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# Antioxidant activity according to bioactive compounds content in dried pumpkin waste

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*Abstract:* In this study, for the preparation of dried pumpkin waste, freeze-drying and oven-drying (at 50 °C and 65 °C) were applied. Effect of drying methods on physical properties (moisture content, water activity, hygroscopicity, water solubility, bulk and tapped density, flowability and colour), content of bioactive compounds (carotenoids and phenolics) and antioxidant activity was investigated. Also, influence of one-year storage at –20 °C on the bioactive compounds content and antioxidant activity of dried pumpkin waste was examined. Results indicated that drying method showed a significant impact on the investigated characteristics. Bioactive compounds content and antioxidant activity by DPPH test of freeze-dried were higher than of oven-dried pumpkin waste, while higher antioxidant activity in reducing power assay was determined by oven-dried pumpkin waste. An artificial neural network model was developed, for the anticipation of antioxidant activity according to bioactive compounds content (phenolics and carotenoids), in oven-dried (at 50 °C and 65 °C) and in freeze-dried pumpkin waste after one-year storage. These models showed good prediction properties (the $r^2$ value during training cycle for output variables was 0.999). It was demonstrated that pumpkin waste is potentially an important source of bioactive compounds, which can be used after extraction in suitable forms in the development of functional food products.

*Keywords: Cucurbita moschata*; carotenoids; polyphenols; antioxidants; artificial neural network..

## INTRODUCTION

*Cucurbita moschata* pumpkin is one of the most commonly cultivated pumpkin species.[1,2] It contains numerous bioactive compounds (such as

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carotenoids, phenolic compounds and vitamins) and possesses a wide range of pharmacological activities.[3] During processing of pumpkin into various products, such as purées, dishes and juices, a large amount of wastes (*i.e.* thick rind, large seeds and pressed residues) is generated.[1,4] Unutilized wastes can lead to potential problems for their handling and disposal.[4] Also, these waste materials can be considered as potential source of valuable bioactive compounds.[5,6]

A limiting factor of using of wastes as raw materials for the extraction of bioactive compounds may be their deterioration. For inhibition of microbial growth and facilitating storage of plant materials different drying methods have been employed.[7] The most commonly used drying method includes convection. This method is not expensive, but has the disadvantage of reducing the content of some valuable compounds which often undergo oxidation at higher temperature.[8] Freeze drying, also known as lyophilization, is based on sublimation process where the product is frozen first.[9] In this process, drying is performed at low temperature and pressure, i.e. under conditions that allow removal of water by sublimation, which involves direct phase transition of water from solid to vapor without passing through the liquid phase.[9] Therefore, freeze drying is one of the most useful processes for drying thermosensitive substances.[9,10] Freeze-drying provides excellent quality characteristics such as colour, flavour and chemical composition of the product, but this method includes very high expenses and requires a lot of time.[8] Although, in most cases, freeze dried products exhibit much higher contents of bioactive compounds than hot air dried products, some studies have shown that freeze drying can led to greater loss of bioactive compounds than hot air drying.[7] The effect of a particular drying method on the retention of raw quality is not predictable and depends on the bioactive compounds and the specific plant material involved.[7] Accordingly, the selection of drying treatment as preservation technique of waste materials can have a great influence on the retention of their bioactive constituents and raw quality.

The isolation and utilization of bioactive compounds from waste are of special interest for the food, cosmetic and pharmaceutical industries.[5,6] However, the effectiveness of bioactive ingredients depends on preserving their stability and bioactivity.[11,12] Since the content of bioactive compounds remain available can be significantly reduced under the adverse conditions during food processing and storage (temperature, oxygen, light), their potential health benefits can be restricted.[11] Therefore, the aim of our study was to determine and compare physical properties, content of bioactive compounds and antioxidant activity of *C. moschata* pumpkin waste subjected to oven and freeze drying. Another objective of this study was to investigate the possibility of predicting the antioxidant activity of oven-dried (50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste after one-year storage, according to the content of phenols and carotenoids in the samples. These tasks were achieved using artificial neural network model.

## EXPERIMENTAL

### *Chemicals*

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), Trolox and trichloroacetic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA), ferric chloride was obtained from J.T. Baker (Deventer, Holland). All other chemicals and solvents used were of the highest analytical grade.

