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The combined effect of time and temperature during oven drying on red grape pomace polyphenols, pigments, and antioxidant properties

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Abstract. This study had as a goal to carry out the drying of red grape pomace (RGP) using a 2 × 3 factorial design. The design of the experiment included combinations of time and temperature in order to achieve the lowest possible moisture levels and examine losses in precious polyphenols, but also the effect on the antioxidant properties of RGP extracts. Drying for 6 hrs at 80°C (D6/80) provided RGP with a satisfactory moisture level (11%). A comparison with untreated (fresh) RGP revealed that drying significantly decreases the polyphenol and anthocyanin pigments' content. This decline was accompanied by a decrease in both the ferric-reducing power and antiradical activity of the RGP extracts. Although necessary for long-term RGP stability, drying should be implemented with caution because improper drying may have severe effects on the polyphenolic composition and antioxidant activity.

Keywords and phrases: anthocyanins, antioxidants, drying, red grape pomace, polyphenols

1. Introduction

The winemaking industry is of paramount importance to the agricultural sector worldwide, as grapes are one of the primary fruit crops cultivated globally. Grape production was about 77.8 metric tons in 2018. Based on the International Organization of Vine and Wine (OIV) statistics, 292 million hectolitres of wine were produced worldwide in 2018 (*Ahmad et al.*, 2020). As a result, the wine industry regularly generates a vast amount of waste, posing serious environmental risks associated with the release of rejected biomass with high chemical and biological oxygen demand. On the other hand, this large pool of underutilized residual material is considered an outstanding bioresource of an array of compounds that could be valorized for the production of high value-added products.

Vinification wastes are mainly composed of vine shoots, grape pomace, and stems. The two latter side-streams are particularly rich in polyphenols, including several classes such as hydroxycinnamates, flavanols, flavonols, and anthocyanins. Many of these compounds may possess biologically important bioactivities, including antioxidant activity, antimicrobial action, anti-inflammatory properties, etc. (*Georgiev et al.*, 2014; *Teixeira et al.*, 2014). Such scientific evidence has led to the development of numerous valorization approaches for winemaking wastes, encompassing strategies that aimed at optimizing raw material handling and the optimization of polyphenol recovery, through improved solid-liquid extraction techniques (*Hogervorst et al.*, 2017; *Makris*, 2018).

One of the critical handling steps taken to prepare grape pomace for subsequent extraction is undisputedly the drying process (*Rajha et al.*, 2014; *Sui et al.*, 2014). Drying is commonly performed to increase preservation time, but it also facilitates pulverization, which is crucial for an effective extraction. The generation of a fine powder out of the dried plant material allows for high mass transfer during solid-liquid extraction and significantly increases extraction yield (*Sridhar et al.*, 2021). However, in procedures destined to acquire a properly prepared plant material for subsequent extraction, drying is performed rather empirically. Thus, its effect on the target substances is not thoroughly appraised. Improper drying may significantly deteriorate grape pomace, with detrimental consequences to the polyphenolic composition and antioxidant activity (*Marchante et al.*, 2018).

On this ground, the present investigation was performed to identify the most suitable conditions that would result in an effective grape pomace drying and to examine losses in precious polyphenolic compounds. To this purpose, several combinations of time and temperature were tested to identify the optimal set of conditions by recording changes in thermolabile polyphenol groups such as flavanols and anthocyanins. The impact on typical in vitro antioxidant properties was also evaluated to further assess the drying conditions used. The composition of the optimally dried material was investigated by carrying out liquid chromatography-mass spectrometry analyses.

2. Materials and methods

2.1 Chemicals and reagents

Solvents used for chromatographic analyses were of HPLC grade. The gallic acid hydrate was from Panreac (Barcelona, Spain). The L-Ascorbic acid (99.5%), quercetin, Folin-Ciocâlteu reagent, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), rutin (quercetin 3-*O*-rutinoside) (> 94%), catechin, *p*-dimethylaminocinnamaldehyde (DMACA), and 2,2-diphenylpicrylhydrazyl (DPPH) were from Sigma-Aldrich (Darmstadt, Germany). Pelargonin (pelargonidin 3,5-di-*O*-glucoside) chloride was from Extrasynthese (Genay, France). Sodium carbonate anhydrous (99%) was from Penta (Praha, Czechia).

