

THE OVEN-DRYING METHOD FOR DETERMINATION OF WATER CONTENT IN BRAZIL NUT

O MÉTODO DE ESTUFA PARA DETERMINAÇÃO DO TEOR DE ÁGUA DA CASTANHA-DO-BRASIL

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ABSTRACT: The official methods adopted by different worldwide agencies for determination of water content of Brazil nut is the dissication in drying oven at 105 °C during 3 or 24 hours and dissication until constant height of samples. However, applying these methods for Brazil nut, may result in inconsistent values, possibly due to lipid oxidation. Thus, the objective of this study was to evaluate the accuracy of the oven-drying methods, recommended by the official agencies, and to determine the oven-drying correct parameters, such as temperature and exposure time. For this purpose, samples were placed in drying ovens set at 85, 90, 95 and 105 °C and weighed hourly, between 3 and 12 hours and after 24 hours of exposure, and the results were compared to Karl Fisher titration, considered as a reference method in this study. The temperature of 105 °C, for any exposure time, resulted in overestimated water content compared to reference method. However, there was no difference between the water content values obtained by oven-drying assay at 90 °C for 6 hours and by the reference method, allowing to conclude that the determination of water content in Brazil nut samples, in drying oven under these conditions, can be performed with the same accuracy and precision of the Karl Fischer method.

KEYWORDS: *Bertholletia excelsia*. Karl Fischer. Nut.

INTRODUCTION

The Brazil nut, fruit of *Bertholletia excelsa* HBK, a native plant of the Amazon region, has a very pleasant taste and is an important source of nutrients (LOUREIRO; SILVA, 1968; LOUREIRO et al., 1979; SOUZA et al., 2004). Its proximate chemical composition is 3.42% of carbohydrate, 3.84% of ash, 8.02% of total fiber, 14.29% of protein and 67.30% of lipids (SOUZA; MENEZES, 2004). In addition, it has a high content of unsaturated fatty acids (85% of total lipids) (FERREIRA et al., 2006), it has a high level of selenium, approximately 2 mg/kg and is rich in essential amino acids including sulfur containing amino acids (SOUZA; MENEZES, 2004).

During the harvest, the Brazil nut has approximately 26% of water content on wet basis (% w.b.) (NOGUEIRA, 2011). 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 is required.

The water content in seeds correspond to the amount of water available for degradation reactions, and also the possibility of interaction with

microorganisms, and its level are determinant in deciding the time of harvesting, drying, processing, and storage (LUZ et al., 1993; ELÍAS et al., 2009). Basic research is still preliminary on Brazil nuts, including proper methodologies for determining the water content. Following the recommendations for products of the same food group, the oven-drying method results in unexpected water contents and so has arose the interest for this fact (NOGUEIRA, 2011). As an example, the methodology for determining water content at 105 °C for 24 h, recommended in RAS (Seed Analysis Rule) by the Ministry of Agriculture, Livestock, and Supply (MAPA) (BRASIL, 2009), Nogueira (2011) suggests that the assay conditions can result in inconsistent values because of the visible oxidation of the product, indicated by darkening, and changes in taste and odor. Thus, the determination of water content in Brazil nut by this methodology and others that expose this product to high temperatures, such as the National Forage Testing Association (NFTA) (2006) which recommends the drying oven temperature at 105 °C for 3 h, shall be tested due to the high content of lipids that can undergo decomposition.

The lipid oxidation as a result of exposure to high temperatures, particularly in products with high level of lipids, in the presence of unsaturated fatty acids, provides favorable conditions to break the

unsaturated bonds leading to the formation of free radicals, which are the initiators to form aldehydes, ketones, alcohols, and hydrocarbons (BOBBIO; BOBBIO, 1992; ALLEN; HAMILTON, 1994; TOMAINO et al., 2005). Many of these substances are volatile and evaporate with water in the drying oven, overestimating the value of water content of the product. Considering that drying oven is recommended as an official standard by Brasil (2009) and also is used as a reference method for calibrating indirect methods, such as electronic devices, widely used by the industries, efforts should be focused to adapt this methodology.

Karl Fischer titration method is an alternative to these fatty products, since it is based on the titration of water molecules with the Karl Fischer reagent (I_2 , SO_2 and an organic base) (ISENGARD, 2001) and therefore does not consider other volatiles compounds. This is a very reliable method and has been used as a reference method to verify the performance of oven-drying method for determining water content in vegetable products (BENJAMIN; GRABE, 1988; TILLMAN; CÍCERO, 1996). Thus, this study evaluates the accuracy of the oven-drying method as recommended by NFTA (2006) and MAPA (BRASIL, 2009), and adapts these methods to determine the water content of the Brazil nut using the Karl Fischer method as the reference.