### *Plant material and dried pumpkin waste preparation*

Fresh *Cucurbita moschata* pumpkin waste material was obtained after pressing the pulp as by-product from the baby food industry ("Juvitana", Inđija, Serbia) and dried by different methods, in oven (model ST-06, Instrumentaria, Zagreb, Croatia) at 50 °C for 14 h and at 65 °C for 12 h without air flow and in freeze drier (model Christ Alpha 2-4 LSC, Martin Christ, Osterode am Harz, Germany) at –40 °C for 72 h (until moisture content was reduced to 9–11 g/100 g). Dried pumpkin waste was ground, packed in plastic bags, vacuumed and kept at –20 °C until further analysis. Dry matter content was determined by weighing the initial and final weight, and calculated percentage of dried weight.

### *Physical characterization*

Water activity ($a_w$), moisture content, hygroscopicity, bulk density, tapped density and colour parameters ($L^*$, $a^*$ and $b^*$) were determined as reported previously.[13] Colour parameters ($C^*$ and $h°$) and browning index (BI) was calculated as described by Phuon et al.[14] The classification of the flowability was made as described by Shishir et al.[15] Solubility was determined according to the method of Yamashita et al.,[16] with some modifications.

*Water activity.* The water activity ($a_w$) was determined by placing approximately 3 g of dried pumpkin waste in the sample holder of a LabSwift-aw metre (Novasina, Switzerland) at 25 °C.

*Moisture content.* The moisture content of dried pumpkin waste was measured using an air oven method at 105 °C until a constant weight was achieved.

*Hygroscopicity.* For hygroscopicity 2 g of dried pumpkin waste from the Petri dishes were placed at 25 °C in an airtight plastic container filled with NaCl saturated solution (75.29 % RH). After 1 week, hygroscopic moisture (hygroscopicity) was weighed and expressed as g of moisture per 100 g dry solids.

*Solubility.* The dried pumpkin waste (0.1 g) was dissolved in 10 mL of distilled water, stirred (Unimax 1010, Heidolph Instruments GmbH, Kelheim, Germany) at 150 rpm and room temperature for 30 min, and then centrifuged (centrifuge Lace 24, Colo Lab Experts, Novo Mesto, Slovenia) at 4000 rpm for 5 min. The supernatant was transferred to a pre-weighed Petri dish and dried at 105 °C until constant weight was achieved. After drying, the dried weight of the soluble solid was measured and used to calculate the percentage solubility.

*Bulk and tapped density.* For determination of bulk density ($D$b), the sample (10 g) was poured into a measuring cylinder and the initial volume was noted as the bulk volume ($V$b). The $D$b was calculated according to the formula:

$$D\mathrm{b} = m/V\mathrm{b} \quad (1)$$

where $m$ is the mass of the sample and $V$b is the bulk volume of the powder.

For determination of tapped density ($D$t), the sample was tapped 250 times and then the volume was measured. Tapping was continued until the difference between successive volumes was less than 2 % and this value was registered as the tapped volume ($V$t) and $D$t was calculated by the formula:

$$Dt = m/Vt \quad (2)$$

Bulk and tapped density of the samples were expressed in g $mL^{-1}$.

*Flowability.* The values of the Carr index and Hausner ratio indicate the quality of the powder in terms of the flow property. Carr index and Hausner ratio were calculated according to the equations:

$$\text{Carr index} = ((Dt–Db)/Dt) \cdot 100 \quad (3)$$

$$\text{Hausner ratio} = Dt/Db \quad (4)$$

*Colour*. The colour parameters (CIE $L^*$, $a^*$ and $b^*$) were determined with a Minolta reflectance colorimeter (Minolta ChromaMeter CR-400, Konica Minolta Inc., Tokyo, Japan) using D65 illumination source at observer angle of 2°. The colour of dried pumpkin waste was measured using attachment for granular materials CR-A50. The liquid extracts were measured in a 10 mm glass cell CM-A98 fixed using specimen holder CM-A96, while standard white calibration plate was fixed behind the cell because of the sample transparency. Chroma ($C^*$) and hue angle ($h°$) were calculated according to the formulas:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (5)$$