2.2 Red grape pomace (RGP)

RGP was obtained from the industrial vinification of *Vitis vinifera* cv. Muscat of Hamburg grapes, and it was provided by a winery located in Karditsa (central Greece). The collected material was transferred to the laboratory within 2 hrs after collection and stored at -40°C until used.

2.3 Determination of moisture content

RGP was thawed and placed on a tray in layers of approximately 0.5 cm thickness. The material was dried in a laboratory oven (Binder BD56, Bohemia, NY, USA) at 105°C for 48 hrs. The % moisture content was then determined as follows:

$$u\% = 100 \cdot \frac{f_m - d_m}{d_m} \quad (1)$$

The terms f_m and d_m correspond to fresh and dry mass (g), and u% is the moisture content in mass percentage.

2.4 Drying assay

The aim was to provide RGP with the lowest possible moisture level by appropriately combining the two critical drying variables, temperature (T) and time (t). In this framework, several combinations were tested by using T levels varying from 50 to 80°C and t processing time ranging from 3 to 9 hrs. After drying, RGP was ground in a ball mill to provide a powder with an average particle diameter of dp = 384 μm. The obtained powder was filled in airtight plastic vessels and stored in the dark at 4°C.

2.5 Extraction of RGP

An amount of 0.5 g of RGP powder was placed in a 50-mL round-bottom flask with 20 mL of 70% ethanol containing 0.1% HCl. The mixture was extracted under magnetic stirring at 500 rpm, for 180 min, and at 40°C by a thermostated hotplate (Witeg, Wertheim, Germany). After extraction, 1 mL of the sample was transferred in a 1.5 mL Eppendorf tube and centrifuged for 10 min at 10000 × g. The clear supernatant was used for all further analyses.

2.6 Design of experiment

The purpose was to assess the combined effect of drying time (t) and temperature, (T) (independent variables) on the residual moisture content, u (%) (response). These two variables were considered because of their profound influence on the drying of plant materials. To accomplish this, a 2 × 3 factorial design was implemented as previously described (*Khiary et al.*, 2009). This statistical approach was used to identify the relationship between the response function and process variables and to determine the optimal conditions for the drying process (minimization of the residual moisture content). The two independent variables, t and T, varied from 3 to 6 hrs and from 50 to 80°C respectively (*Table 1*). These variation ranges were chosen based on both preliminary experiments and bibliographic data. All experiments were performed in triplicate.

Table 1. Selected combinations of process variables of oven drying (experimental design)

Design point	t (h)	T (°C)
1	3	50
2	3	65
3	3	80
4	6	50
5	6	65
6	6	80

2.7 Total polyphenol (TP) determination

Samples were first diluted 1:50 with 0.5% (v/v) aqueous formic acid, and analysis was carried out according to a previously described micro-scale methodology (*Cicco et al.*, 2009). Briefly, 0.1 mL Folin-Ciocâlteu reagent and 0.1 mL of diluted

sample were mixed in a 1.5 mL Eppendorf tube and allowed to react for 2 min. Then 0.8 mL of Na_2CO_3 solution (5% w/v) was added, and the mixture was incubated in a water bath for 20 min, at 40°C. After incubation, samples were cooled down with tap water and measurement of the absorbance at λ = 740 nm was performed.

The total polyphenol concentration (C_{TP}) was determined from a calibration curve, using gallic acid as standard (10 – 80 mg·L^{-1}, R^2 = 0.9996). Results were given as mg gallic acid equivalents (GAE) L^{-1}. Yield in TP (YTP) was expressed as mg GAE g^{-1} dry mass (DM) (*Grigorakis et al.*, 2020).