MATERIAL AND METHODS

This research was developed in Energy and Postharvest Laboratory of the Federal University of Mato Grosso, Campus of Sinop, Mato Grosso, Brazil. The samples of Brazil nuts were obtained from a processing industry in Sinop, Mato Grosso, Brazil. The water content was randomly determined for samples collected from the initial, intermediate, and final stages of the production chain. The nuts were selected by quartering and then crushed in an industrial processor (Spolu, Industrial model, Itajobi, Brazil). It was classified after sieving using a nine Mesh sieve and used for determining water content by Karl Fischer and oven-drying methods.

This study was divided in optimization and validation steps. In the optimization step, the tests were performed to define the optimal conditions (appropriated exposure time, temperature and sample size) to be used on the oven-drying assay in the validation step. Then, in the validation step, the optimal condition determined in the early step were tested to validate the working range of water content for samples and to confirm the precision

(repeatability and reproducibility), and accuracy of the oven-drying method.

The levels of water content were randomly obtained, getting samples from different stages of processing, like harvesting, processing, and marketing of Brazil nuts. Four water content levels, from 2.30 to 23.10 % w.b., was used in the optimization step, and other six different water content levels, from 1.90 to 19.28 % w.b., were used in the validation step.

The reference method for water content determination was the Karl Fischer titration. The analysis were performed in triplicate by placing 1 g of crushed Brazil nut samples in an automatic titrator (Labindia, Karl Fischer Titrator KAFI Automatic V 2.06, Navi Mumbai, India). Dry methanol was added to the crushed samples into the titration flask, to extract the water from solid phase. This solution was titrated with the Karl Fischer reagent, and the final point was automatically detected by the machine.

The same samples subjected to Karl Fischer titration were taken to four forced circulation drying ovens (Nova Ética, 400 / ND, São Paulo, Brazil) adjusted to temperatures of 85, 90, 95, and 105 °C, during the optimization phase. Samples of 15 g of shelled and crushed Brazil nuts were placed in 6.5 cm of diameter and 5.5 cm height metal dishes and kept in a drying oven for 24 h. Twenty replicates for each oven-drying temperature were used in this experiment. During the drying, each sample was weighted hourly between the 3rd and the 12th hour exposure time, and also after the last exposure time of 24 h. The samples taken from the drying oven were cooled in a desiccator and immediately weighted to avoid further dehydration. The water content of the Brazil nut was calculated by mass difference, according to equation 1:

1)

$$\text{Water content (\% w.b.)} = 100 \frac{\text{initial mass (g)} - \text{final mass (g)}}{\text{initial mass (g)}}$$

The data for water content obtained in this step were compared to the data obtained by Karl Fischer titration, and analyzed by descriptive statistical methods and depicted in Boxplot charts. After the statistical analysis, appropriated exposure time, temperature and sample size for Brazil nuts were established for oven-dry method. This set of experimental conditions were employed in the validation phase.

For testing repeatability, five replicates in four water content levels with approximately 15 g of crushed Brazil nuts were subject to analysis in drying oven at the temperature and time previously

determined. The water content values allowed to calculate the coefficient of variation (CV) for evaluation of the repeatability of the adjusted method. The procedure for the reproducibility was similar to repeatability test, using the same procedure, however under different environmental conditions (room temperature), using other equipment (scales and drying ovens) operated by other professionals.

To confirm the accuracy of the oven-drying method, the results were compared with those obtained by Karl Fischer titration, using the Wilcoxon test with $\alpha < 0.05$ and the linear correlation test.

RESULTS

The samples of Brazil nut submitted to 85, 90, 95 and 105°C and analyzed by Karl Fischer titration method in the optimization phase resulted in water contents of 2.30, 4.50, 7.25 and 23.10% w. b., represented by dashed line in Figures 1 to 4.

