DRYING TREATMENT EFFECTS ON ANTHOCYANINS OF ORGANIC RASPBERRY (CV. HERITAGE) FRUIT

Liliana BĂDULESCU[1], Aurora DOBRIN[2], Andreea STAN[2], Andrei MOȚ[2], Oana-Crina BUJOR[2]

[1]*University of Agronomic Sciences and Veterinary Medicine of Bucharest*
Faculty of Horticulture, 59 Mărăşti Boulevard, Bucharest, 011464, Romania
Email: lilib_20@yahoo.com

[2]*University of Agronomic Sciences and Veterinary Medicine of Bucharest*
Research Center for Studies of Food and Agricultural Products Quality
59 Mărăşti Boulevard, Bucharest, 011464, Romania
Email: oana_crin@yahoo.com

Keywords: anthocyanins, freeze drying, hot-air drying, HPLC, Rubus idaeus L.

ABSTRACT

The aim of this study is to compare the effects of some drying processes (hot-air drying and freeze drying) on total anthocyanin content (TAC) for organic raspberry (cv. *Heritage*) fruits as measured by spectrophotometric method and UPLC technique. The total anthocyanin content was determined in powders obtained from fruits and juice of organic raspberry dried in a hot-air dehydrator at 70 ºC and a freeze dryer at –55 ºC for 45 h.

Qualitative analysis revealed the similar anthocyanin profiles in all raspberry powders and showed a clear anthocyanin pattern with the presence of two major compounds. In both fruit and juice of organic raspberry, freeze drying produced a better extraction of the total anthocyanin content either by spectrophotometric method or UPLC and this could be attributed to the thermal degradation and/or oxidation of these compounds during hot-air drying. As matter of fact, the highest

aspberries compared to fresh dried fruits.

The results presented in this work indicate that the most appropriate drying method in terms of the anthocyanin content is freeze drying. However, the detailed qualitative analysis of the raspberry powders should help understanding the effects of different drying treatments.

INTRODUCTION

Raspberry fruits are among the most frequently consumed berries in the world as fresh and processed into various products like preserves, jam, jelly, puree, bakery products, frozen products, dried

products, juices, extracts, ice cream and yogurt (Kim et al., 2016). In addition, raspberries are an excellent source of nutritional and bioactive compounds including vitamins, essential minerals, fatty acids, dietary fiber, carotenoids and phenolic compounds (Bobinaitė et al., 2016; Tănase et al., 2016). Among these, anthocyanins are a class of phenolic compounds, which are responsible for the red to purple color of the raspberry fruit. The major anthocyanins in raspberries are derivatives of cyanidins, with the major constituents being cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3--rutinoside, and cyanidin-3-glucoside (Bobinaitė et al., 2016; Sparzak et al., 2010; de Ancos et al,. 2000).

Anthocyanins have been traditionally used as natural food additives and colourants as well as pharmaceutical ingredients that can be used for preventing several diseases, including CVDs, cancers, diabetes, some metabolic diseases, and microbial infection (Khoo et al., 2017). Anthocyanins are designated E163 and allowed for food applications in the European Union, while in the United States, it is approved in foods as extracts from grape color or skin, vegetable juice, and fruit juice, exempt from certification (CFR 2016) (Sigurdson et al., 2017).

The use of anthocyanin extracted from natural souces as food additives is a challenging process due to their low stability which depend on structure, pH, light, temperature, enzymes, oxygen, sugars and their degradation products (Mazza and Miniati, 1993). Several methods, such as controlled atmospheres, encapsulation, hot-air drying, spray drying, freeze-drying, hard-panned coating method, and enzymatic methods, are proposed for the stabilization of natural anthocyanin pigments to overcome their utilization as food colorants (Cortez et al., 2017). Drying is the most common food preservation process who help to provide products of higher shelf life (Raponi et al., 2017). Hot-air drying is the conventional drying method, but provides drastically reduction of quality of dried products (Ratti, 2001). By contrast, freeze drying is one of the best methods to preserve flavor, color and nutritional compounds due the absence of water, low pressure and temperature (Raponi et al., 2018).

In the last years, considerable interest on organic food–based diet has been developed. Moreover, a recent study supports the claim that a higher frequency of organic food consumption was associated with a reduced risk of cancer (Baudry et al, 2018). Furthermore, restrictions in the usage of additives and the need of phasing out contentious materials further increase pressure on organic food producers, while the full potential of natural organic additives and colourants is unexplored.