$$h° = \arctan(b^*/a^*) \quad (6)$$

Browning index (*BI*) was calculated using the following expression:

$$BI = ((X - 0.31)/0.17) \cdot 100 \quad (7)$$

where

$$X = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*) \quad (8)$$

*Extraction procedure*

For determination of the content of bioactive compounds and antioxidant activity previously described extraction procedure with modification was used.[10] Briefly, dried pumpkin waste was extracted using acetone : ethanol mixture (36:64 v/v) in solid to solvent ratio 1:10 (w/v) using a laboratory shaker (Unimax 1010, Heidolph Instruments GmbH, Kelheim, Germany) at 300 rpm, under light protection, at room temperature. The extraction was performed three times with the same volume of solvents. The obtained three extracts were filtered (Whatman paper No.1), combined, and stored in dark bottles at -20 °C till further analysis.

*Determination of β-carotene and total phenolic content*

The content of β-carotene was analyzed spectrophotometrically using a Multiskan GO microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the method of Nagata and Yamashita,[17] adapted for 96 well microplate. The β-carotene content was expressed as mg of β-carotene equivalents per 100 g sample (DW). The total content of phenolics was determined spectrophotometrically by Folin-Ciocalteau method,[18] adapted for 96 well microplate. Results of total phenolics content were expressed as gallic acid equivalents (GAE) per 100 g sample (DW).

*HPLC analysis*

Qualitative and quantitative analysis of flavonoids and phenolic acids was performed by HPLC analysis.[19] Also, analysis of carotenoids was done according to the HPLC method described previously.[13]

*Determination of antioxidant activity*

Antioxidant activity on DPPH radicals (DPPH assay) was estimated spectrophotometrically using a 96-well microplate reader, following the method described by Girones-Vilaplana et al.[20] Reducing power (RP assay) was performed with the method of Oyaizu[21] adapted for 96 well microplate. The calibration curves were made with Trolox and results were expressed as mg Trolox equivalents (TE) per 100 g of sample (DW).

*Storage stability test*

Dried pumpkin waste samples were stored at –20 °C in high-density polyethylene bags for one year. The effect of storage on the stability of bioactive compounds and antioxidant activities were measured using the appropriate methods described above.

*ANN modelling*

A multi-layer perceptron model (MLP), with three layers was applied for artificial neural network (ANN) modelling, to investigate the antioxidant activity of oven-dried (50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste samples after one-year storage, on the basis of the bioactive compounds content. The experimental database was normalized in order to improve the behaviour of the ANN. Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was employed in solving nonlinear problems during the network modelling.[22] A series of different topologies (more than 100,000) were tested during the modelling, changing the number of neurons in the hidden layer (from 5 to 20), randomly setting initial weights and biases.[23]

*The accuracy of the model*

The numerical investigation of the developed ANN model's accuracy was performed applying the common used statistical tests, such as coefficient of determination ($r^2$), reduced chi-square ($\chi^2$), mean bias error (MBE), root mean square error (RMSE), mean percentage error (MPE), average absolute relative deviation (AARD) and sum of squared errors (SSE).[24]

*Global sensitivity analysis*

The Yoon's interpretation method was used to determine the relative influence of bioactive compounds content on antioxidant activity of oven-dried and freeze-dried pumpkin waste samples. This method was applied using the weight coefficients of the developed ANN model.[25]

*Statistical analysis*

All experiments were done in triplicate. The results were expressed as mean value ± standard deviation (± SD, n = 3). The calculations were performed using StatSoft Statistica 2010 software.

## RESULTS AND DISCUSSION

*Physical properties of dried pumpkin waste*

In recent years, the need for dried products of high quality has been increased. Much attention has been focused to the quality of dried materials.[26] In our study, oven-drying and freeze-drying were applied to obtain dried pumpkin waste materials. After oven-drying (at 50 °C and 65 °C) and freeze-drying, dry matter content was determined to be 15.42 %, 15.40 % and 15.37 %, respectively. The influence of drying methods and temperatures on the physical properties of dried pumpkin waste is shown in Table I.

