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Characterization of pseudo-fruits of *Hovenia dulcis* T. at different maturation stages and drying methods

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ABSTRACT. The vast majority of fruits and unprocessed vegetables, have a very short shelf life, mainly due to its high-water activity. Drying technology is an effective process, used to increase the shelf life of these products. It also can provide an effective method of consumption of fruits in food products. In this study, the influence of the maturation stage and drying method on the phenolic compounds and antioxidant activities of Japanese grape pseudo-fruits (*Hovenia dulcis* T.) were studied. The centesimal composition, reducing sugars and nonreducing sugars, ratio, color, phenolic compounds and antioxidant activity by oxygen radical absorbance capacity (ORAC) of the unprocessed pseudo-fruits, at different maturation stages (immature, in maturation and mature), were analyzed. Two drying methods were employed in the pseudo-fruits (oven with air circulation and freeze drying) obtaining flour from which moisture content, instrumental color, phenolic compounds and antioxidant activity were determined. The unprocessed pseudo-fruits of Japanese grape showed significant content of phenolic compounds at the early stages of maturation and high antioxidant capacity when mature (at the final stages of maturation). In addition, they showed a high content of fibers and sugars, and can be used as raw material in the food industry. After dehydration, the freeze-drying process provided a more stable color, because this process minimizes the enzymatic browning reactions, and preserved a greater number of phenolic compounds. The antioxidant activity, however, showed to be associated with the drying method, being higher in the flour obtained from lyophilized immature pseudo-fruits.

Keywords: Japanese grape; phenolic compounds; antioxidant activity.

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Introduction

The *Hovenia dulcis* T. tree, commonly known as Japanese grape, is very widespread in the southern Brazil, due to the favorable climate and soil conditions in that region (Cardoso et al., 2015). Its blossoming occurs between August and February and its fructification from March to October. Its pseudo-fruit is a small dry rounded capsule containing from 2 to 4 seeds attached to a dark brown stalk, which is fleshy, juicy and rich in sugar when ripe (Bampi, Bicudo, Fontoura, & Ribani, 2010).

Its leaves and pseudo-fruits have been used in traditional medicine from East Asian countries, where several studies have been conducted, showing their potential anti-inflammatory effect (Park et al., 2016; Choi, Woo, Ham, & Lee, 2017) and their hepatoprotective, antioxidant, antimicrobial, and antidiabetic properties (Hyun, Eom, Yu, & Roitsch, 2010; Liu, Qiang, Sun, & Du, 2015; Kim et al., 2017).

Even having its pharmacological benefits recognized, the pseudo-fruit is not yet used in the food industry. Bampi et al. (2010), Maieves, Ribani, Morales and Sánchez-Mata (2015a) and Maieves et al. (2015b) studied the composition of extracts and pseudo-fruits and observed a considerable amount of dietary fiber, sugars and phenolic compounds, which represents a promising characteristic for its use in food production. However, just as the vast majority of fruits and unprocessed vegetables, the pseudo-fruit of Japanese grape has a very short shelf life, mainly due to its high-water activity. Thus, the drying technology is an effective method to increase the storage time of the pseudo-fruits and as an alternative for its use in foods.

Several authors have studied drying processes in the dehydration of fruits and vegetables: Sogi, Siddiq, Greiby and Dolan (2013) studied the total phenolic content, antioxidant activity and functional properties of the peel and the seed of the 'Tommy Atkins' mango affected by the methods such as freeze-drying, hot air drying, vacuum drying and infrared drying. Wojdylo, Figiel and Oszmiański (2009) studied the effect of drying methods with the application of vacuum microwave drying on the bioactive compounds, color and antioxidant activity of Strawberry tree fruits. Dermikol and Tarakci (2018) studied the use of freeze-drying, and of different temperatures of hot air drying for dehydration of grape to be used in yogurts. Nemzer, Vargas, Xia, Sintara and Feng (2018) evaluated the chemical composition and the physical properties of blueberries, cherries, strawberries and cranberries affected by different drying methods (freeze drying, hot air drying, and refractance window).