While research regarding the anthocyanins of organic raspberry has not been reported before, the aim of this study is to compare the effects of two drying processes, hot-air drying and freeze drying, on total anthocyanin content of organic raspberry (cv. *Heritage*) fruits as measured by spectrophotometric method and UPLC technique.

MATERIALS AND METHODS

Reagents

The anthocyanin standards cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside (kuromanin chloride) were purchased from Extrasynthese (Genay, France).

HPLC gradient grade acetonitrile and methanol were purchased from Honeywell Riedel-de Haën (Seelze, Germany), 37% hydrochloric acid from Merck (Darmstadt, Germany) and formic acid from Honeywell Fluka (Steinheim, Germany). Ultrapure water with resistivity 18.2 $M\Omega.cm^{-1}$ at 25 °C was obtained with a Milli-Q water purification system.

Raspberry samples

Organic raspberry fruits (*Rubus ideaus* L., cv. Heritage) were provided by a farm in second year of conversion to organic production (SC Livada de Zmeura SRL, Mihăeşti, Argeş, Romania), in August 2018 at the ripening stage. The moisture content of 83.44% for fresh samples was determined using a PARTNER MAC 50 moisture analyzer.

Drying treatments

Fresh and milled to juice form raspberries were submitted to two type of drying experiments: hot-air dehydration (HAD) and freeze drying (FD). For hot-air dehydration the raspberries were dried in a hot-air dehydrator (Escalibur 9) at 70 ºC, whereas the freeze drying were performed in a Christ Alpha 2-4 PLUS (Germany) freeze dryer at –55 ºC for 45 h. The dried raspberries were ground in a knife mill (Retsch Grindomix GM 200) in order to obtain fine powders.

Extraction of anthocyanins

Raspberry powders (0.3 g) were extracted with 5 mL of 1% hydrochloric acid in methanol (v/v) (Jung et al., 2011). The mixture was homogenised for 15 min at 500 rpm and then centrifuged (5000 rpm, 5 min, 4 °C). The supernatant was removed and the residue reextracted two times. The combined supernatants were adjusted to a final volume of 15 mL. The extractions were made in triplicates.

Total anthocyanin content (TAC) by spectrophotometric method

The total anthocyanin content was determined according to the pH differential method (AOAC,. 2005). The absorbance of the raspberry extracts was measured at 530 and 700 nm for extracts diluted in pH 1.0 and pH 4.5 buffers (Specord 210 Plus spectrophotometer). The dilution factor was 5. The results were expressed as cyanidin-3-glucoside equivalents per 100 gram of fresh weight (mg/100g FW) using an extinction coefficient of 34300 Lcm^{-1} mol^{-1} (Giusti and Wrolstad 2001).

Anthocyanins content (AC) by UPLC analysis

The determination of AC by UPLC was carried out according to a procedure described by Bujor et al. (2016) with some modifications. Before UPLC analysis, preliminary purification of anthocyanins from the raspberry extracts was performed using solid-phase adsoption. Firstly, 10 mL of acidified methanolic extracts of raspberry were concentrated under nitrogen in order to evaporate methanol. The remaining aqueous extract was make up to 3 mL with 0.3% aqueous hydrochloric acid and purified by a C18 Bond Elute cartridge (200 mg, 3 mL, Agilent, Santa Clara, CA). Firstly the cartridge was conditioned with two column volumes of 0.01% HCl in methanol followed by two volumes of 0.01% aqueous HCl (v/v) to remove remaining methanol. Afterwards, the extracts were loaded onto the mini-column and then washed with one volumes of 0.01% aqueous HCl to remove compounds not adsorbed. Finally, the anthocyanins were eluted with 0.01% HCl in methanol and immediately analyzed by UPLC (Ultra Performance Liquid Chromatography).
Anthocyanins separation was made according to the conditions described by Bujor et al. (2016). A Waters ACQUITY UPLC chromatograph (Waters, Milford, MA) coupled to an UV−VIS diode-array detector was used. Separation was performed on a reverse-phase Eclipse Plus C18 column (100 mm x 2.1 mm i.d., 1.8 µm; Agilent) at 30 °C. A binary solvent system with solvent A (1% formic acid in water, v/v) and solvent B (1% formic acid in acetonitrile) and the following elution gradient was used: 0-15 min, linear 0-20% B; 15-20 min, linear 20-40% B; 20-20.5 min, linear 40-100% B; 20.5-20.6 min, linear 100-0% B; 20.6-24 min, isocratic 0% B. The volume of extract injected was 2 µL at a flow rate of 0.17 mL/min. The detection was at 520 nm. All samples were injected in triplicate.
For the quantification of anthocyanins, cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside standards prepared in MeOH acidified with 1% HCl (v/v) were used for 5 point-calibrations. The results were expressed as milligram per 100 gram of fresh weight (mg/100g FW).

