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Effect of temperature on the degradation of bioactive compounds of *Pinot Noir* grape pomace during drying

Efeito da temperatura na degradação de compostos bioativos do bagaço de uva Pinot Noir durante a secagem

Aline Soares Cascaes Teles[1]*, Davy William Hidalgo Chávez[1], Flávia dos Santos Gomes[2], Lourdes Maria Corrêa Cabral[2], Renata Valeriano Tonon[2]

[1] *Universidade Federal Rural do Rio de Janeiro (UFRRJ), Departamento de Tecnologia de Alimentos (DTA), Seropédica/RJ - Brazil*

[2] *Embrapa Agroindústria de Alimentos, Rio de Janeiro/RJ - Brazil*

***Corresponding Author**

Aline Soares Cascaes Teles, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Departamento de Tecnologia de Alimentos (DTA), Rodovia BR 465, km 07, s/n, Zona Rural, CEP: 23890-000, Seropédica/RJ - Brazil, e-mail: aline_cascaes@yahoo.com.br

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Abstract

The objective of this study was to evaluate the effect of temperature and drying time on the total phenolic content and antioxidant capacity of grape pomace. Experimental data of the drying kinetics were fitted to six mathematical models and the Page model was selected as the most suitable to represent the drying of grape pomace. The best preservation of the phenolic compounds and antioxidant capacity was observed at 60 °C, suggesting that the temperatures of 40 °C and 50 °C were not sufficient to inactivate the enzyme polyphenol oxidase, being responsible for the greater degradation of these compounds.

Keywords: *Antioxidant capacity; Drying kinetics; Mathematical modeling; Water activity; Polyphenol oxidase.*

Resumo

O objetivo deste estudo foi avaliar o efeito da temperatura e do tempo de secagem sobre o conteúdo de compostos fenólicos totais e a capacidade antioxidante do bagaço de uva. Os dados experimentais das cinéticas de secagem foram ajustados a seis modelos matemáticos. O modelo de Page foi selecionado como o mais adequado para representar a secagem do bagaço de uva. A melhor preservação dos compostos fenólicos e da capacidade antioxidante foi observada a 60 °C, sugerindo que as temperaturas de 40 °C e 50 °C não foram suficientes para inativar a ação da enzima polifenoloxidase, sendo estas as responsáveis pela maior degradação desses compostos.

Palavras-chave: *Capacidade antioxidante; Cinética de secagem; Modelagem matemática; Atividade de água; Polifenoloxidase.*

1 Introduction

Grape pomace is the main by-product of the grape juice and wine industries, consisting of skin, seeds and stalks. According to FAO (2013), the world generation of winemaking waste in 2013 was approximately 3.8 million tons, resulting in a large volume that may become an environmental and economic problem.

According to Ratnasooriya and Rupasinghe (2012), about 70% of the phenolic compounds present in grapes are trapped in the grape pomace after the winemaking process, resulting in a waste rich in bioactive compounds with anti-inflammatory, anticarcinogenic, antimicrobial, antimutagenic and antioxidant potential (PÉREZ-JIMÉNEZ et al., 2008). In addition, winemaking by-products are a good source of fibers, which, compared to cereal fibers, are of better quality, due to the presence of natural antioxidants (JARA-PALACIOS et al., 2013).

Due to the high content of phenolic compounds present in grape pomace, considerable effort has been employed to optimize the extraction of these compounds and their

use in food, pharmaceutical and cosmetic industries (MONAGAS et al., 2006; LLOBERA; CANELLAS, 2007).

However, even after extraction, a large volume of solid waste is generated, which still represents an environmental and economic problem. In this sense, drying can be suggested as an alternative to grape pomace processing, resulting in a product rich in phenolic compounds and fibers, which can be used as an ingredient in the formulation of other food products.

Convective drying using hot air is one of the most common drying techniques, being considered less expensive when compared to other dehydration methods such as freeze-drying or vacuum-drying. One of the most important process variables during convective drying is the air temperature. This variable is directly related to mass and heat transfer and, in the case of thermo-sensitive compounds, can be responsible for the preservation or degradation of these compounds.