This study aimed to evaluate the chemical composition, the content of phenolic compounds and the antioxidant activity of pseudo-fruits of Japanese grape harvested at different maturation stages, as well as to verify whether the oven-drying methods with forced air convection and freeze-drying affect the chemical characteristics of the flour produced from these pseudo-fruits.

Material and methods

Harvest of pseudo-fruits

The pseudo-fruits of Japanese grape were harvested in 2018, in the city of Pinhalzinho, Santa Catarina state, Brazil (Latitude: 22° 46' 46'' S, Longitude: 45° 35' 26'' W), at three maturation stages, determined through the ratio (total soluble solids/total titratable acidity). Immature pseudo-fruits were harvested in January (ratio = 2.15), maturing pseudo-fruits in March (ratio = 4.70), and mature pseudo-fruits in April (ratio = 19.31).

The pseudo-fruits were sent to the Laboratory of Food Quality of the Santa Catarina State University (UDESC), Campus Pinhalzinho, where they were cleaned by removing branches and leaves, sanitized with a 200 ppm sodium hypochlorite solution for 15 min, rinsed under drinkable running water, and then the excess water was removed using paper towels.

Characterization of unprocessed pseudo-fruit of Japanese grape at different stages of maturation

The pseudo-fruits were evaluated after harvest, in triplicate, by determining: moisture (oven, 105°C to constant weight); lipids (948.22 method); protein (920.152 method); ashes (967.04 method); total dietary fiber (985.29 method); total soluble solids (°Brix) (932.14 (c) method); reducing sugars (% glucose) (923.09 method); nonreducing sugars (906.03 (c) method); pH (981.12 method); total titratable acidity (942.15 (a) method) of the Association of Official Analytical Chemists (AOAC, 2016). Total carbohydrates were calculated by difference. The evaluation of color was performed by colorimetry using a MiniScan EZ-Hunterlab® portable colorimeter, through the CIELAB system; in which the values of L^*, a^* and b^* were used to define a three-dimensional color space. The value of chroma was calculated using the formula $C^* = (a^{*2} + b^{*2})^{1/2}$; and the hue angle, using $h° = \tan^{-1} b^*/a^*$. The device was previously calibrated with black and white patterns (Commission Internationale de l'Éclairage [CIE], 2004, 2007). For the measurements, the pseudo-fruits were slightly crushed in a high-speed industrial blender (Spolu, Brazil), and packed in Petri dishes for analyses.

The total phenolic compounds were evaluated through the extract obtained according to the method described by Bochi et al. (2014), which uses an aqueous solution with 20% (v v-1) acetone and 0.35% (v v-1) formic acid for extraction of compounds. From this extract, the total content of phenolic compounds was evaluated (Singleton, Orthofer, & Lamuela-Raventos, 1999), through the reduction of the phosphotungstic and phosphomolybdic acids by phenolic hydroxyls, forming a blue complex (molybdenum), which absorbs energy at a maximum wavelength of 760 nm. The antioxidant activity was determined by oxygen radical absorbance/ORAC, using photosynthetic fluorescein protein, fluorescence marker, as target of the radicals, according to the method described by Prior et al. (2003).

Drying and characterization of pseudo-fruits of Japanese grape

The unprocessed pseudo-fruits were lightly crushed to be opened, and went through two drying processes to obtain a moisture content below 10%: a) drying at 50°C (± 1°C) in an oven with forced air convection system (EC-205) (Cienlab, Brazil); b) freeze-drying in a TFD 5503 lyophilizer (IlShin, Netherlands) at a pressure of 50 mTorr, and capacitor at -50°C, being the specimens previously frozen in a vertical Ultrafrezzer ULT 335/710 D (Indrel, Brazil) for 12 hours at -86°C.