RESULTS AND DISSCUSSION

In this study hot-air dehydration and freeze drying treatments applied to fresh and milled organic raspberries were used and compared in order to assess their quality on the basis of anthocyanins content and composition. For this, UPLC and a spectrophotometric method were used to identify and quantify anthocyanins.

Anthocyanin profile of raspberry

The anthocyanins identification was confirmed by comparison with external standards, UV–VIS spectra (520 nm) and literature. Chromatographic separation of the anthocyanins of raspberry extracts is presented in Figure 1. The chromatografic analysis by UPLC shows the predominant presence of two anthocyanins, cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside, which in agreement with the findings of Ancos et al., 2000 and Sparzak et al., 2010 who funded a similar anthocyanin profile for Heritage variety of raspberry.

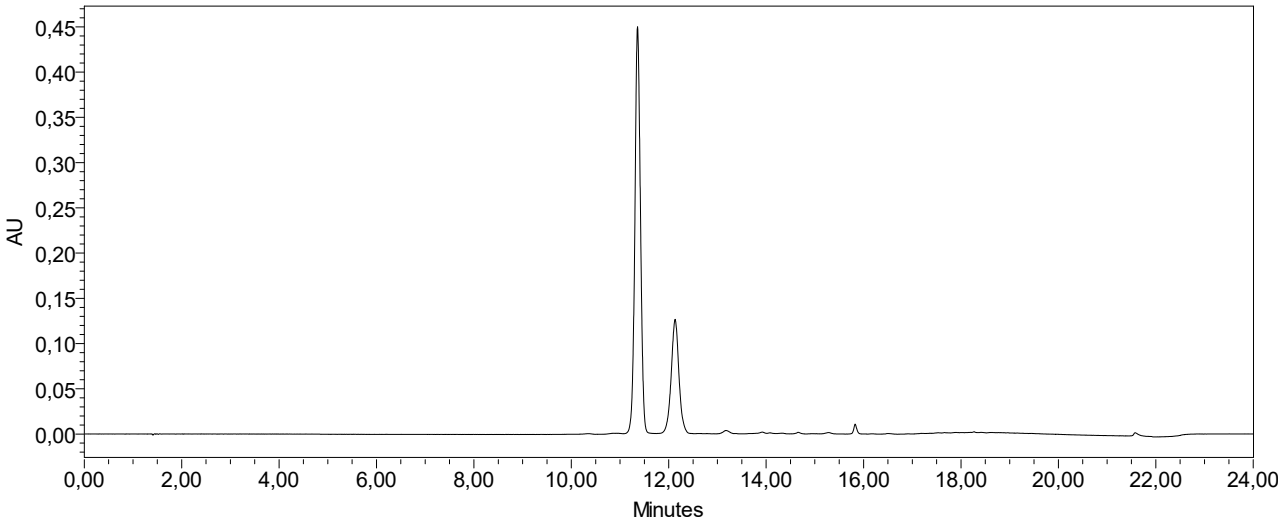

Figure 1. Chromatographic profile of anthocyanins of raspberries at 520 nm.

Effects of drying on raspberry anthocyanins

The anthocyanins content of raspberries are shown in Figure 2.