2.8 Total monomeric anthocyanin (TA) determination

TA were estimated with the pH-differential method (*Lee et al.*, 2005). A volume of 0.1 mL RGP extract was combined with 0.9 mL KCl buffer (pH = 1.0), and the absorbance was obtained at both λ = 520 nm and λ = 700 nm. Similarly, RGP extract was also combined with CH_3COONa buffer (pH = 4.5), and the corresponding absorbances were read. The total anthocyanin concentration (C_{TA} expressed in mg·L^{-1}) was calculated as follows:

$$C_{TA} = \frac{A \cdot MW \cdot F_D \cdot 10^3}{\varepsilon} \quad (2)$$

A corresponds to $(A_{520} - A_{700})_{pH = 1} - (A_{520} - A_{700})_{pH = 4.5}$, and MW is the molecular weight of malvidin 3-*O*-glucoside (MW = 529 g·mol^{-1}). F_D is the dilution factor (1:10), and λ is the molar absorptivity of malvidin 3-*O*-glucoside (λ = 28,000). Results were given as malvidin 3-*O*-glucoside equivalents (MvE).

2.9 Total flavanol (TF) determination

The *p*-dimethylaminocinnamaldehyde (DMACA) assay (*Makris et al.*, 2008) was used, with some modifications. Samples were diluted with methanol (1:50), and an amount of 0.02 mL of diluted sample was transferred in a 1.5 mL Eppendorf tube, along with 0.1 mL DMACA (0.1% w/v in methanol) and 0.88 mL HCl (2 M in methanol). After exactly 15 min, absorbance readings were obtained at λ = 640 nm. Total flavanol concentration (C_{TF}) was determined from a catechin calibration curve (1 – 80 mg·L^{-1}, R^2 = 0.9999) and given as catechin equivalents (CtE).

2.10 Antioxidant properties

The antiradical activity (A_{AR}) determination was performed with a stoichiometric assay, using as chromophore probe the stable DPPH radical, as described elsewhere (*Chakroun et al.*, 2021). Results were given as μmol DPPH g^{-1} DM. Reducing

power (P_R) determination was carried out according to a previously published protocol (*Chakroun et al.*, 2021), and results were expressed as μmol ascorbic acid equivalents (AAE) g^{-1} DM.

2.11 Liquid chromatography-diode array-mass spectrometry (LC-DAD-MS)

The devices were a Finnigan (San Jose, CA, USA) MAT Spectra System P4000 pump, a Finnigan AQA mass spectrometer, and a UV6000LP diode array detector. Chromatography was performed on a Fortis RP-18 column (150 mm × 2.1 mm, 3 μm) at 40°C, with a 10 μL injection loop. Mass spectra acquisition was carried out with electrospray ionization (ESI) in both positive and negative ion mode. Details on mass spectrometry setting and elution have been described elsewhere (*Makris & Kefalas*, 2013).

2.12 High-performance liquid chromatography (HPLC) analysis

The equipment was a Shimadzu CBM-20A (Shimadzu Europa GmbH, Germany) coupled to a Shimadzu SPD-M20A detector and interfaced by a Shimadzu LC solution software. Chromatographic analyses were accomplished on a Phenomenex Luna C18(2) column (100 Å, 5 μm, 4.6 × 250 mm) (Phenomenex, Inc., Torrance, CA, USA), with the same guard column, at 40°C. Analytical details concerning the elution and solvents used have been previously reported (*Lakka et al.*, 2020). Quantification was carried out with the calibration curves of gallic acid (R^2 = 0.9990), caffeic acid (R^2 = 0.9980), catechin (R^2 = 0.9999), quercetin (R^2 = 0.9990), and pelargonin (R^2 = 0.9999). All calibration curves were constructed using methanolic solutions with concentrations in the range of 0–50 $\mu g \cdot mL^{-1}$.

2.13 Statistics

All extractions were accomplished at least twice, and all determinations were done in triplicate. The results were given as means with standard deviations. Distribution analysis was performed with JMP™ Pro 13 and linear regressions with SigmaPlot™ 12.5. At least a 95% significance level was applied to all statistical analyses.