Several studies have evaluated the drying kinetics of grape pomace using different drying methods, as well as the effect of drying on the bioactive compounds and antioxidant capacity of the final products. Vashisth et al. (2011) evaluated the use of three drying technologies (vacuum belt drying, hot air drying and freeze drying FD) as applied to muscadine pomace, and their impact on drying time, moisture content, water activity, total phenolic content and antioxidant activity of the dried pomace. Sui et al. (2014) studied the effects of infrared drying and/or convective drying on the drying kinetics of wine grape pomace, evaluating the drying characteristics, sterilizing efficacy, and the effects on the pomace polyphenol and pro-anthocyanidin contents. Tseng and Zhao (2012) evaluated the effects of conventional, vacuum oven and freeze drying on the phenolic content and antiradical scavenging activity stability of grape pomace. However, none of these works reported the degradation of bioactive compounds throughout the drying process, which could be useful to detect fluctuations in the contents of these compounds during drying, as well as to determine the time at which significant changes in this parameter begin, if any.

Thus the objective of this work was to evaluate the effect of drying temperature on the drying kinetics and bioactive compound contents and antioxidant capacity of Pinot noir grape pomace during drying.

2 Materials and methods

2.1 Material

Grape pomace was obtained from Pinot Noir grape winemaking processed by Aurora (Bento Gonçalves, RS). After manual seed separation, the pomace (skin and stems) was ground in a laboratory grinder.

2.2 Convective drying

For drying, a convective tray dryer with perpendicular air flow and an air velocity of 0.42 m/s was used. The system consisted of a dryer with input and output air flow promoted by the rotation of an internal blower, with air heating promoted by incandescent lamps. The grape pomace was arranged in one tray (0.50 × 0.75 m), forming a layer with approximately 0.015 m in height. Since the sample thickness was considerably smaller than the tray dimensions (thickness: diameter ratio <0.1), the sample behaviour was considered as a semi-infinite plate for the modelling of the experimental data (AZUARA-NIETO et al., 2003). Drying was carried out at three temperatures (40 °C, 50 °C and 60 °C). The samples were weighed on a semi-analytical balance (resolution of 0.001g) every 15 minutes during the first hour of processing, every 30 minutes for the next two hours and then every hour until the weight changes were insignificant. The drying kinetics was evaluated as the dimensionless moisture, MR (Equation 1).

$$MR=\frac{X^{*}-X_{e}^{*}}{X_{i}^{*}-X_{e}^{*}} \quad (1)$$

Where: X* is the average moisture content at a given time; X^{*}_{e} is the equilibrium moisture content (dry basis) and X^{*}_{i} is the initial moisture content (dry basis).

2.3 Mathematical modelling

The experimental data were adjusted using six models (Table 1). The Fick model (Equation 2) was applied considering that water migration to the surface occurred only by diffusion, that the temperature and water diffusivity were constant and that shrinkage was negligible (CRANK, 1975). Due to the long drying period (25 hours), only one term of the equation was used. The other empirical and semi-empirical models (Equations 3 to 7), commonly used to fit drying kinetics data, are also based on the Second

Table 1. Mathematical models used to fit the grape pomace convective drying data.

Model	Equation	
Fick	$Ln(MR)=Ln\left(\frac{8}{\pi^2}\right)-\frac{\pi^2 D_{ef} t}{4L^2}$	(2)
Herderson & Pabis	$MR=a\exp(-kt)$	(3)
Page	$MR=a\exp(-kt^{n})$	(4)
Logarithmic (One term)	$MR=a\exp^{(-kt)}+c$	(5)
Logarithmic (Two terms)	$MR=a\exp^{(-kt)}+c\exp^{(-gt)}$	(6)
Verma	$MR=a\exp^{(-kt)}+(1-a)\exp^{(-gt)}$	(7)

MR = moisture ratio (dry weight); D_{ef} = effective diffusivity (m^2s^{-1}); t = time (s); L = thickness of the material to be dried; a, c, g, k and n = model constants.

Fick's law and involve the dependence of a drying constant (k) on the process time (t).