TABLE I. Physical properties of oven-dried (at 50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste

| Physical properties | Oven-dried (50 °C) | Oven-dried (65 °C) | Freeze-dried (–40 °C) |
|---|---|---|---|
| Moisture content, % | 10.78±0.33$^{b}$ | 10.18±0.44$^{b}$ | 9.10±0.32$^{a}$ |
| Water activity ($a_w$) | 0.274±0.010$^{c}$ | 0.126±0.005$^{b}$ | 0.035±0.001$^{a}$ |
| Hygroscopicity, g/100 g | 13.29±0.47$^{a}$ | 15.08±0.71$^{b}$ | 15.49±0.48$^{b}$ |
| Bulk density, g mL$^{-1}$ | 0.40±0.02$^{c}$ | 0.36±0.02$^{b}$ | 0.11±0.00$^{a}$ |
| Tapped density, g mL$^{-1}$ | 0.58±0.04$^{c}$ | 0.50±0.03$^{b}$ | 0.17±0.01$^{a}$ |
| Carr index, % | | | 33.35±0.93$^{c}$ |
| Hausner ratio | | | 1.50±0.02$^{c}$ |
| Flowability | Poor | Poor | Very poor |
| Solubility, % | 31.19±1.12$^{a}$ | 33.30±1.47$^{b}$ | 33.76±1.38$^{b}$ |
| | | | 29.91±0.01$^{c}$ |
| Chroma ($C^*$) | | | 30.45±0.01$^{c}$ |
| Hue angle ($h$°) | 67.71±0.06$^{a}$ | 73.64±0.06$^{b}$ | 79.17±0.01$^{c}$ |
| Browning index (*BI*) | 74.49±0.03$^{c}$ | 70.36±0.05$^{b}$ | 63.46±0.01$^{a}$ |

The results are presented as mean ± SD; different letters (a-c) in rows indicate that there is significant difference at $p \leq 0.05$, according to Tukey-s HSD test

Moisture content, *i.e.* quantity of water contained in a food system, is an important characteristic of dried powdered products, which is associated to the efficiency of drying process.[27,28] Also, moisture content of powders has a significant influence on storage stability.[29] Similarly as in our study, moisture content of oven-drying pumpkin material was higher than that in freeze-dried pumpkin material.[3,30] Aydin and Gocmen[31] reported that moisture contents in hot-air oven-dried and freeze-dried pumpkin flour were 12.64 % and 12.56 %, respectively. Probably, crust formed during hot air drying did not allow removing the moisture from material.[30] Water activity ($a_w$) represents the availability of free water in a food system which has a crucial role in biochemical reactions.[29] Basically, the most of the adverse changes in food system during storage such as lipid oxidation, enzymatic reactions, non-enzymatic browning, and microbial growth are almost completely obstructed when the water activity value is below 0.4.[32] Therefore, water activity has been considered as one of the most important quality parameters for long term storage of dried products.[30] In our study, water activity values of dried pumpkin waste samples provide their good storage stability. Previously, oven-dried pumpkin materials also had higher water activity (0.408) than freeze-dried pumpkin material (0.239).[30] Hygroscopicity, *i.e.* capacity to adsorb ambient moisture, is one of the major factors which affect product stability.[27,33] A prominent property of freeze-dried products is great hygroscopicity.[34] Hygroscopicity is related to the gradient of water concentration

between the product and the surrounding environment, which is greater for the less moist powder.[33] Indeed, higher hygroscopicity was determined in freeze-dried pumpkin waste in comparison with oven-dried.

Tapped density represents the real solid density, whereas the bulk density takes into account all the spaces between the particles of powdered products. Lower bulk density implies that more occluded air exists within the powder, thus there is a greater possibility for oxidation and reduced stability of product during storage.[28] The bulk and tapped densities were higher in oven-dried in relation to freeze-dried pumpkin waste, which is in agreement with the study of Que et al.[3] According to Carr index and Hausner ratio, oven-dried and freeze-dried pumpkin waste are classified,[15] in terms of flow property as poor and very poor, respectively. Solubility is a property referring to the product behaviour in an aqueous phase and is an indicator for its reconstitution quality. Quick and complete reconstitution of powder is common requirement for its application.[35] The powder structure with more cavities and pores allows easier passage of water, thus facilitates dissolution. Pumpkin waste dried at 65 °C showed higher solubility compared to the waste dried at 50 °C. Solubility can be associated with the amount of starch degradation, since at higher temperature during oven-drying more starch can be decomposed.[3]