3. Results and discussions

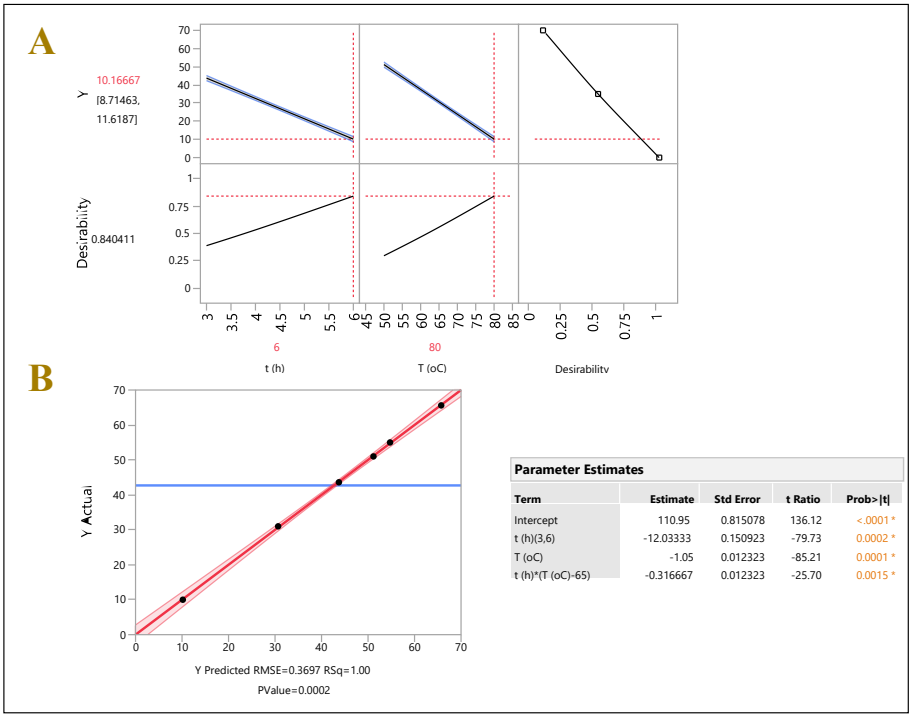
3.1 Drying optimization

The process was designed to assess the effect of two key drying variables, *t* and *T*, and to detect possible synergistic effects between them.

Table 2. The results of the drying experiments (in triplicate) in conformity with the chosen experimental design

***t* (h)**	***T* (°C)**	***u* (%)**
3	50	65.6 ± 3.2
3	65	55 ± 3.0
3	80	43.6 ± 2.7
6	50	51 ± 3.1
6	65	31 ± 2.1
6	80	10 ± 1.4

Assessment of the fitted model and response surface suitability was done by considering the closeness of the measured and predicted values (*Figure 1B*).



Notes: Graphs A and B correspond to the desirability function and actual-to-predicted diagram. Asterisk (*) in the inset table "Parameter Estimates" shows statistically significant terms, at least at a 95% significance level.

Figure 1. Model fitting and evaluation obtained by implementing a 2 × 3 factorial design.

The second-degree polynomial equation (mathematical model) derived was as follows:

$$u\% = 110.95 - 12.03 \cdot \left(\frac{t-4.5}{1.5}\right) + 0.33 \cdot T \cdot \left(\frac{t-4.5}{1.5}\right) \cdot (T - 65) \quad (3)$$

Since the total correlation coefficient of the model was $R^2 = 1.00$, and the $p = 0.0002$ (assuming a confidence interval of 95%) was highly significant, it could be supported that equation (3) represents a good fitting to the experimental data. The three-dimensional plot constructed on the basis of the model (*Figure 2*) is the graphical representation of residual moisture (response) dependence on the drying process parameters, the drying temperature (T), and the drying process duration (t).