The fit of the models to the experimental data was evaluated using the sum of the squared residual (SSR) and the determination coefficient (R^2) as the statistical parameters. Modelling was carried out by non-linear regression using the SigmaPlot 8.0 software.

2.4 Moisture content and water activity

The moisture content was determined by drying in a vacuum oven at 65 °C to constant weight (HELRICH, 1990). The water activity (a_w) was determined using an Aqualab digital hygrometer (Decagon Devices, Pullman Inc. USA) at 25 °C.

2.5 Degradation of bioactive compounds

In order to evaluate the effect of convective drying on the bioactive compounds of the grape pomace, the levels of total phenolic compounds and the antioxidant capacity of the samples taken at predetermined times throughout the drying process, were evaluated.

The phenolic compounds were extracted with two aqueous solutions: one with 70% acetone and the other with 50% methanol, in a ratio of grape pomace: acetone: methanol of 1:20:20. The phenolic compound content was determined by the spectrophotometric method proposed by Singleton and Rossi (1965) and modified by Georgé et al. (2005), using the Folin-Ciocalteu reagent, reading the absorbance at 760 nm. The results were expressed as g/100g of gallic acid, on a dry weight basis.

The antioxidant capacity was measured according to the methodology described by Re et al. (1999). Absorbance readings were performed at 734 nm, 6 minutes after the addition of the extract, and results were expressed in µmol TEAC/g (Trolox Equivalent Antioxidant Capacity), in a dry basis.

2.6 Statistical analysis

All the experiments were carried out in triplicate and the data reported on a dry basis as the mean ± standard deviation. The treatments were analyzed in relation to significant differences by the ANOVA and Tukey tests in order to compare the means at a significance level of 5% ($p < 0.05$) using the Statistica software, version 6.0.

3 Results and discussion

3.1 Drying kinetics and mathematical modeling

Figure 1 shows the effect of different temperatures (40 °C, 50 °C and 60 °C) on the drying kinetics of the grape pomace. As expected, there was a decrease in moisture content (initial moisture on a dry weight basis

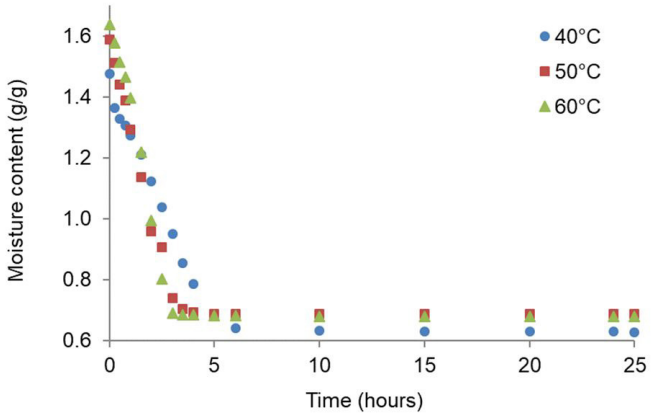

Figure 1. Moisture content (dry weight basis) during the drying of grape pomace at different temperatures.

1.47 ± 0.01; 1.59 ± 0.01 and 1.64 ± 0.01, respectively) throughout the three treatments.

The equilibrium moisture content was reached at different process times, depending on the drying temperature: samples dried at 40 °C, 50 °C and 60 °C showed no variation in the moisture content values after 360, 240 and 180 minutes, respectively. The highest temperature resulted in the shortest drying time (Figure 1), due to the higher heat and mass transfer occurring under this condition. This ehaviour was also observed during the drying of tomato slices, pears and grape pomace (DJENDOUBI et al., 2012; FERREIRA et al., 2012; SANTOS-SÁNCHEZ et al., 2012). A reduction in the drying time is associated with an increase in the drying rate, which depends on the heat transfer between the drying air and the material to be dried, resulting in better evaporation of the water from the material (AMIRI CHAYJAN et al., 2015).

All the treatments showed significant differences in relation to the final a_w values, which were 0.601 ± 0.01, 0.270 ± 0.01 and 0.135 ± 0.01 for the pomace dried at 40 °C, 50 °C and 60 °C, respectively. The initial grape pomace a_w was 0.971 ± 0.01, and hence reductions of 38.11%, 72.16% and 86.13% were observed in the processes carried out at 40 °C, 50 °C and 60 °C, respectively, demonstrating that higher temperatures allowed for greater reductions in the available water present in the raw material.