Colour is a significant factor for consumers in food choice.[36] It can be used as an indicator of the chemical and quality changes as a consequence of thermal processing.[36] In agreement with our study, oven-drying in comparison to freeze-drying resulted in a darker colour (lower $L^*$ values).[3,30] The lower $L^*$ value and higher $a^*$ value could be indicative of the browning reaction.[31,37] Compared to oven-dried waste samples, freeze-dried waste showed less yellowness and less redness (indicated by lower $b^*$ and $a^*$ values, respectively). Browning is an important colour reaction in food processing such as drying because it affects appearance quality.[36] As an indicator of browning, browning index (*BI*) is often used especially in conventional drying since it represents the purity of brown colour and is considered to be an important parameter associated with browning.[30] In this study, the values of browning index of dried waste samples showing that oven-drying caused more brown compounds. The difference in browning may be related to the removal of water by sublimation and prevention of enzymatic browning reactions during freeze-drying.[38,39] The yellowish colour of dried pumpkin waste powders could be mainly attributed to the presence of carotenoids naturally found in this vegetable. Drying conditions, including high temperature, light and oxygen exposure can cause changes in food surface that lead to colour changes, and also to degradation of carotenoids.[32] The lighter yellowness of freeze-dried in relation to oven-dried waste is shown by $C^*$ and $h°$ values. Similar colour values as in our study, lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) of convective dried (55.57, 22.67, and 35.25, respectively) and freeze-dried (77.96, 18.54, and 31.22, respectively) slices *of C. moschata* pumpkin were found.[30] In

addition, Que et al.[3] reported that the freeze-drying process can significantly decrease the brownish appearance of pumpkin flour and can produce pumpkin powders of high-quality colour.

*Bioactive compounds and antioxidant activity of dried pumpkin waste*

Effect of drying methods, drying temperatures and storage on the content of bioactive compounds and antioxidant activity of dried pumpkin waste samples is presented in Table II.

TABLE II. The content of bioactive compounds (phenolics and carotenoids) and antioxidant activity (DPPH and RP assays) of oven-dried (at 50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste

| Bioactive compounds/ antioxidant activity | Oven-dried (50 °C) | Oven-dried (65 °C) | Freeze-dried (–40 °C) |
|---|---|---|---|
| | | | 7.89±0.22$^{c}$ |
| | | | 9.217±0.43$^{c}$ |
| Catechin* | | | 1.672±0.067$^{c}$ |
| Caffeic acid* | 0.128±0.005$^{c}$ | 0.096±0.003$^{a}$ | 0.116±0.003$^{b}$ |
| Syringic acid* | | | 1.835±0.076$^{c}$ |
| | | | 12.606±0.598$^{c}$ |
| Vanillic acid* | 0.171±0.006$^{c}$ | 0.117±0.004$^{a}$ | 1.385±0.060$^{b}$ |
| Myricetin* | | | 1.385±0.056$^{c}$ |
| Rutin* | | | 0.313±0.011$^{c}$ |
| Total phenolics* | 13.492 | 10.352 | 31.365 |
| Lutein* | | | 0.174±0.006$^{c}$ |
| Zeaxanthin* | 1.598±0.069$^{b}$ | 0.403±0.017$^{a}$ | 1.660±0.081$^{b}$ |
| β-Carotene* | 4.406±0.187$^{c}$ | 1.303±0.057$^{a}$ | 3.247±0.158$^{b}$ |
| β-Cryptoxanthin* | n.d. | n.d. | 2.820±0.134 |
| Total carotenoids* | 6.037 | 1.743 | 7.901 |
| DPPH - after drying | 99.27±1.52$^{b}$ | 78.87±0.55$^{a}$ | 131.64±6.40$^{c}$ |
| DPPH - after storage | 50.56±1.75$^{b}$ | 30.41±0.34$^{a}$ | 65.66±1.55$^{c}$ |
| RP - after drying | 66.23±1.97$^{b}$ | 140.94±0.77$^{c}$ | 17.39±0.28$^{a}$ |
| RP - after storage | 60.40±2.68$^{b}$ | 140.20±3.10$^{c}$ | 16.41±0.46$^{a}$ |