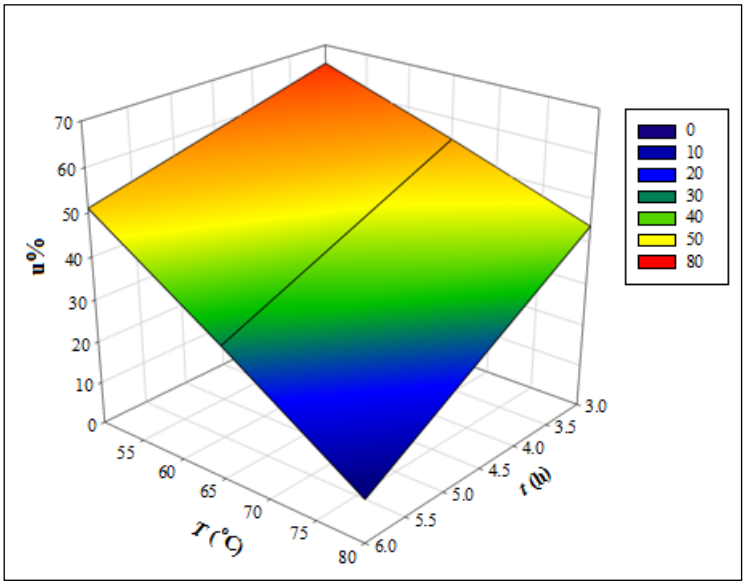


Figure 2. Effect of the drying process parameters (t and T) on residual moisture content (u)

Using the desirability function (*Figure 1A*), it could be estimated that to achieve a sufficiently low moisture level of around 10% (*Marchante et al.*, 2018), the values of the process parameters should be $t = 6$ h and $T = 80$°C. Under these conditions, the predicted moisture level was 10.2 ± 1.5%. This theoretical value was confirmed by performing three individual drying experiments under optimal conditions, where a value of 10.1 ± 2.0% was found. Thus, the process with these parameters ($t = 6$ h and $T = 80$°C), marked as D6/80, was considered for further investigation.

3.2 Effect of drying on polyphenols

As can be seen in *Figure 3*, D6/80 generated a decrease in total polyphenol content (Y_{TP}) by 18.3% compared with the fresh (non-dried) sample. Other studies on RGP drying showed that RGP oven-dried at 80°C for 24 hrs retained significantly more total polyphenols than when dried at 40°C for 72 hrs (*Demirkol & Tarakci*, 2018). Similarly, RGP dried at 60°C was found to better retain polyphenols compared with drying at either 40 or 50°C (*Teles et al.*, 2018). On the other hand, it has been reported that fluidized-bed drying of RGP at $T < 70$°C for 90 min was more effective in preserving polyphenolic content compared with 80°C for 180 min (*Planinić et al.*, 2015). In the same line, drying at $T < 60$°C for 3 days generated significantly less reduction in total polyphenols than at higher temperatures for 8 hrs (*Khanal et al.*, 2010). Furthermore, large increases in the drying temperature, from 60 to 140°C, have also been proven to be detrimental for total polyphenol retention (*Larrauri et al.*, 1997).

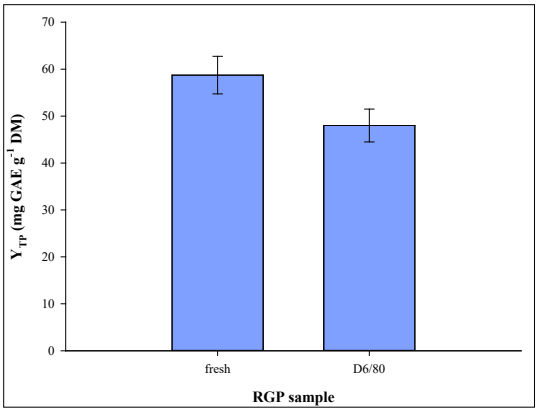


Figure 3. The effect of drying (D6/80) on the total polyphenol content (Y_{TP}) of the RGP in comparison with the fresh samples

To obtain a deeper insight into the effect of D6/80 drying on RGP polyphenols, two other indices were also considered: the yield in total flavanols (Y_{TF}) and the yield in total monomeric anthocyanins (Y_{TA}). These two major polyphenolic RGP constituents were selected according to their sensitivity to drying (*Çoklar & Akbulut*, 2017). Therefore, it would be expected that any destructive effect of drying could be clearly reflected on the changes in these indices. Indeed, it was a Y_{TF} decrease by 54.4% (*Figure 4A*); likewise, the reduction of Y_{TA} was 56.2% (*Figure 4B*).