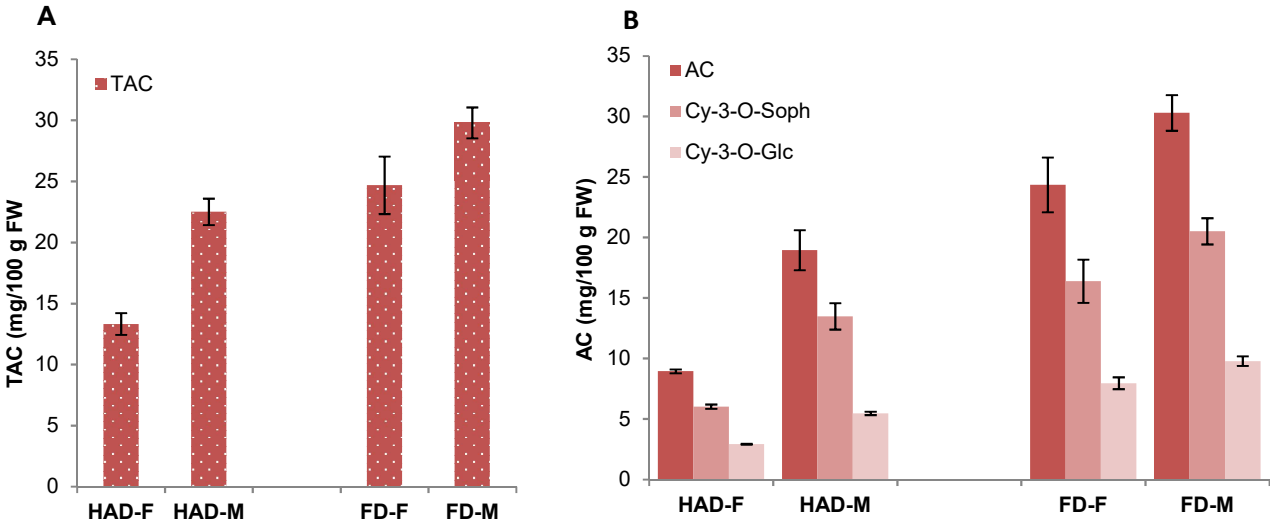

Figure 2. Effect of drying treatments on total anthocyanin content (A) and anthocyanins content (B) of organic raspberry samples. Hot-air dried fresh raspberries: HAD-F. Hot-air dried milled raspberries: HAD-M. Freeze dried fresh raspberries: FD-F. Freeze dried milled raspberries: FD-M. Values are mean ± SD (n = 3).

In both fresh and milled raspberries, FD provided high amounts of anthocyanins in all samples than HAD, whatever was the method of quantification. Indeed, these results are in accordance whith the fact that the FD is the most efficient method to preserve nutritive value and chemical constituents in fruits and vegetables compared to HAD (Ratti, 2001). Similarly, Mejia-Meza et al. (2010) and Michalczyk et al. (2009) found that the dehydration led to a reduction in anthocyanins content in

comparison with FD of raspberries. Also, it should be noted that the absence of liquid water and the low temperatures in the freeze drying process contributed to stopping the deterioration and microbial reactions which gives to final product a good quality (Ratti, 2001).
There was no difference in TAC and AC for both fresh and milled FD raspberries. On the other hand, the raspberry samples milled before drying had higher TAC and AC fresh samples. This probably may be attributed to better extraction of anthocyanins from milled raspberries because of cellular disruption.
Cyanidin-3-*O*-sophoroside was the predominant anthocyanin in all raspberry samples representing 67-71% of the AC, which is in agreement with the results of Sparzak et al., 2010 who determined a level of 61% in Heritage variety of raspberry. This compound had found in high concentration in FD raspberries compared to HAD samples.

CONCLUSIONS

The influence of hot-air dehydration and freeze drying were used in this study in order to evaluate the anthocyanin profile and content of organic raspberry fruits through UPLC analysis and spectrophotometric method. Results of qualitative analysis revealed similar anthocyanin composition (cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside) for all raspberry samples tested. Levels for each compound and the anthocyanin content were varied depending on drying treatments. Cyanidin-3-*O*-sophoroside was the most abundant anthocyanin in raspberries. Freeze drying was more effective in extraction of anthocyanins that hot-air drying. It was also showed that processing such as milling before drying resulted in higher anthocyanin contents. Given these results, organic raspberries are valuable raw material for the development of innovative natural pigments such as anthocyanins. However, new data are necessary on others quality papameters of organic raspberry to understand better the effects of different drying treatments.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Authority for Scientific Research and Innovation, CCCDI - UEFISCDI, project number ERANET-COREORGANIC-SusOrgPlus, within PNCDI III. We are grateful to thank Mrs Gabriela Vinograschi (SC Livada de Zmeura SRL) for providing the organic raspberry fruits.

REFERENCES

de Ancos, B., Ibañez, E., Reglero, G. and Cano, M.P. (2000), Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit, Journal of Agricultural and Food Chemistry, Vol. 48, pp. 873-879.

AOAC. (2005), Official Method of Analysis. Total monometric anthocyanin pigment content of fruit juice, beverage, natural colorants, and wine by pH difference method, Int. 88.