The results for water content determined by oven-drying at 85, 90, 95, and 105 °C over a 24 hours period, in the optimization step, are shown in Figures 1 to 4:

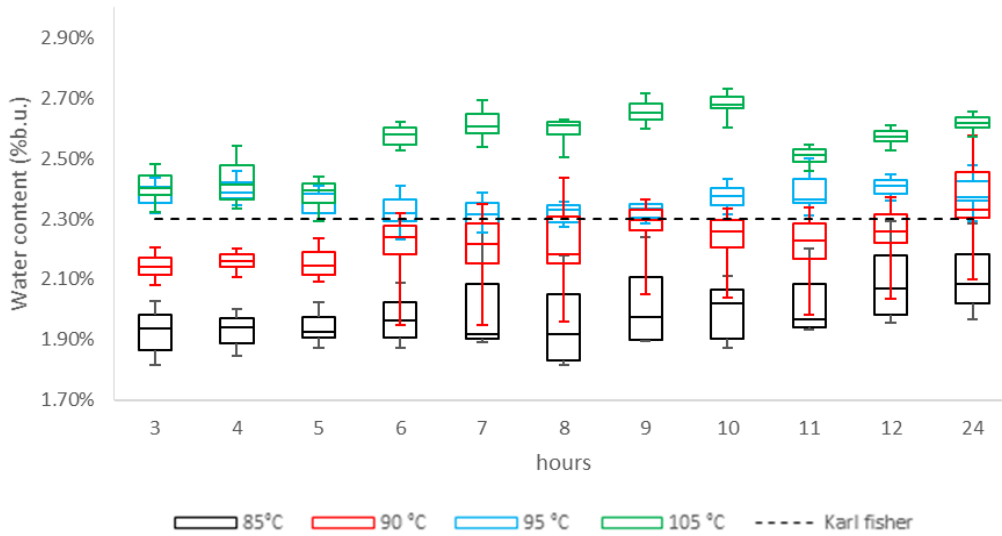


Figure 1. Water contents determined by the oven-drying at 85, 90, 95, and 105 °C in the range of 3 to 24 h for the sample with 2.30% w.b. of water content by Karl Fischer method.

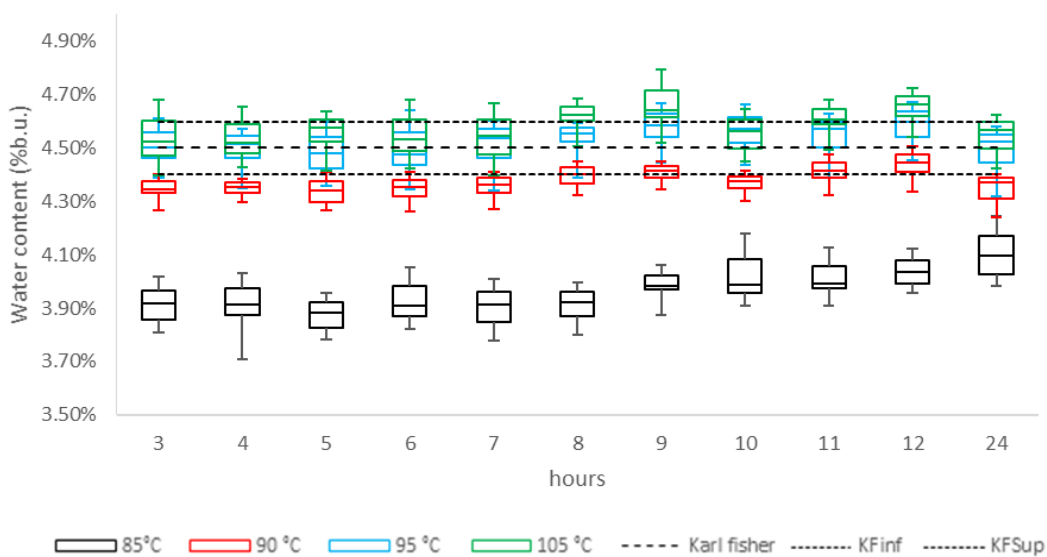


Figure 2. Water contents determined by the oven-drying at 85, 90, 95, and 105 °C in the range of 3 to 24 h for the sample with 4.50% w.b. of water content by Karl Fischer method.