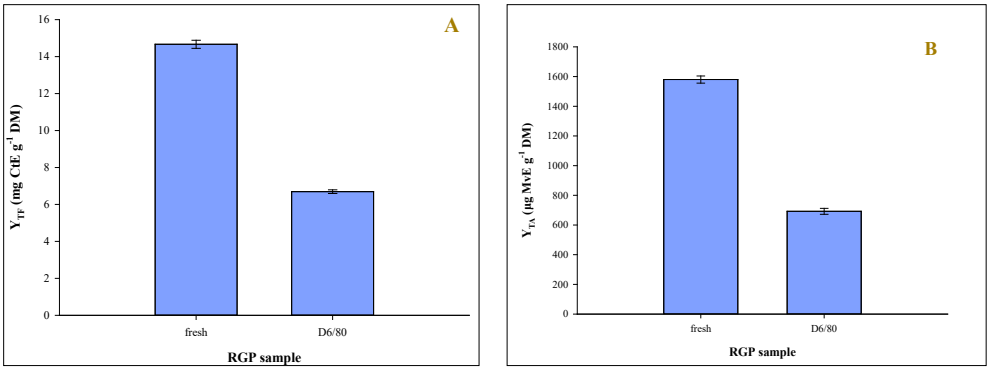
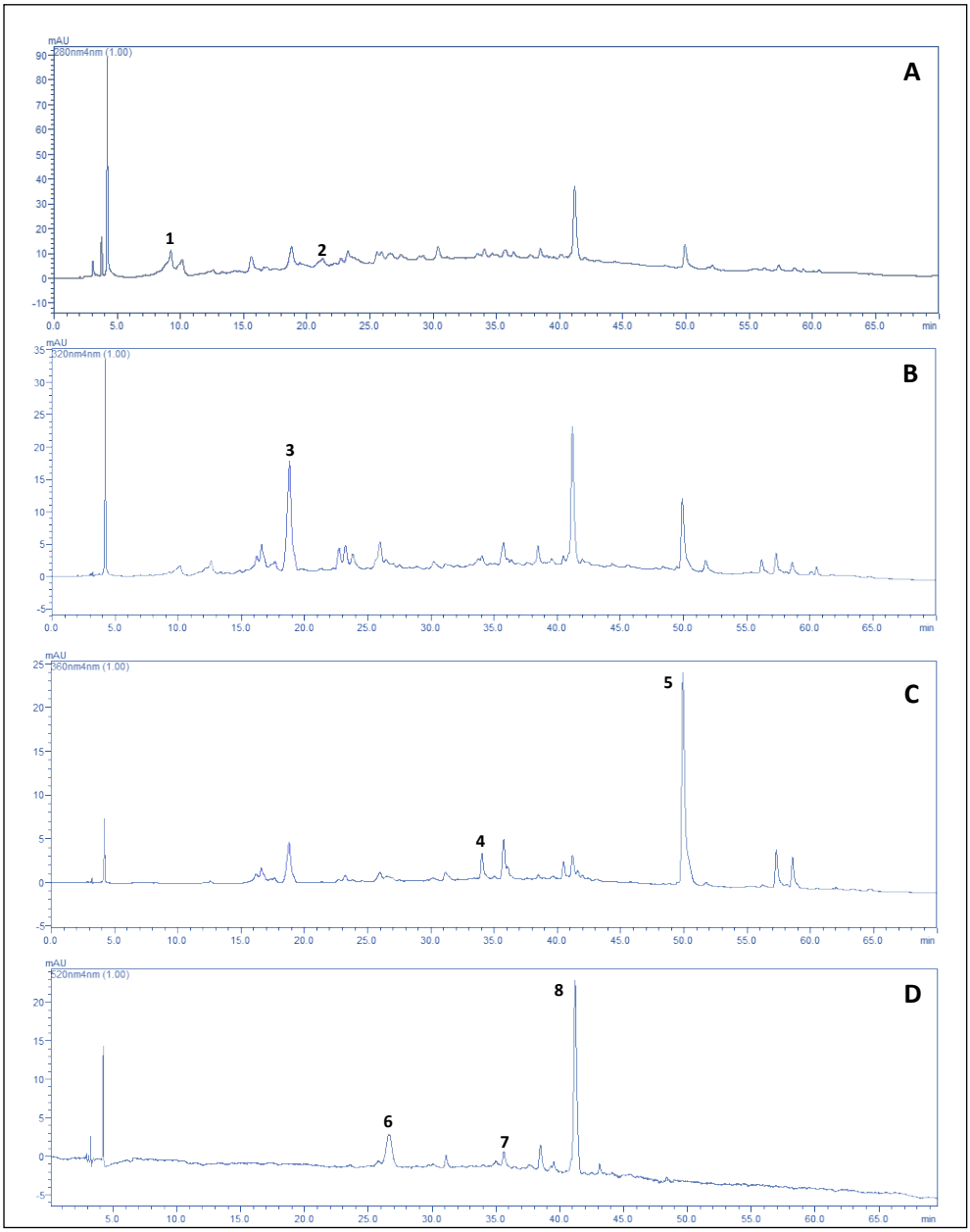