The spectrophotometric determinations were done after drying of pumpkin waste and after storage at –20 °C (during one year) of dried pumpkin waste samples, while HPLC analysis (indicated by *) was performed after drying of pumpkin waste; the results are presented as mean ± SD; different letters (a-c) in rows indicate that there is significant difference at $p \leq 0.05$, according to Tukey-s HSD test; total and individual contents of carotenoids and phenolics are presented

in mg/100 g DW, where total phenolics determined spectrophotometically are expressed in mg GAE/100 g DW; antioxidant activity by DPPH and RP assays is presented in mg TE/100 g DW; n.d. means not detected

Higher content of total phenolics determined by spectrophotometric and HPLC methods was determined in freeze-dried pumpkin waste in comparison to oven-dried samples. In the study of Aydin and Gocmen,[31] higher level of phenols (236 mg GAE/100 g DW) was found in oven-dried (at 60 °C) pumpkin (*Cucurbita moschata* Duch.) flour compared to freeze-dried (226 mg GAE/100 g DW). Also, Que *et al*.[3] reported that content of phenolic compounds (1.64 mg $g^{-1}$) in hot air-dried (at 70 °C) pumpkin (*Cucurbita moschata* Duch.) flour was 4.6 times higher than that in freeze-dried pumpkin (0.39 mg $g^{-1}$) flour, indicating the formation of phenolics during drying at 70 °C. Higher degree of heating could have a great impact on the increase of total phenolics of pumpkin flour.[3] Beside the difference in sample, extraction processes, drying conditions, cultivar, maturation stage, geography and climate may cause difference in determination of phenolics.[30,40] In the study of Nawirska-Olszanska et al.,[41] the content of phenolics in different varieties of *C. moschata* pumpkin was ranged from 141.16 mg/100 g DW to 390.61 mg/100 g DW. Potosi-Calvache et al.[42] determined the content of total phenolics (from 27.7 mg GAE/100 g to 79.9 mg GAE/100 g) in samples of *C. moschata* pumpkin dried under different temperature (45-65 °C) and air flow (4-7 m/s). During processing and storage phenolics can be degraded or formed from the availability of their precursors or by non-enzymatic inter conversions between them.[42] In our study, in investigated pumpkin waste powders, *p*-hydroxybenzoic and protocatechuic acid were determined in highest content of all identified phenolic acids, while catechin was determined in highest content of identified flavonoids. Kulczyński and Gramza-Michałowska[1] reported that profile of bioactive compounds in pumpkin (*C. moschata*) is considerably diversified in its cultivars. In agreement with our study, content of identified flavonoids were lower than content of phenolic acids, and high contents of *p*-hydroxybenzoic and protocatechuic acids were also found.[1]

Higher β-carotene content, determined spectrophotometrically, was observed in freeze-dried than in oven-dried pumpkin waste samples. Different factors (temperature, oxygen and light) during drying process (*i.e.* drying conditions) may cause carotenoid degradation.[32,40] In agreement with in our study, higher total carotenoid content (between 5 mg/100 g and 160 mg/100 g) was obtained with freeze-drying than by convective drying of 12 pumpkin cultivars.[8,40] Previously, higher drying temperatures also produced greater pigment losses.[32] Colour values, $L^*$, $a^*$ and $b^*$ of extracts obtained after extraction of oven-dried at 50 ° (59.43, –5.27, and 66.53, respectively), oven-dried at 65 ° (59.27, –5.61, and 67.16, respectively) and freeze dried (56.01, 1.79, and 65.57, respectively) *C. moschata* pumpkin waste, indicated yellow colour that could be attributed to the presence of

carotenoids. It is interesting to notice that colour parameter a* had negative values in oven-dried samples, indicating degradation of reddish pigments in comparison to freeze-dried sample, which had positive a* values. In our study, higher content of total carotenoids determined by HPLC method was found in freeze-dried pumpkin waste in comparison to oven-dried samples. β-Criptoxanthin was not even identified in oven-dried waste. Interestingly, somewhat higher β-carotene content was found in dried pumpkin waste after oven-drying at 50 °C than after freeze-drying. Kulczyński and Gramza-Michałowska[1] reported high variability of carotenoids among pumpkin (*C. moschata*) cultivars, and on average the content of zeaxanthin, lutein and β-carotene of different *C. moschata* cultivars was 2.64 mg/100 g DW, 6.87 mg/100 g DW and 2.92 mg/100 g DW, respectively.