The values obtained from the mathematical modelling at the different drying temperatures, as well as the parameters (R^2 and SSR), are shown in Table 2. The R^2 value was higher than 0.95 for all settings, except for the Fick model at 60 °C (0.940), while the SSR values were lower than 0.4 for all models and treatments.

Since the Page model presented the highest R^2 values (0.986 to 0.996) and the lowest SSR values (0.012 to 0.036), it showed the best fit and was selected to represent the experimental data of the grape pomace drying kinetics (Figure 2). This model was also used by several authors to describe the drying processes of grape

Table 2. Results of the mathematical modelling of the experimental data at different temperatures.

Model	Temperature (°C)	Model constants	R^2	SSR
Fick	40	D_{ef}= 6.341x10^{-9}	0.965	0.190
	50	D_{ef}= 1.156x10^{-8}	0.963	0.251
	60	D_{ef}= 1.143x10^{-8}	0.940	0.370
Henderson & Pabis	40	a= 1.017; k= 0.336	0.976	0.056
	50	a= 1.095; k= 0.623	0.975	0.065
	60	a= 1.131; k= 0.629	0.954	0.129
Page	40	k= 0.242; n= 1.292	0.986	0.036
	50	k= 0.400; n= 1.568	0.996	0.012
	60	k= 0.296; n= 2.020	0.996	0.014
Logarithmic (One term)	40	a= 1.040; k= 0.320; c= -0.026	0.977	0.053
	50	a= 1.120; k= 0.585; c= -0.031	0.976	0.058
	60	a= 1.164; k= 0.587; c= -0.039	0.955	0.118
Logarithmic (Two terms)	40	a= 0.515; k= 0.335; c= 0.503; g= 0.338	0.976	0.056
	50	a= 0.572; k= 0.623; c= 0.523; g= 0.623	0.975	0.065
	60	a= 0.593; k= 0.630; c= 0.537; g= 0.630	0.954	0.129
Verma	40	a= 0.662; k= 0.329; g=0.329	0.976	0.057
	50	a= 0.230; k= 0.566; g= 0.565	0.976	0.084
	60	a= 0.873; k= 0.555; g= 0.555	0.955	0.167

R^2 = coefficient of determination; SSR= sum of the residual squares; D_{ef} = effective diffusivity (m^2s^{-1}); a, c, g, k and n = models constants.

pomace, apricot and fresh pepper (TUNDE-AKINTUNDE, 2011; FERREIRA et al., 2012; IGUAL et al., 2012).

The other models were not as predictive as the Page model, with lower R^2 and SSR values. One explanation for these results may be the assumption of the grape pomace layer as a homogeneous layer, which is not totally true, since there are some "spaces" between the pomace particles, which can affect the drying process, making it less homogeneous.

However, the mathematical Fick model (Equation 2), which is the basis for all the other empirical and semi-empirical models, can also be used to provide relevant information on the drying process, such as the effective diffusivity of water from the product. This information can be useful for the optimization of drying processes and construction of industrial dryers. The Fick model is related to the behaviour of the exit of water from the material. Therefore, the effective diffusivity values indicate the speed with which the water leaves the product, being higher at higher temperatures. In the present study, drying at 50 and 60 °C resulted in very similar effective diffusivities, considerably higher than that observed at 40 °C, indicating that higher temperatures favoured water diffusion inside the product. The values found for diffusivity were within the range from 10^{-12} to 10^{-8}, which is common for biological materials (DOYMAZ, 2017), and were higher than those observed by Arslan and Özcan (2010) (10^{-11} to 10^{-9}) for the convective air drying of food materials.

3.2 Degradation of bioactive compounds

The bioactive compounds were evaluated during 6 hours of drying, since after this time, no significant variations were observed in the sample moisture contents. The initial phenolic content of the grape pomace was 2.82 ± 0.24 g GAE.100g^{-1} (d.b.) and the antioxidant capacity was 165.05 ± 15.20 µmol Trolox.g^{-1}(d.b.).

