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ORIGINAL ARTICLE

Effect of drying methods on nutritional constitutes of fermented grape residue

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Abstract One of the biggest hurdles faced by the wine industry is the disposal of residual biomass generated after vinification. Although this residue is biodegradable, it constitutes a potential source of environmental pollutants. To alleviate this issue, this biomass may be used in alternative applications; for example, it may be transformed into an enriched flour that can be used to improve the nutrient content in different foods. In this study, were evaluated the effects of drying processes on the relevant nutritional components in dry extracts obtained from the residue of fermented grape pomace. The concentrations of phenolic compounds and anthocyanins were higher when drying the flour by the traditional oven procedure than by freeze-drying. The highest difference (approximately 40%) was observed for tannin compounds. Therefore, drying in an oven is recommended due to the lower loss of bioactive compounds, in addition to being simple and cheap.

Keywords Wine residue · Grape pomace · Drying processes · Antioxidant compounds

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Introduction

The world's largest wine producers in 2019 were Italy, France, and Spain with 5.4, 4.9, and 4.4 billion liters produced in that year, respectively (CONAB 2019).

The wine industry generates residues both before (leaves and stems) and after (husks and seeds) the vinification process, where the production of 100 L of wine generates 31.7 kg of residue containing 20 kg of bagasse. The contents of the residue depend upon the vinification process and the type of extraction process employed. The main by-product of vinification is fermented grape pomace, which contains 16.8–24.2% lignin, 37–54% pectic substances, 27–37% cellulose, 72–76% unsaturated fatty acids (m/m) tocopherols from the oil present in the seeds and phenolic compounds (Rani et al. 2020).

Although post-winemaking biomass residue is biodegradable, its disposal is of concern as it contains a considerable level of phenolics, which act as inhibitors in seed germination. The aerobic and anaerobic degradation processes of the residue can also release greenhouse gases. Furthermore, the liquid part of the bagasse (containing a high amount of organic matter, biological and chemical oxygen demand, and total organic carbon) is a source of environmental pollutants (Silva et al. 2018; Rani et al. 2020). This problem could be solved with the transformation of fermented grape pomace into flour. The produced flour could be used in the food industry because it contains high amounts of dietary fiber (glycans, cellulose, and pectins) as well as polyphenols, which are relevant sources of phytochemicals (Silva et al. 2018).

During the winemaking process, although some of the phenolic compounds get transferred to the wine, most of the compounds reside in the rigid structures of the fruits (peels and seeds) and thus remain in the final residue

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(Barcia et al. 2014). Phenolic components are important because they remove free radicals, thus preventing the associated initiation, propagation and β-cleavage reactions. Phenolic compounds are classified as flavonoids (catechins, epigallocatechin, spicetachins, tannins, kaempferol, quercetin, myricetin, and anthocyanins) and non-flavonoids (phenolic, hydroxybenzoic, and hydroxycinnamic acids). Resveratrol and polyphenols belong to the class of stilbenes (Acosta-Estrada et al. 2014).

In the literature, studies using the freeze-drying process have been reported without investigating how much this process interferes with the loss of bioactives, such as Rockenbach et al., 2011 in Castas Cabernet Sauvignon, Merlot and Bordopara grape pomaces in the analysis of compounds phenolics and antioxidants.

In this study, the effects of different drying processes were evaluated on the relevant nutritional components present in dry extracts obtained from the residue of fermented grape pomace.

Materials and methods

Dry extract preparation

The residue employed in these studies was taken from the production of dry red table wine from the *Vitis labrusca* species, Bordo variety. The batch of grapes had been harvested in January 2019, where went through the traditional winemaking process of a winery located in Piracicaba (22° 43′ 30″ S, 47° 38′ 51″ W, southeast of Brazil).

An oven with forced air circulation (Marconi, MA 037) at 60 °C for 24 h and a freeze-dryer (LIOTOP, L108) with a capacity of 8 kg of ice programmed to a pressure of 0.12 mbar and a temperature of − 40 °C for 96 h were employed in the drying process. AOAC (2005) methodologies were used for oven drying and Rockenbach et al., 2011 for freeze-drying.

The samples obtained by drying in an oven (denoted GMFO) and a freeze-dryer (denoted GMFL) were ground in a knife mill, passed through a sieve (mesh 20), packed in polypropylene bags, and sealed. The water loss was found to be 80% in both samples. To maintain the humidity, the GMFO and GMFL samples were stored in a refrigerator (8 °C) and a freezer (− 18 °C), respectively, due to the hydrophilic character of the grape pomace. The ideal humidity being below 15% according to current legislation in Brazil (Botelho et al. 2018) and as performed by Rockenbach et al., 2011 that store the freeze-drying samples in a freezer.