The dry material was crushed in a high-speed industrial blender (Spool, Brazil), sieved (32 mesh), and from the flour obtained, moisture, color, total phenolic compounds and antioxidant activity were determined in triplicate, according to methods previously described.

Statistical analysis

The results of the physical and chemical measurements were submitted to analysis of variance (ANOVA), F-test, followed by comparison of means using the Tukey test, at a probability of 5%, with the software Statistica® 13.2 (Tulsa, USA).

Results and discussion

Characterization of unprocessed pseudo-fruits of Japanese grape

Table 1 shows the results of the analyses of unprocessed pseudo-fruits at three stages of maturation (R1, R2 and R3).

Table 1. Characterization (dry weight) of unprocessed pseudo-fruit of Japanese grapes at three stages of maturation.

Analyses	R1	R2	R3
Moisture (%)	69.94 ± 0.45^{a}	69.47 ± 0.68^{a}	62.70 ± 0.69^{b}
Ash (%)	6.01 ± 0.03^{a}	4.63 ± 0.13^{b}	4.51 ± 0.05^{b}
Protein (%)	20.12 ± 0.88^{a}	10.75 ± 0.99^{b}	9.34 ± 0.25^{b}
Ether extract (%	0.65 ± 0.18^{a}	0.34 ± 0.01^{b}	0.32 ± 0.01^{b}
Total dietary fiber (%)†	50.01 ± 4.09^{b}	73.30 ± 4.11^{a}	53.35 ± 8.61^{b}
Total carbohydrates (by difference) (%) β	73.22 ± 0.90	84.28 ± 1.00	85.83 ± 0.25
Total soluble solids (°Brix)	9.00 ± 0.29^{b}	9.20 ± 0.12^{b}	25.00 ± 0.62^{a}
Total titratable acidity (%)	4.20 ± 0.28^{a}	1.96 ± 0.12^{b}	1.29 ± 0.29^{c}
Ratio	2.14	4.69	19.38
Reducing sugars (%)	5.94 ± 0.04^{c}	9.05 ± 0.24^{b}	20.53 ± 1.24^{a}
Non-reducing sugars (%)	4.02 ± 0.54^{c}	9.10 ± 1.06^{b}	31.22 ± 2.74^{a}
Hue Angle°	97.49 ± 0.12^{a}	89.47 ± 0.14^{b}	79.79 ± 0.96^{c}
*L**	54.54 ± 0.65^{a}	52.09 ± 0.53^{b}	42.95 ± 0.44^{c}
*C**	27.72 ± 0.16^{a}	25.60 ± 0.83^{b}	21.78 ± 0.82^{c}

Mean ± standard deviation. R1, R2 and R3: respectively, immature, maturation, mature stage. †Fraction included in the carbohydrates. Hue Angle°, *L** and *C**: Color parameters. β standard deviation for propagation error. Means with different lowercase letters in the same column indicate significant difference ($p < 0.05$).

The moisture content of the samples ranged from 62.70 to 69.94%, and the lowest content was found in the most advanced stage of maturation (R3). A decrease in the ashes content, protein and ether extract was observed at the beginning of the maturation process of the pseudo-fruits ($p < 0.05$), but they remained constant until the last harvest (R3). As reported by Chitarra and Chitarra (2005), the analysis of ash is related to the amount of minerals such as N, K, P, Mg, and Ca present in the pseudo-fruit, which are influenced by climatic conditions, humidity and soil composition. According to these authors, the protein content measure also the enzymes that catalyze metabolic processes, which are important in the maturation. When the fruits are still "green", a greater protein synthesis occurs, in which new enzymes are synthesized to catalyze the maturation process, explaining the highest value of this parameter in R1 (20.12%).

All stages of maturation (R1, R2 and R3) showed high total dietary fiber content (ranging from 50.01 to 73.30%). These values are higher than those found by Maieves et al. (2015a), who observed total fiber content of about 57% in mature pseudo-fruits. According to the authors, most of this fiber content is in the form of soluble fiber, such as pectin. Considering the frequent search for products with high contents of these compounds, for their benefits are recognized, the Japanese grape is promising to use in food formulations rich in fibers due to its high total dietary fiber content.