Figure 3 shows slight reductions in the phenolic compound contents and antioxidant capacities at all temperatures throughout the drying process. Some differences can also be seen between the processes carried out at different temperatures. The phenolic compound contents of samples dried at 60 °C were slightly higher than the values observed for samples dried at the other temperatures. Regarding the antioxidant activity, the differences were more pronounced: samples dried at 60 °C showed the highest antioxidant activity, followed by samples dried at 50 °C, while the grape pomace dried at 40 °C showed the lowest values. These results are contrary to some studies that reported higher degradation of bioactive compounds and antioxidant activity with increase in temperature during the drying of fruits, as well as negative effects of heat drying on the antioxidant capacity of plants (GARCIA-PEREZ et al., 2010; SUVARNAKUTA et al., 2011; DJENDOUBI et al., 2012).

According to Figure 3, in general, the process performed at 60 °C resulted in greater phenolic compound retention and antioxidant capacity throughout the process,

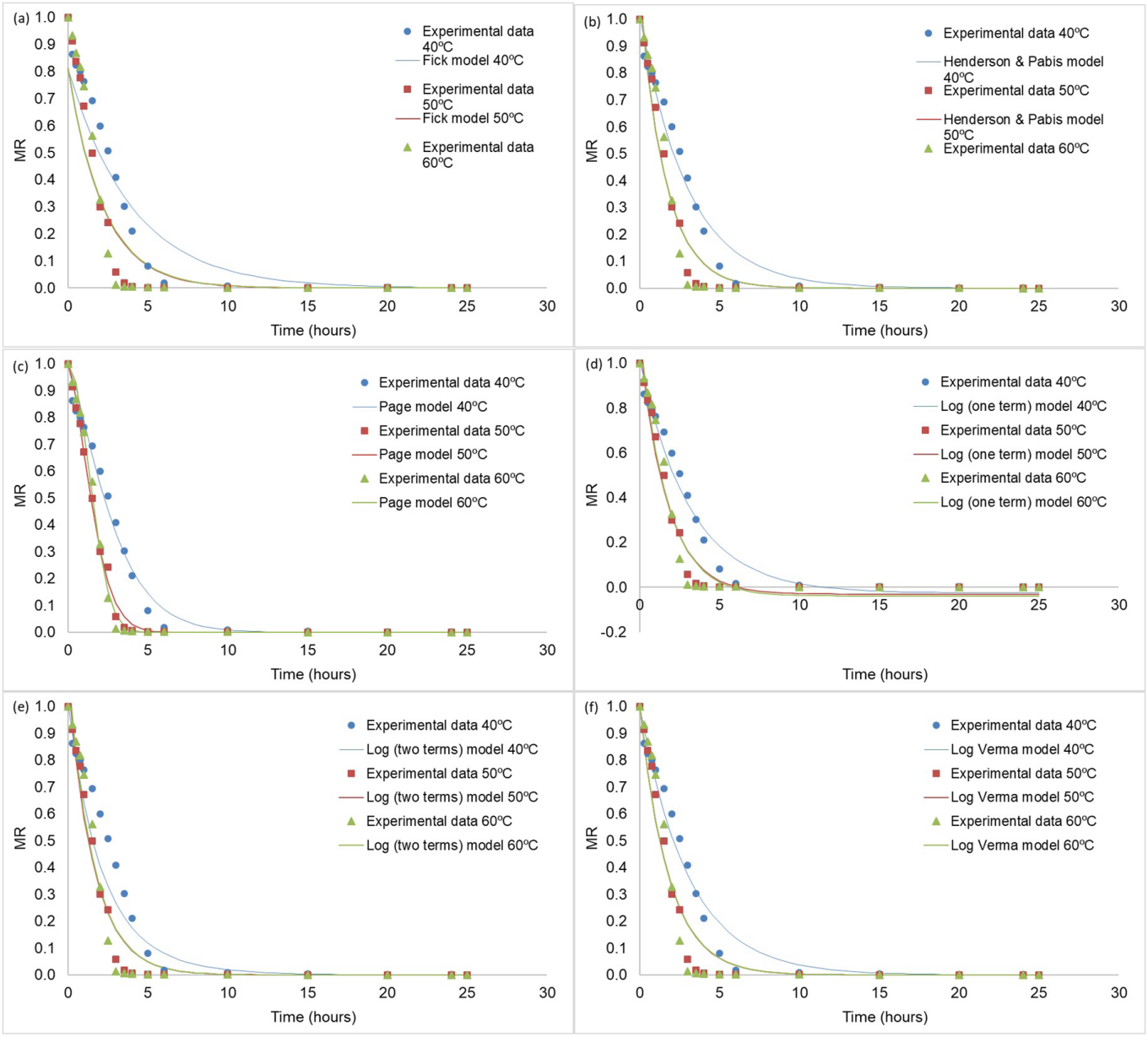

Figure 2. Experimental and estimated values during the drying of grape pomace at different temperatures: (a) Fick, (b) Henderson & Pabis, (c) Page, (d) Logarithmic (one term), (e) Logarithmic (two terms), (f) Verma.

which is possibly related to the inhibition of the polyphenol oxidase activity in grape pomace.