Figure 4. The effect of the drying processes (D6/80) on the yield of total flavanol (A) and total monomeric anthocyanin (B) of RGP

To obtain an integrated picture concerning the impact of drying on RGP polyphenols, the extract prepared after implementing D6/80 drying was analysed by HPLC and compared to the extract obtained from fresh (non-dried) sample. The analysis of both extracts revealed the presence of eight principal polyphenols (*Figure 5*), three of which were anthocyanin pigments. Catechin, rutin, gallic acid, and quercetin could be easily identified through a comparison of the corresponding retention times with those of commercial standards. Caftaric acid (caffeoyltartaric acid) was identified by its molecular ion at m/z = 311 using negative ion mode operation, based on previously published data (*Makris et al.*, 2003). Likewise, malvidin 3-*O*-glucoside was tentatively identified by its molecular ion at m/z = 493 and the diagnostic fragment at m/z = 331 (aglycone), in positive ion mode operation. Similarly, malvidin 3-*O*-glucoside acetate gave a molecular ion at m/z = 535 and a fragment at 331, and malvidin 3-*O*-glucoside *p*-coumarate a molecular ion at m/z = 639 and a fragment at 331 (*Kefalas & Makris*, 2006).

As can be seen in *Table 3*, all polyphenol contents decreased significantly as a result of drying. Catechin was the most thermolabile substance exhibiting a decrease by 93.1%, whereas rutin content decreased by 79.2%. Overall, the decrease of the non-pigment polyphenols was 86.2% and of the anthocyanins 88.3%. These values are in accordance with those previously reported for RGP drying (*Goula et al.*, 2016), highlighting the detrimental effect of drying on several major polyphenolic compounds. Although the decreasing amount in non-pigment and anthocyanin compounds was almost equal, other examinations demonstrated a higher sensitivity of anthocyanins to oven drying (*Çoklar & Akbulut*, 2017; *Marchante et al.*, 2018).



Chromatograms A, B, C, and D were detected at λ= 280, 320, 365, and 520 nm respectively. Peak assignment: 1, gallic acid; 2, catechin; 3, caftaric acid; 4, rutin; 5, quercetin; 6, malvidin 3-*O*-glucoside; 7, malvidin 3-*O*-glucoside acetate; 8, malvidin 3-*O*-glucoside *p*-coumarate.

Figure 5. Representative chromatograms of the extract of RGP dried for 6 hrs at 80°C (D6/80).