Regarding the total soluble solid content, an increase was observed at the end of the maturation period (R3), in which the Brix of the pseudo-fruit was 25°. This value is similar to those found by Fernandes et al. (2015) and Garrido-Bañuelos et al. (2019) for different types of grape, which the pseudo-fruit of the Japanese grape becomes a possible raw material for fermentation processes, such as the production of wines.

For the reducing and non-reducing sugars, the R1 showed the lowest values (5.94 and 4.02%, respectively); however, a significant increase ($p < 0.05$) during the maturation process was observed, reaching percentages of 20.53 and 31.22%, respectively, in R3. These values are higher than those described by Bampi et al. (2010) (12.57% for reducing sugars and 6.89% for non-reducing sugars). The highest concentration of sugars in R3 explains the sweetness, which, associated with the degradation of acids observed in the significant reduction ($p < 0.05$) in the total titratable acidity along the stages (R1 > R2 > R3), evidences the maturation of the pseudo-fruit. In a study conducted by Maieves et al. (2015a), a higher concentration of sucrose (non-reducing sugars) has also been observed when compared with the concentration of glucose and fructose (reducing sugars). Thus, sucrose, can be considered the main sugar present in the Japanese grape.

The instrumental color of all the unprocessed pseudo-fruits was yellow (Hue angle of 97.49°, 89.47° and 79.79° for R1, R2 and R3, respectively); however, they were different ($p < 0.05$) regarding the intensity of their color (C^*) and lightness (L^*), higher for R1, R2 and R3, respectively (Figure 1). This behavior was already expected, because changes in coloration due to partial or total loss of green color (degradation of chlorophyll), development of the colors red, blue and/or yellow (by increasing the content of carotenoids), and a decrease in brightness, evaluated through the chromaticity (C^*) can be indicators of maturation of fruits (Williams & Benkeblia, 2018).

Figure 1. Unprocessed pseudo-fruit of Japanese grapes at the three stages of maturation (R1, R2, R3: respectively, immature, in maturation, mature).

Figure 2 shows the total phenolic compounds and antioxidant activity of unprocessed pseudo-fruits of Japanese grape. The contents of total phenolic compounds decreased significantly along the period of maturation ($p < 0.05$). In R1, the mean contents were 2176.93 mg of gallic acid 100 g^{-1} of sample and showed a reduction of more than 55% in R3 (1249.87 mg of gallic acid 100 g^{-1} of sample). However, even with this reduction, the contents were higher than those of fruits such as: gabiroba (275 mg of gallic acid 100 g^{-1} of pulp), cerrado cherry (*Eugenia calycina*) (327 mg of gallic acid 100 g^{-1} of pulp), and different varieties of grape (18.9 to 113.4 mg of gallic acid 100 g^{-1} of pulp) (Rocha et al., 2011; Fu-xiang, Fu-hua, Ya-xuan, Ran, & Jian, 2019).

