observed differences between the methods, if the water content determination was not appropriate, mainly for products with higher waters, processing steps may be taken by wrong way or on inadequate time. Other important point is that, as absolute numbers it is questionable whether a difference of 1% on the water content for product is significant. Otherwise, considering the high market prices for Brazil nuts, 1% of an industry's production, state's production or country's production, may represents a significant amount of money. It is also important to note that it is essential to apply a reliable methodology in research laboratories, because when it is considered the necessary precision for science, 1% may be unacceptable, mainly when the postharvest steps are studied.

Likewise, in Figure 2 it is observed that throughout the 24 hours that samples were in the oven, the water content in the majority samples were overestimated in comparison to those obtained by Karl Fischer titration. Only for the water content 3 (4.50% w.b.), the values obtained by the oven method were similar to those found by titration. This observation is significant because it indicates that a temperature of 105°C is unsuitable for the use of oven as a technique to determine water content in Brazil nut, regardless of the considered time.

However, the overestimation of water content determined by oven, may indicates that the oxidation process in lipid portion of product, when it is in contact with extreme conditions of temperature, is the most probable source of methodological error, since on the oven the volatile compounds and the water may be evaporated together. These volatile compounds are formed during the oil oxidation. For Brazil nut, that has approximately 70% of oil on its composition, being 75.2% of those, insaturated fatty acid represented by oleic and linoleic acids (GONÇALVES et al., 2002), the oxidation may be favored in relation to other oleaginous or other nuts because the presence of instaurations promotes the reactivity of fatty acids, by free radicals formation. In this case, the energy necessary to break C-H bonds and form free radicals on double bonds is relatively low, comparing to single bond. The free radicals formed are indicators of oxidation process of lipids (BOBBIO; BOBBIO, 1992).

Peroxide values in samples of Brazil nuts in natura, that is, before sent to oven, were considered low and are nearby those founded by Santos et al. (2012) studying the oil extracted from Brazil nuts in natura, that were from 2.90 to 4.06 meq.kg-1. For Brazil nuts submitted to oven, the values indicates a high level of oxidation, with peroxide values considered high when compared to those recommended by RDC 270/2005, maximum of 15 meq.kg-1 for almonds on marketing, showing that changes on fatty acids presents in the samples were substantial and that the secondary compounds formed during the oxidation reactions could be evaporated together with water during water content determination, overestimating the contents founded by oven. Aquino et al. (2009) studying the influence of drying process on quality of oil from pequi (*Caryocar brasiliense* Camb.) mentions that when the product is in contact to high temperatures for a long period, its lipid portion may be oxide. The same information may be founded in the results from Adeeko; Jibola, (1990), that working with groundnut (*Arachis hypogaea* L.) oil extraction, by different processing methods, showed that, a direct association between temperature and time is decisive to oxide the oil presents in the product.

Based on the points discussed above, it may be conclude that water contents obtained by Karl Fisher titration and by oven at 105°C for 24 hours were different for majority of studied samples, showing that applying the method of oven in studied conditions, as used to all of types of grains, seeds, nuts and other food product, when used to Brazil nut, it overestimate the results. This behavior may be a result of the evaporation of secondary compounds formed during the lipid oxidation which was confirmed by the presence of peroxide in the samples submitted to oven. So, the official method of water content determination by oven at 105°C for 24 hours should not be applied to Brazil nut.

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