Quantification of compounds

Spectrophotometry analyses (AGILENT, Cary 60) were performed to quantify the anthocyanins, tannins, and phenolic compounds in the samples. The quantification of resveratrol was conducted by high-performance liquid chromatography (HPLC, AGILENT, 1100 Series). All solutions were prepared with analytical grade chemicals and deionized water (conductivity of 18.2 MΩ cm at 25 °C).

Anthocyanins

To the GMFO and GMFL samples (0.250 and 0.050 g, respectively), 25 mL of 0.05 mol L^{-1} of potassium chloride, pH 1.5 was added to obtain Extract I. To the same weight of the samples, 25 mL of 0.66 mol L^{-1} of sodium acetate, pH 4.5 was added to give Extract II. After 30 min without light exposure, the extracts were centrifuged for 15 min at 4000 rpm and spectrophotometric measurements were performed at 510 and 700 nm (Fuleki and Francis 1968). The quantity of anthocyanins in each sample was expressed as cyanidin-3-glucose in grams per 100 g of sample.

Tannins

The quantification of tannins was based on the methodology reported by Price et al. (1980), who performed the extraction with methanol and the colorimetric reaction with 1% (m/v) vanillin solution and 8% (v/v) HCl in a 1:1 ratio.

The GMFO and GMFL sample extracts were obtained at 0.051 and 0.202 g, respectively, in 10 mL of methanol. After stirring using an orbital shaker (200 rpm) and centrifugation (4000 rpm) for 20 min each, a 100 µL aliquot of the sample was added to 5 mL of the reagents. After 20 min, spectrophotometric measurements (500 nm) were performed. The standard curve was prepared with catechin solution, and the results were expressed in grams per 100 g of sample.

Total Phenolic

The sample extracts (GMFO and GMFL) were obtained by adding 1.00 g of the sample to 20 mL of methanol. The mixtures were stirred using an orbital shaker (200 rpm) and centrifuged (4000 rpm) for 20 min each. After 5 min, to 600 µL of extract, 3,000 µL of 10% (v/v) Folin-Ciocalteu reagent and 2,250 µL of 7.5% (w/v) potassium carbonate were added. Spectrophotometric measurements (770 nm) were carried out after keeping the samples in the dark for 40 min. The results were expressed in grams of gallic acid per sample (Singleton et al. 1999).

Resveratrol

The methodology of Ribeiro de Lima et al. (1999) was employed for the quantification of resveratrol. First, 0.500 g of each sample (GMFO and GMFL) was taken in 20 mL of ethyl acetate. After solvent evaporation over approximately 24 h, the sample was resuspended in 7 mL of methanol and filtered through a cellulose acetate membrane (0.45 μm).

Chromatographic analyses were carried out with 20 μL of sample in a C18 column (4.6 × 250 mm, 5 μm) and a mobile phase of water—the pH of which was adjusted using phosphoric acid (pH 2.50 ± 0.05)—and acetonitrile (60:40 v/v) in isocratic elution. The peak at 306 nm was monitored and the results were expressed in milligrams of resveratrol per 100 g of sample (Souto et al. 2001).

Statistical analysis

Once the content of the investigated compounds was calculated, the results were subjected to Shapiro–Wilk, Levene, and Student's t-tests at a significance level of $\alpha = 0.05$, using the RStudio software (Version 1.3.1093).

Results and discussion

The chromatographic profile obtained from the analytical standard allowed for the identification and quantification of resveratrol in the samples. As shown in Fig. 1, the compound of interest identified to be resveratrol was eluted in 4.39 min.

Calibration curves were constructed based on analytical standards for known concentrations of tannins, total phenolics, and resveratrol, and the detection and quantification limits were estimated (Table 1).

The results obtained in the analysis in triplicate expressed in grams per 100 g of sample, are shown in Table 2. Based on the Shapiro Wilk test, the results obtained were found to have a normal data distribution. In the Levene test, the data obtained for the levels of anthocyanins, tannins, and total phenolics showed homogeneous population variance, while it showed heterogeneous population variance in the case of the resveratrol analysis data. Thus, it was necessary to apply this information to the Student's test using the RStudio software. Averages followed by the same letter, on the line, do not differ by Student's t-test at the 95% confidence level ($\alpha = 0.05$). The concentrations of the evaluated compounds (anthocyanins, tannins, total phenolics, and resveratrol) in the residue obtained by different drying processes showed significant differences, with higher values observed in the residue obtained after oven drying at 60 °C.

The values of anthocyanins in the residues of both drying processes in this research, as shown in Table 2, did show significant differences, at 89.15 and 82.32 mg 100 g^{-1} for the oven-dried and freeze-drying sample, respectively.