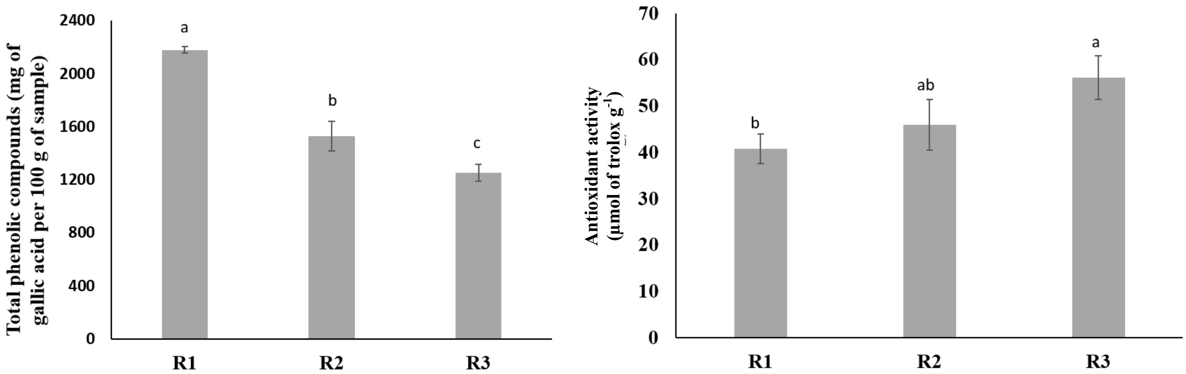

Figure 2. Phenolic compounds and antioxidant activity of unprocessed pseudo-fruit of Japanese grapes at the three stages of maturation (R1, R2, R3: respectively, immature, in maturation, mature). Different letters between the columns indicate significant difference ($p < 0.05$).

The influence of maturation is one of the factors described in the literature that influences the composition of fruits and vegetables (Chitarra & Chitarra, 2005), as this study confirmed with the results of physicochemical composition and phenolic compounds. In addition, other factors such as cultivation practices, geographical origin, stage of growth, conditions of harvest and storage process also contribute to variation in the composition of vegetables (Radović et al., 2020; Chitarra & Chitarra, 2005). Moreover, the reduction in phenolics during maturation can be related to the conversion of these soluble compounds into insoluble compounds, because they are esterified to polysaccharides in the cell wall of the fruits, and related to the loss of astringency, associated with an increase in the polymerization of leucoanthocyanidins and the hydrolysis of D-arabinose ester of hexahydroxy diphenic acid (Lewak, 1968; Benchikh, Louaileche, George, & Merlin, 2014).

The maturation of Japanese grape in R3 (mature) showed the highest antioxidant activity when compared with R1 (immature stage). Maieves et al. (2015b) also found an increase in antioxidant activity (analyzed by methods of FRAP, CUPRAC and ABTS) in mature pseudo-fruits of Japanese grape. In advanced stages, an increase in the antioxidant activity, even with the decrease in total phenolics compounds, can be attributed to the increase in carotenoids content, probably synthesized at the least stage of ripening (Maieves et al., 2015b), as explained to the increase in color yellowness. It is worth noting that the pseudo-fruits showed values of antioxidant activity higher than those of fruits such as apples, pears, and green grapes (25.72, 18.56 and 11.18 μmol of trolox g^{-1}, respectively) and lower than those of fruits such as blueberry and cranberry (61.84 and μmol of 92.56 trolox g^{-1}, respectively) (Wu et al., 2004).

Characterization of flour obtained from pseudo-fruits of Japanese grape after drying

Table 2 shows the results of the analysis of pseudo-fruits of Japanese grape after drying in an oven with air circulation (O) and lyophilizer (L), at the three stages of maturation (R1, R2 and R3).

Table 2. Moisture and color of flour of pseudo-fruits of Japanese grape at three stages of maturation and different drying methods.

Method of drying/maturation stage	Moisture (%)	° Hue	*L**	*C**
OR1	5.64 ± 0.03^c	86.45 ± 0.34^e	55.90 ± 0.38^d	22.87 ± 0.13^e
OR2	8.61 ± 0.14^b	87.27 ± 0.13^d	65.15 ± 0.22^c	21.16 ± 0.32^f
OR3	9.67 ± 0.41^a	79.35 ± 0.35^f	53.74 ± 0.80^e	28.12 ± 0.37^b
LR1	4.36 ± 0.11^d	100.51 ± 0.17^a	71.55 ± 0.25^b	26.62 ± 0.18^c
LR2	5.56 ± 0.02^c	98.08 ± 0.08^b	75.58 ± 0.54^a	24.20 ± 0.36^d
LR3	9.51 ± 0.31^a	89.62 ± 0.16^c	64.83 ± 0.51^c	29.46 ± 0.23^a

Mean ± standard deviation; OR1, OR2, OR3: in an oven dried at the three stages of maturation; L1, L2, L3: in a lyophilizer at the three stages of maturation. *L**, *C** and Hue°: Color parameters. Means with different lowercase letters in the same column indicate significant difference ($p < 0.05$).