Polyphenol oxidase is an enzyme that can be present in grape pomace (FORTEA et al., 2009) and is directly related to the degradation of phenolic compounds (O'DONNELL et al., 2010). This enzyme is responsible for the hydroxylation of monophenols to o-diphenols and oxidation to their corresponding quinones, therefore polymerizing to form undesirable dark coloured pigments (CHISARI et al., 2007).

According to Cheng et al. (2013), polyphenol oxidase does not show a significant loss of activity when exposed to temperatures below 55 °C for a short period of time. However, at higher temperatures it is denatured, losing its activity in a short time with a reduction of up to 92%. Terefe et al. (2015) found similar results evaluating the activation/inactivation of the polyphenol oxidase present in blueberry at temperatures of 40 °C to 100 °C. The authors observed that the temperatures of 60 °C to 70 °C activated the enzyme, but that exposure of the enzyme to these temperatures for a prolonged time (more than 30 minutes) was sufficient for its inactivation.

The similar behaviour of the phenolic content and the antioxidant capacity is related to the existence of a strong correlation between these two responses, since the antioxidant capacity is a property very characteristic of these compounds (BARTOSZEK; POLAK, 2012;

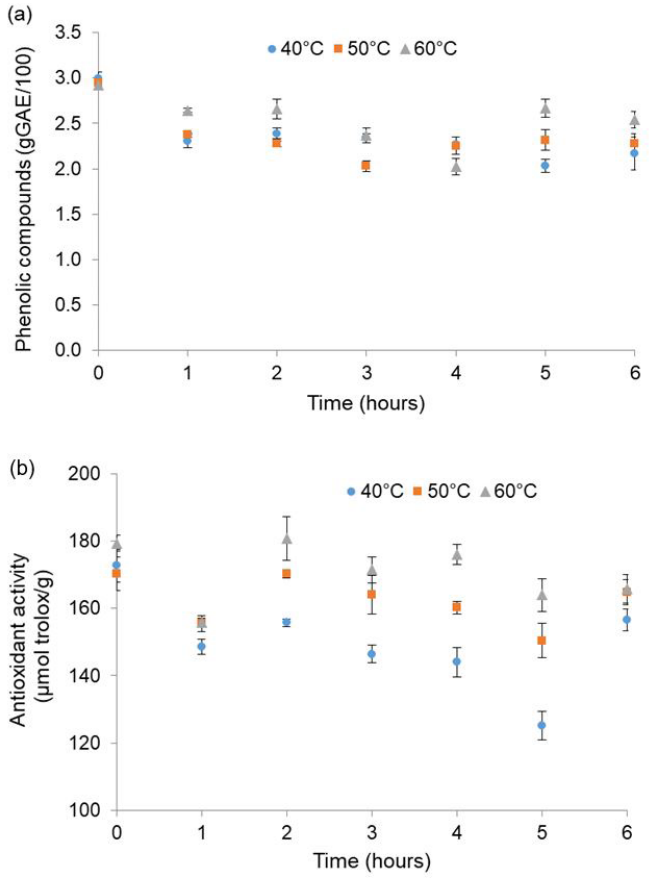

Figure 3. The effect of drying temperature on the phenolic compounds (a) and antioxidant capacity (b) of grape pomace.