Anthocyanins are natural compounds found in abundance (0.1–1.0%) dry weight (Pojer et al. 2013) in red, blue, or purple fruits and vegetables, which can decrease

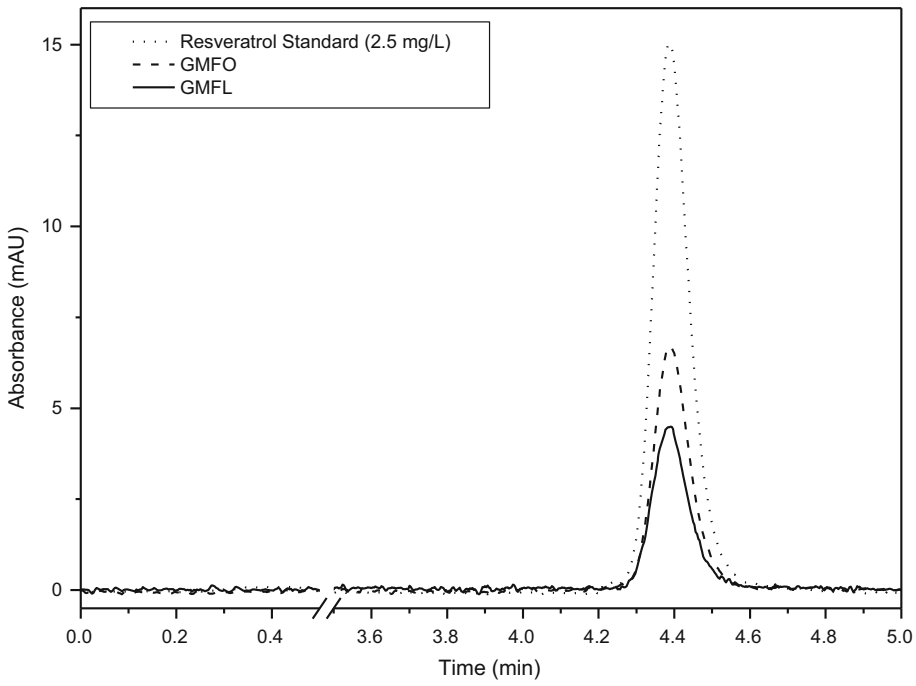

Fig. 1 Chromatographic profile obtained for sample and identification of the resveratrol at 306 nm. GMFO: dry sample in an oven and GMFL: freeze-dried sample

Table 1 Description of the reference solutions evaluated by spectrophotometry and chromatography used to quantify the compounds in the samples

Compound	Linear response	Equation	R^2	LQ	LD
Tannins (mol L^{-1})	0.00–0.16	A = 0.041 + 2.69 C (mol L^{-1})	0.999	0.031	0.010
Total Phenolic (mg L^{-1})	0.00–50.00	A = 0.032 + 0.0099 C (mg L^{-1})	0.994	8.52	2.81
Resveratrol (mg L^{-1})	0.50–2.50	Peak area = 0.10 + 44.47 C (mg L^{-1})	0.998	0.57	0.19

R^2: correlation coefficient; LD: detection limit and LQ: quantification limit

Table 2 Mean values and standard deviations (n = 3) for the determination of chemical compound in dry samples

Compounds quantification	Shapiro–Wilk (*p*-value)	Levene (*p*-value)	Student t (*p*-value)	Mean values	
				GMFO	GMFL
Anthocyanins (mg 100 g^{-1})	0.44	4.99×10^{-1}	2.25×10^{-2}	89.15 ± 1.08a	82.32 ± 3.09b
	0.22				
Tannins (g kg^{-1})	0.14	6.62×10^{-1}	1.93×10^{-8}	25.70 ± 0.20a	9.30 ± 0.10b
	0.66				
Total Phenolics (g 100 g^{-1})	0.80	8.88×10^{-1}	8.53×10^{-5}	8.37 ± 0.21a	7.04 ± 0.19b
	0.09				
Resveratrol (mg 100 g^{-1})	0.10	3.18×10^{-4}	3.67×10^{-3}	2.43 ± 0.56a	1.68 ± 0.12b
	0.45				

GMFO: dry sample in an oven and GMFL: freeze-dried sample

the number of free radicals in the cells of the human body, thus preventing aging (Lila et al. 2016). These compounds also act as neuroprotectors in the cardiovascular system and against inflammation (He and Giusti 2010; Tsuda 2012).

The amount of anthocyanin in the freeze-dried samples (GMFL) was equivalent to 82.32 mg 100 g^{-1} of fermented bagasse, which is smaller than that found by Rockenbach et al (2011) of 1,122 mg 100 g^{-1} using the same process. The use of an air circulation pump in the fermentation reassembly process (mixing of the lower and the upper parts) may be responsible for this difference; the wine used for this work, the reassembly was manual, impairing the anthocyanin content in the samples. However, the anthocyanin found in the oven-dried samples (GMFO) was 89.15 mg 100 g^{-1}, which was similar to that reported by Bennemann et al (2016) (0.9–114.7 mg 100 g^{-1}). In this work, the reassembly process and different factors such as terroir and physicochemical factors (temperature and pH) that can influence the concentration of this chemical species were not considered.