All samples showed moisture content below 10%, compatible with microbiologically stable dehydrated foods (Gava, Silva, & Frias, 2009). The flour resulting from the pseudo-fruits freeze dried at the early stages of maturation (LR1 and LR2) showed higher values for lightness (*L**) than those dried in an oven, and yellow-greenish color (Hue angle of 100.51° and 98.08°, respectively) (Figure 3). Freeze drying removes water by sublimation of ice and prevents enzymatic browning reactions, resulting in a relatively stable color (Jia et al., 2019). The most mature pseudo-fruits (OR3 and LR3) showed the lowest values for the Hue angle, among the drying methods (79.62° and 89.62°, respectively), and the highest intensity in color (*C**). This behavior is opposite to that observed in the unprocessed pseudo-fruits (R3).

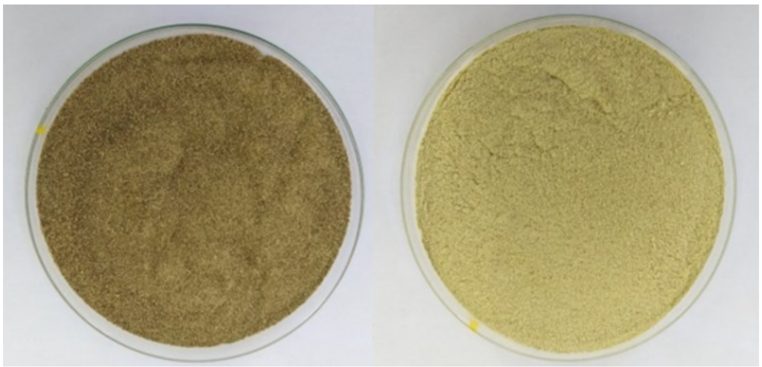

Figure 3. Flour of pseudo-fruit of Japanese grape dried in an oven (left) and in a lyophilizer (right) in R1 (Ratio 2.14).

According to Dermikol and Tarakci (2018), the variations in the parameters of color can be attributed mainly to structural changes caused by thermal degradation, such as porosity and shrinkage. This author also reports that the browning of fruits and vegetables during the drying process is caused by reactions of enzymatic and non-enzymatic browning. However, the presence of sugars in fruits could cause relative stability in the color parameters due to inactivation of the enzyme system.

Figure 4 shows the average of total phenolic compounds and antioxidant activity of flour obtained from the pseudo-fruits submitted to different drying methods during the 3 stages of maturation: in an oven dried (OR1, OR2 and OR3), and in a lyophilizer (LR1, LR2 and LR3). The content of phenolic compounds was higher ($p < 0.05$) in freeze-dried samples (9925.19, 15151.15 and 8265.04 mg of gallic acid 100 g^{-1} of sample for LR1, LR2 and LR3, respectively) when compared with the oven-dried samples (5918.34, 7276.39 and 6650.00 mg of gallic acid 100 g^{-1} of sample for O1, O2 and O3, respectively). In addition, despite a greater quantity of total phenolic compounds (Figure 2) in the unprocessed sample R1 (pseudo-fruits still green/immature), one noticed that the phenolic compounds present in the samples R2 and R3 (intermediate stage and final stage of maturation, respectively) were able to support the dehydration process and still preserve high amount in flour.