Table 3. Impact of the drying process (D6/80) on major RGP polyphenols

Compound	Extraction yield (μg·g^{-1} DM)*	
	Fresh RGP	Dried RGP (D6/80)
Non-pigment polyphenols		
Gallic acid	838.36 ± 12.58	151.33 ± 2.72
Caftaric acid	842.32 ± 10.63	98.16 ± 1.77
Catechin	971.71 ± 14.58	66.69 ± 1.20
Rutin	247.20 ± 3.71	51.41 ± 0.93
Quercetin	947.71 ± 14.22	165.14 ± 2.97
Total leuco-polyphenols	3847.30	532.73
Anthocyanin pigments		
Malvidin 3-*O*-glucoside	2036.93 ± 30.55	177.98 ± 3.20
Malvidin 3-*O*-acetyl glucoside	458.41 ± 6.88	48.67 ± 0.88
Malvidin 3-*O*-*p*-coumaroyl glucoside	6643.87 ± 99.66	844.62 ± 15.20
Total anthocyanins	9139.21	1071.27

*Values reported are means (n = 3) ± standard deviation.

3.3 Effect of drying on antioxidant properties

The effect of drying on the antiradical activity (A_{AR}) was dramatic, as D6/80 brought about a decrease of 82.4% (*Figure 6A*). The effect observed for the ferric-reducing power was milder (P_R), as it was decreased by 37.2% (*Figure 6B*). Modifications in the antioxidant properties of RGP extracts as a result of drying should be normally anticipated, since they are tightly associated with the polyphenolic composition, which is largely impacted. Such a phenomenon has been dealt with by early studies, which demonstrated that increasing drying temperature resulted in the lower radical scavenging potential of RGP extracts (*Larrauri et al.*, 1997). The studies indicated herein were in concurrence, suggesting that drying may entail a significant loss of antiradical activity in RGP extracts (*Marchante et al.*, 2018), but also in P_R (*Chikwanha et al.*, 2018; *Çoklar & Akbulut*, 2017). These changes were linked with changes in the polyphenolic content. Results from investigations into other tissues, such as strawberries, were in accordance with the related observations (*Wojdyło et al.*, 2009; *Méndez-Lagunas et al.*, 2017). It should be stressed that the 82.4% decrease in A_{AR} found for the sample that received the D6/80 treatment coincided with the 86.2% decrease found for non-pigment polyphenols and the 88.3% decrease for anthocyanins. This finding might suggest

that the drop in A_{AR} actually reflected the loss of polyphenolic substances, and therefore the determination of A_{AR} could be an additional criterion in assessing drying processes.

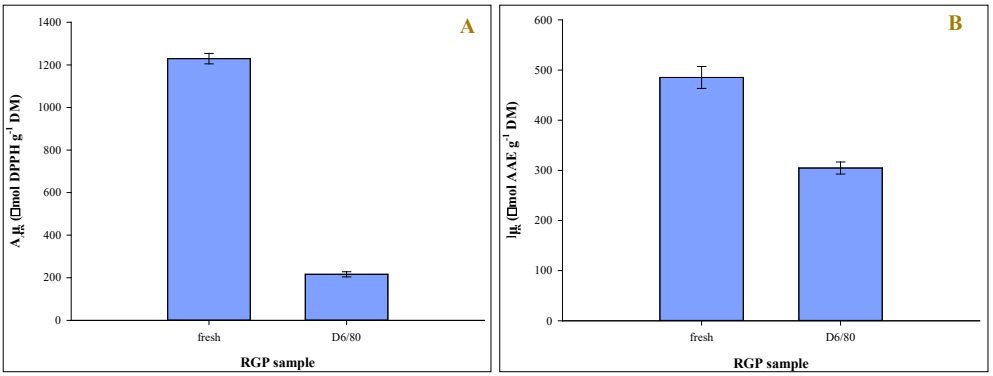


Figure 6. The effect of the drying processes (D6/80) on the antiradical activity (A) and ferric-reducing power (B) of RGP extracts

4. Conclusions

The testing of several combinations of time and temperature for RGP drying showed that D6/80 might facilitate a very effective moisture removal. However, a comparison of the sample undergone the D6/80 process with the fresh (untreated) sample revealed a drastic decline in several major polyphenols and antiradical activity. Thus, although drying is a salient compromise for long-term RGP stability, drying processes should be implemented with caution because an improper implementation of drying variables may have extremely severe effects on the polyphenolic composition and antioxidant activity. It is believed that this study could be a guide for future laboratory-scale but also large-scale studies and for the application of drying procedures with minimal impact on thermosensitive metabolites.

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