The values of tannin concentration were estimated to be 9.3 and 25.7 g kg^{-1} in grape pomace for freeze-drying and oven-dried samples, respectively, demonstrating the negative effect of the freeze-drying process. Tannins are found predominantly in the seeds and bark of plants and provide defense against pests. Tannins are abundant in tree trunks, owing to which the use of the latter in wooden barrels is important for the wine aging process (Serrano et al. 2009). The tannin concentrations in studies reported in the literature range from 4.3 to 70.5 g kg^{-1} in grape pomace depending on the type of grape and the process of wine production (Alipour and Rouzbehan 2007; Abarghuei et al. 2015).

The biological properties of tannins depend on their bioavailability because their absorption through metabolism occurs in different parts of the gastrointestinal system. Tannins act as antioxidant, antimicrobial, and antiviral agents. In addition to the ability to induce intracellular, modular signaling pathways for genes, tannins also have systemic effects on some organs (Serrano et al. 2009).

In general, phenolic compounds have several biological effects on the human body, including antioxidant, antimicrobial, anti-inflammatory, and vasodilatory effects. As mentioned by Beres et al. (2019), more than 70% of the polyphenols present in grapes remain in the bagasse after fermentation, similar to some flavonoids such as tannins, phenolic compounds, and anthocyanins (Bennemann et al. 2016).

The amount observed in this study, shown in Table 2 (8.37 and 7.04 g 100 g^{-1} for oven-dried and freeze-drying, respectively), was above the value found in solar-dried

fermented residues of Bordo by Bennemann et al. (2016) of 2.4 g 100 g^{-1}. Since these species are easily degraded, differences in the amounts of these compounds can be observed with the use of different drying processes.

Studies have shown that resveratrol has antimicrobial, anti-inflammatory, neuroprotective, anti-aging, anti-cancer, and antioxidant activities, relieves oxidative stress, elevates autophagy, prevents the accumulation of body fat, and reduces the risk of cardiovascular disease and metabolic diseases (Salehi et al. 2018; Breuss et al. 2019; Schlich et al. 2020). It is reported in the literature that resveratrol can decrease the occurrence of cell death caused by hypoxia by reducing the number of reactive oxygen species, thus preventing neurodegeneration (Akyuva and Nazıroğlu 2020).

The levels of resveratrol in this study 2.43 (GMFO) and 1.68 mg 100 g^{-1} (GMFL) showed significant differences depending upon the type of drying (Table 2). However, the values estimated were within the range cited by Rockenbach et al. (2011), 1.18 mg to 6.40 mg 100 g^{-1} of grape marc. The concentrations found by Careri et al. (2003) and Brezoiu et al. (2019) were 6 µg g^{-1} and 0.033 to 0.049 mg g^{-1} of resveratrol in *Nero d'Avola* and *Cabernet Sauvignon* or *Feteasca neagra* grape marc, respectively. These differences are associated with the type of grape used in the production of wines.

The product's porosity is associated with the solvent diffusion process, thus allowing the solvent to have greater contacting the sample and performing a more efficient extraction. Although the freeze-drying process produces dry products with greater porosity (80 to 95%) than in conventional drying (> 80%), the effect of temperature was responsible for the decrease of bioactive compounds present in the GMFL sample. Other factors that could interfere in the experiment were kept constant for both process, such as solvent type; product structure; particle size and extraction temperature (Çoklar and Akbulut 2017).

Furthermore, Larrauri et al. (1997) evaluated the over-dried process (at temperatures of 60, 100 and 140 °C) in grape pomace and concluded that the temperature of 60 °C was the most adequate. Çoklar and Akbulut (2017) evaluated the process of freeze-drying; over and sun drying on fresh grapes and the drying process in an oven was a more viable alternative due to lower losses of bioactive compounds and lower cost of the process.

Conclusion

Flour produced from wine residue is useful in the food industry because of its richness in several components beneficial to human health, and can be used to fortify various foods, characterizing them as functional supplements.

The drying method used to proves the samples can significantly influence the concentrations of the available components in the residue. Samples dried by freeze-drying contained lower concentrations of all components (anthocyanins, tannins, phenolic compounds, and resveratrol) than those dried in an oven at 60 °C. Therefore, drying in an oven is preferable; in addition to preserving the components, it also requires cheaper instrumentation and can be used to process several samples at the same time.

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Declaration

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