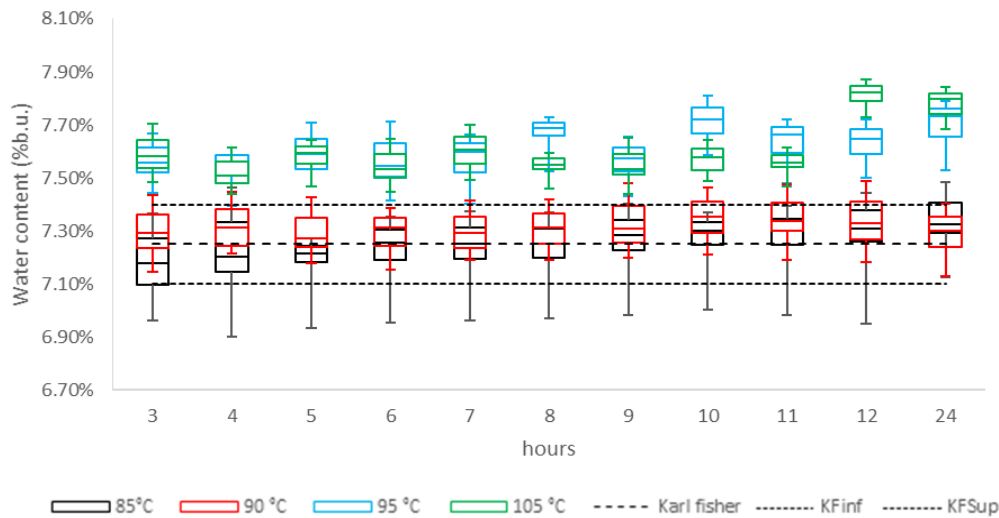


Figure 3. Water contents determined by the oven-drying at 85, 90, 95, and 105 °C in the range of 3 to 24 h for the sample with 7.25% w.b. of water content by Karl Fischer method.

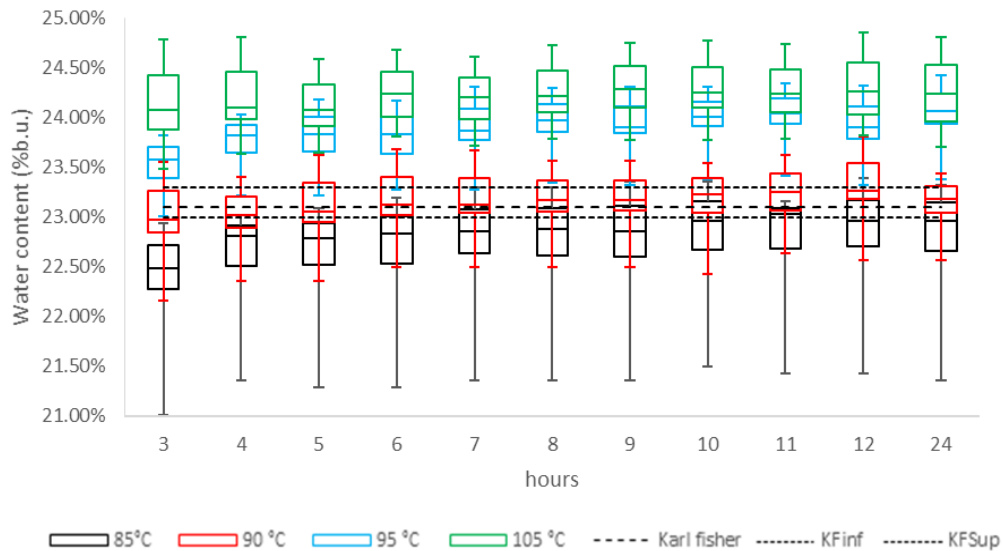


Figure 4. Water contents determined by the oven-drying at 85, 90, 95, and 105 °C between 3 and 24 h of drying for the sample with 23.10% w.b. of water content by Karl Fischer method.

For the lowest water content (2.30% w. b.), the drying conditions of 90 °C for 6 h and 95 °C between 6 and 9 h, and after 24 h resulted in similar values to those from Karl Fischer method. For the water content of 4.50% w.b., the drying conditions of 90 °C for 5 h of exposure in the drying oven and temperatures of 95 to 105 °C during the whole time showed values similar to the reference method. For the water content of 7.25% w.b. determined by Karl Fischer, drying at 85 and 90 °C showed similar values to the Karl Fischer method. Finally, for the highest water content (23.30% w.b.) samples dried in drying oven at 85 °C for 4 h of exposure; 90 °C throughout the whole sampled interval, and 95 °C

from 3 to 7 h, were similar to those obtained by Karl Fischer titration.