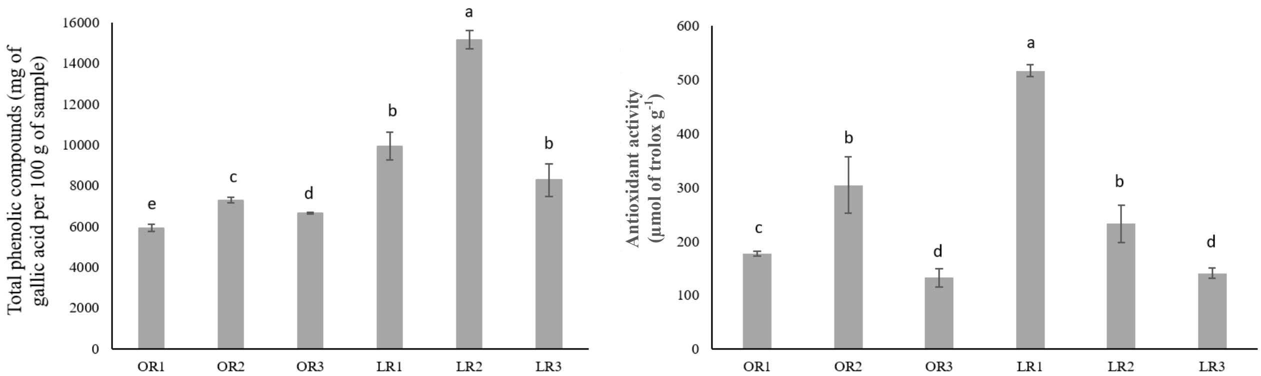

Figure 4. Phenolic compounds and antioxidant activity of processed pseudo-fruit of Japanese grapes at the three stages of maturation (OR1, OR2, OR3 in an oven dried; L1, L2, L3 in a lyophilizer, respectively). Different letters between the columns indicate significant difference ($p < 0.05$).

According to a study by Lopez et al. (2017), flour of grape wastes was submitted to two drying methods: freeze-drying and drying in oven, in which the flour dried by the freeze-drying method showed contents of total phenolic compounds higher than the oven-dried one, a trend already expected due to losses while drying in an oven caused by the sensitivity of these compounds to the temperature. According to Barcia et al. (2015), most of it accelerates degradation when the temperature increases.

For antioxidant activity, the flour obtained by the freeze-drying method (LR1) showed the highest value (516.74 µmol of trolox g^{-1} of sample), followed by OR2 and LR2 (304.29 and 232.56 µmol of trolox g^{-1} of sample, respectively), obtained at the second stage of maturation (R2). One highlights that, although the method of drying in an oven showed lower content of phenolic compounds and antioxidant activity, it shows a large amount of these compounds, proving to be a simple and less expensive method for obtaining dehydrated Japanese grape.

Nemzer et al. (2018) also found higher values of antioxidant activity, analyzed through the oxygen radical absorbance capacity (ORAC), in blueberry, cherry, cranberry and strawberry dried by the freeze-drying process when compared with those dried using the air circulation drying method. Moreover, the antioxidant activity found by these authors (ranged from 273.75 to 444.50 µmol of trolox g^{-1} of sample for freeze-drying and from 210.67 to 278.67 µmol of trolox g^{-1} of sample for air circulation drying method) was similar, and even lower than the one found in in some cases of this study. According to Nicoli, Anese, Parpinel, Franceschi and Lerici (1997), although some of the endogenous antioxidants in a sample can be destroyed during drying, the antioxidant properties of foods can be increased by the release of new antioxidant species resulting from the processing. They may increase through the formation of Maillard reaction secondary products (in Strecker degradation step), which, in this study, was probably more evident in oven drying (Dermikol & Tarakci, 2018).

Conclusion

The unprocessed pseudo-fruits of Japanese grape harvested at three stages of maturation differed regarding the chemical composition, especially the contents of fibers and sugars, and they show a considerable content of phenolic compounds present in the early maturation stages with higher antioxidant activity when mature, being interesting raw materials for application in the food industry. In general, the freeze-drying method provided a more stable color and was able to preserve a greater number of bioactive compounds in the flour from the pseudo-fruits compared with drying in an oven.

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