Baudry, J., Assmann, K.E., Touvier, M., Allès, B., Seconda, L., Latino-Martel, P., Ezzedine, K., Galan, P., Hercberg, S., Lairon, D. and Kesse-Guyot, E. (2018), Association of frequency of organic food consumption with cancer risk: findings from the NutriNet-Santé prospective cohort study, JAMA Internal Medicin, Vol. 178(12), pp.1597-1606.

Bobinaitė, R., Viškelis, P., and Venskutonis, P.R. (2016), Nutritional Composition of Fruit Cultivars, 1st edition, Chapter 29 - Chemical Composition of Raspberry (*Rubus* spp.) Cultivars, Monique Simmonds, M.S.J. and Preedy, V.R., Academic Press, United Kingdom.

Bujor, O-C., Le Bourvellec, C., Volf, I., Popa, V.I. and Dufour, C. (2016), Seasonal variations of the phenolic constituents in bilberry (*Vaccinium myrtillus* L.) leaves, stems and fruits and their antioxidant activity, Food Chemistry, Vol. 213, pp. 58-68.

Cortez, R., Luna-Vital, D.A., Margulis, D., and Gonzalez de Mejia, E. (2017), Natural pigments: stabilization methods of anthocyanins for food applications, Comprehensive Reviews in Food Science and Food Safety, Vol. 16, pp.180-198.

Giusti, M.M. and Wrolstad R.E. (2001), Anthocyanins. Characterization and measurement with UV-Visible spectroscopy. In: Wrolstad RE, editor. Current protocols in food analytical chemistry. New York: John Wiley & Sons. pp. F1.2.1–F1.2.13.

Jung, E.J., Bae, M.S., Jo, E.K., Jo, Y.H. and Lee, S. C. (2011), Antioxidant activity of different parts of eggplant. Journal of Medicinal Plants Research, Vol. 5(18), pp. 4610-4615.

Khoo, H.E., Azlan, A., Tang, S.T. and Lim. S.M. (2017), Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits, FOOD & Nutrition Research, Vol. 61, pp. 1-21.

Kim, M.J., Sutton, K.L. and Harris, G.K. (2016), Raspberries and Related Fruits, Reference Module in Food Science Encyclopedia of Food and Health, Pages 586-591.

Mazza, G. and Miniati, E. (1993), Anthocyanins in fruits, vegetables and grains. CRC Press, Ino; Boca Raton.

Mejia-Meza, E.I., Yáñez, J.A., Remsberg, C.M., Takemoto, J.K., Davies, N.M. , Rasco, B. and Clary, C. (2010), Effect of dehydration on raspberries: polyphenol and anthocyanin retention, antioxidant capacity, and antiadipogenic Activity, Journal of Food Science, Vol. 75(1), pp. H5-H12.

Michalczyk, M., Macura, R. and Matuszak, I. (2009), The effect of air-drying, freeze-drying and storage on the quality and antioxidant activity of some selected berries, Journal of Food Processing and Preservation, Vol. 33, pp. 11–21.

Raponi, F., Moscetti, R., Monarca, D., Colantoni, A. and Massantini, R. (2017), Monitoring and Optimization of the Process of Drying Fruits and Vegetables Using Computer Vision: A Review. Sustainability, Vol. 9, 2009, pp. 1-27.

Ratti, C. (2001), Hot air and freeze-drying of high-value foods: A review. Journal of Food Engineering, Vol. 49, pp. 311–319.

Sigurdson, G.T., Tang, P. and Giusti, M.M. (2017), Natural colorants: food colorants from natural sources, Annual Review of Food Science and Technology, Vol. 8, pp. 261-280.

Sparzak, B., Merino-Arevalo, M., Vander Heyden, Y., Krauze-Baranowska, M., Majdan, M., Fecka, I., Głód, D. and Bączek, T. (2010), HPLC analysis of polyphenols in the fruits of *Rubus idaeus* L. (*Rosaceae*), Natural Product Research: Formerly Natural Product Letters, Vol. 24:19, pp. 1811-1822.

Tănase, E.E., Popa, V.I., Popa, M.E., Geicu-Cristea, M., Popescu, P., Drăghici, M. and Miteluț, A.C. (2016), Identification of the most relevant quality parameters for berries - a review, Scientific Bulletin. Series F. Biotechnologies, Vol. XX, pp. 222-233.