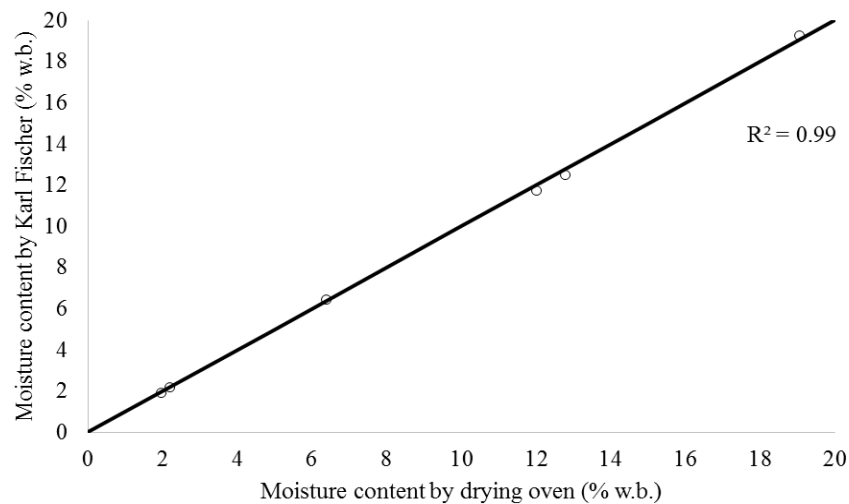
Therefore, based on these results, the drying condition at 90 °C for 6 h was chosen to be tested in accuracy and precision. This choice was justified because it predicted the majority of water content and required shorter oven-drying time.

In the validation phase, the water content obtained by the reference method was 1.90, 2.17, 6.45, 11.73, 12.50, and 19.28% w. b. (Table 1). The water content results obtained in oven-drying at chosen conditions (90 °C for 6 h) are shown in Table 1. Also, adjusts of the method using a linear model is presented in Figure 5.

Table 1. Comparison of the water content determined by Karl Fischer titration method and oven-drying at 90 °C for 6 h, and coefficient of variation (CV) of data from the oven-drying method

| Karl Fischer (Mean ± SD) | (% w. b.) | Drying Oven (Mean ± SD) | (% w. b.) | CV from drying oven (%) |
|--------------------------|-----------|-------------------------|-----------|-------------------------|
| *1.90 ± 0.00 | | *1.98 ± 0.06 | | 3.28 |
| 2.17 ± 0.04 | | 2.18 ± 0.02 | | 0.75 |
| *6.45 ± 0.07 | | *6.39 ± 0.04 | | 0.71 |
| 11.73 ± 0.22 | | 12.00 ± 0.03 | | 0.21 |
| 12.50 ± 0.42 | | 12.79 ± 0.03 | | 0.22 |
| 19.28 ± 0.28 | | 19.06 ± 0.10 | | 0.56 |

*Water content levels determined to test reproducibility.

**Figure 5.** Correlation between water content obtained by Karl Fischer titration and by oven-drying at 90 °C for 6 h. Significant at the 5% probability.

DISCUSSION

The oven drying at 105 °C between 3 and 24 h, as recommended by NFTA (2006) and Brazil (2009), reproduced different results when compared to those using the Karl Fischer method for most of the results, overestimating the water content. Similar results were found by Melo and Almeida-Muradian (2011) comparing different methods for water content of dry bee pollen, verifying the methods that employ heat, mainly drying in drying oven at 100 °C, overestimated this parameter. Also, Garcia-Amoedo and Almeida-Muradian (2002) compared methods for determining water content in royal jelly and concluded that drying in drying oven at 105 °C also overestimated this parameter.

Overestimation of water content levels in Brazil nut analyzed by the oven-drying method, can cause problems in setting the operational conditions in industrial dryers to achieve the adequate water content and, thereby, may result in a low quality

final product. In addition, there may promote economical losses due to over drying of the product and, therefore, reducing product's weight and demanding more resources to dry it out.

It was also observed a tendency for samples with higher water content (7.25 and 23.10% w. b.) to have similar results as the ones obtained by Karl Fischer titration, for the drying in drying oven at temperatures between 85 and 90 °C. This can be explained by the action of enzymes on lipid substrates products with higher water activity, where prooxidant enzymatic reactions can be favored (SILVA et al., 1999). This factor, associated with high temperature, enhances lipid oxidation and volatile substances releasing, promoting an overestimation of the water content in Brazil nut, mainly for samples with high water content levels. However, products with low water content have lower enzymatic activity and lower free water, which requires more energy to release the water. Indeed, the drying at higher temperatures (90 to 95

The oven-drying method...

°C) was necessary to approach the values obtained by Karl Fischer titration.