NOUR et al., 2013). However, the more pronounced reduction in the antioxidant capacity rather than in the phenolic compounds may indicate that other non-phenolic compounds are responsible for the antioxidant capacity, which may have been degraded during drying.

3.3 Influence of water activity on the degradation of phenolic compounds

An inverse relationship was observed between water activity and the degradation of phenolic compounds in the three drying processes. The water activity of grape pomace dried at 60 °C was lower than the values observed for those dried at 50 °C and 40 °C (0.14 ± 0.001, 0.27 ± 0.001 and 0.60 ± 0.001, respectively) (Figure 4).

This result, together with the better preservation of the phenolic compounds at this temperature, indicates that water activity may also be related to the thermal stability of enzymes that degrade phenolic compounds. Korbel et al. (2013) showed that polyphenol oxidase activity was lower at lower water activities in mango pulp, especially when the sample was subjected to heat treatment at 60 °C. Oliviero et al. (2014) reported similar results during the evaluation of myrosinase activity in dried broccoli at different temperatures. They found the

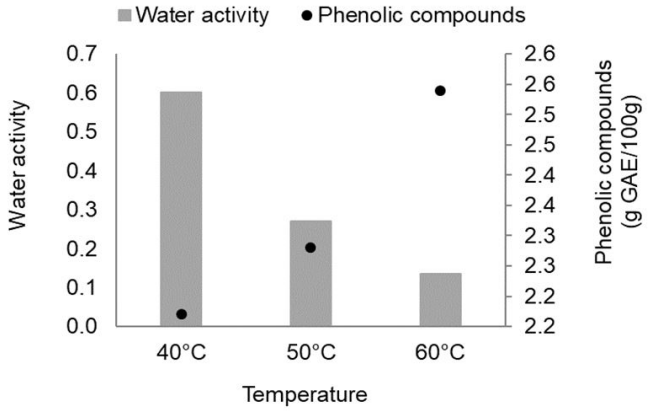

Figure 4. Water activity and phenolic compounds after 6 hours of drying at 40 °C, 50 °C and 60 °C.

highest enzymatic activity (61 ± 3.0) in samples with higher a_w (0.10 ± 0.01), concluding that water activity may have a great influence on enzyme stability and inactivation during thermal treatments. This theory would explain the greater retention of phenolic compounds when drying was carried out at higher temperatures.

According to Kurozawa et al. (2014) a reduction in water activity implies in a reduction in molecular diffusion through the medium, as well as in the chemical reactions occurring therein, including oxidation reactions.

Therefore, the greater retention of phenolic compounds in the process carried out at 60 °C could be related to two factors: the lower molecular mobility associated with lower a_w values, which makes the oxidation reactions difficult, and the loss of enzymatic activity, which can be related to the thermal effects, regarding: (a) the change in the three dimensional conformation of polyphenol oxidase and (b) the lower amount of water available for the enzyme action (GOULA; ADAMOPOULOS, 2010).

4 Conclusions

The experimental data of the grape pomace drying kinetics were most adequately predicted by the Page model. The drying temperature of 60 °C resulted in lower moisture contents and better phenolic retention, consequently with higher antioxidant capacity, being considered the most suitable temperature for the convective drying of grape pomace. This result suggested a possible oxidation of bioactive compounds through the action of polyphenol oxidase in the grape pomace dried at temperatures below 60 °C, since the temperatures of 50 °C and 40 °C were probably not sufficient to inactivate this enzyme. In addition to the influence of temperature, a reduction in water activity may also have contributed to the reduction in enzyme activity and the occurrence of chemical reactions, thus improving the retention of phenolic compounds. These results may be useful for the optimization of the industrial drying of grape pomace, which can be used as an ingredient in

food formulations, representing a source of dietary fiber and bioactive compounds with antioxidant capacity.

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