The lipid oxidation of Brazil nuts with higher moisture content was verified by Prado-Filho (1994) during the storage for 6 days at different water activity values (A_w), where the daily increasing in the peroxide levels were found for nuts with higher A_w values. Several authors refer to lipid oxidation in food relating to increasing of temperature of exposure, as verified during the drying of pequi (*Caryocar brasiliense* Camb.) (AQUINO et al., 2009), and peanuts (*Arachis hypogaea* L.) (ADEEKO; JIBOLA, 1990). For soybean (*Glycine max* L.), Ghaly and Sutherland (1983) found an increased peroxide value of crude oil when submitted to drying temperatures above 50 °C.

For Brazil nut, the use of the drying oven at 90 °C for 6 hours was the most adequate condition for samples with a broad water content level (2.30 to 23.10% w. b.), as confirmed by Karl Fischer titration. This condition ensures economy once the energy consumption for oven-drying is lower than for the drying at 105 °C for 24 h. In addition, reducing the exposure time into the drying oven provides a faster method for water content determination analysis. Jindal and Siebenmorgen (1987) evaluated different drying temperatures and time in oven-dry for water content determination of paddy rice and verified that the reduction on time from 20 h to 11.4 h does not result in errors, and consequently would give flexibility of using this method.

The working range for water content investigated in this study (2.30 to 23.10% w. b.) typically represents moisture contents of unshelled Brazil nut during marketing, processing and harvesting steps. The similarity between the water content found for the oven-drying method at 90 °C for 6 h and the Karl Fischer titration one, determined by the Wilcoxon test, suggests the accuracy of the proposed oven-dry method. Hence, the adoption of Karl Fischer as a reference is already established in literature for validating the oven-dry method (BORMUTH, 1994; TILLMAN; CÍCERO, 1996) and also is used as a reference for

the adjustment of oven-dry condition for plant products (HART et al., 1959; BENJAMIN; GRABE, 1988).

Furthermore, strong correlation achieved between the methods is strengthened by the adjustment of a linear model between the two methods with 0.99 of correlation (Figure 6). A linear fit with 0.99 of correlation was found in the validation study of the water content determination in acetone by gas chromatographic method, using the Karl Fischer as reference (O'KEEFE et al., 2008). The CV of most of the samples varied by less than 1% in five replicates in the oven-dry at each water content and can be inferred about the precision of the method (Table 1). These values were lower than those obtained by Borges et al. (2005) in the validation studies of a methodology for water determination using the infrared water content analyzer for four different herbal drugs.

CONCLUSIONS

The time and temperature for oven-dry methods recommended in RAS by Brasil (2009) and the NFTA (2006) overestimates the water content for Brazil nut and therefore is not indicated for this product.

The drying at temperature of 90 °C by 6 h achieved similar results to those obtained by the Karl Fischer titration method, also confirmed by the accuracy and the precision tests. This new set of drying conditions for the oven-drying method can be used to water content determination for Brazil nut, using 15 g of shelled samples, crushed with particle up to nine mesh. Therefore, when the drying in drying oven is performed using these improved conditions, errors during the water content determination in bromatological analysis, during harvesting, processing, and marketing steps will be reduced.

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RESUMO: Os métodos oficiais adotados por diferentes órgãos ao redor do mundo para a determinação do teor de água da castanha-do-brasil são a dessecação em estufa a 105 °C por 3 ou 24 horas e a dessecação até peso constante. Contudo, a aplicação destes métodos para a castanha-do-brasil pode resultar em valores inconsistentes, possivelmente pela oxidação dos lipídeos. Assim, o objetivo deste estudo foi o de avaliar a acurácia dos métodos de estufa, recomendados pelas agências oficiais, bem como determinar os parâmetros adequados do método, como temperatura e tempo de exposição. Para isto, amostras foram colocadas em estufas ajustadas em 85, 90, 95 e 105 °C e pesadas a cada hora, entre 3 e 12 horas e ao final de 24 horas de exposição, e os resultados foram comparados com aqueles obtidos por titulação de

Karl Fisher, considerado como método de referência neste estudo. A temperatura de 105 °C, para quaisquer tempos de exposição, resultou na superestimação do teor de água comparado ao método de referência. Não houve diferença entre os valores para o teor de água obtidos em estufa a 90 °C por 6 horas e o método de referência, permitindo concluir que a determinação do teor de água em amostras de castanha-do-brasil, em estufas nestas condições, pode ser executada com a mesma acurácia e precisão do método de Karl Fisher.

PALAVRAS-CHAVE: *Bertholletia excelsa*. Karl Fischer. Nut.

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