After storage, the content of phenolics and β-carotene in dried pumpkin waste samples was decreased (Table II). After storage, similar values of phenolics content were found in all dried pumpkin waste samples, while highest content of β-carotene was determined in freeze dried waste in comparison to oven-dried pumpkin waste samples.

Freeze-dried pumpkin waste exhibited higher antioxidant activity by DPPH test, while higher antioxidant activity in reducing power assay was achieved by oven-dried pumpkin waste (Table II). This can be explained by various mechanisms by which different antioxidants exert their action.[26] Previously, hot-air oven dried (at 60 °C and 70 °C) pumpkin flours showed higher antioxidant activities by different assays, than freeze-dried samples.[3,31] Phenolics formed from precursors in hot-air drying treatment or generation of Maillard-type antioxidants might be responsible for higher antioxidant activity of hot-air dried pumpkin flour.[3] Samples of dried pumpkin waste in our study showed somewhat lower, but significant antioxidant activity by DPPH test (from 3.15 μmol TE $g^{-1}$ to 5.26 μmol TE $g^{-1}$), and by reducing power test (from 0.69 μmol TE $g^{-1}$ to 5.63 μmol TE $g^{-1}$) in comparison to the antioxidant activity of dried pumpkin flours (from 5.57 μmol TE $g^{-1}$ to 7.21 μmol TE $g^{-1}$) reported by Aydin and Gocmen.[31]

After storage, DPPH antioxidant activity in dried pumpkin waste powders was decreased, while reducing power was almost unchanged in comparison with levels determined before storage (Table II). After storage, highest reducing power was observed for waste dried at 65 °C, while highest antioxidant activity by DPPH test was determined for freeze-dried waste.

*ANN model*

The acquired optimal neural network model could be used to adequately anticipate the antioxidant activity of oven-dried (50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste samples after one year storage, on the basis of phenolics and carotenoids content. The optimal number of neurons was: 9 (network MLP 14-9-4), while the highest $r^2$ values during the training cycle were 0.999).

The obtained ANN model for the anticipation of output variables was complex (175 weights-biases coefficients) due to the high nonlinearity of the observed system. The goodness of fit between experimental and ANN model calculated values were shown in Table III.

Table III. The "goodness of fit" tests for the developed ANN model

| Output variable | $\chi^2$ | RMSE | MBE | MPE | SSE | AARD | $r^2$ |
|---|---|---|---|---|---|---|---|
| DPPH after drying | 1.042 | 0.589 | 0.168 | 0.589 | 0.957 | 1.766 | 1.000 |
| DPPH after storage | 0.456 | 0.390 | –0.036 | 0.718 | 0.452 | 1.091 | 1.000 |
| RP after drying | 0.267 | 0.299 | –0.193 | 0.277 | 0.156 | 0.601 | 1.000 |
| RP after storage | 0.690 | 0.480 | –0.349 | 0.542 | 0.325 | 1.046 | 1.000 |

The ANN predicted values were very close to the measured values in most cases, in terms of $r^2$ values.[43,44] The SSE values obtained with the ANN model was of the same order of magnitude as experimental errors for output variables reported in the literature.[22,45] The ANN model had an insignificant lack of fit tests, which means the model satisfactorily predicted output variables.

*Global sensitivity analysis- Yoon's interpretation method*

According to the Fig. 1, gallic acid, syringic acid, chlorogenic acid, *p*-hydroxybenzoic acid, vanillic acid, myrcetin, rutin and lutein content were the most influential parameters with approximately relative importance of 9.222–10.337 % for the prediction of DPPH after drying, while the relative influence of these variables for the prediction of DPPH after storage reached the relative importance of 9.583–10.680 %.The RP assay was mostly influenced by the catechin content, 27.82 % after drying, and 28.55 % after the on-year long storage.

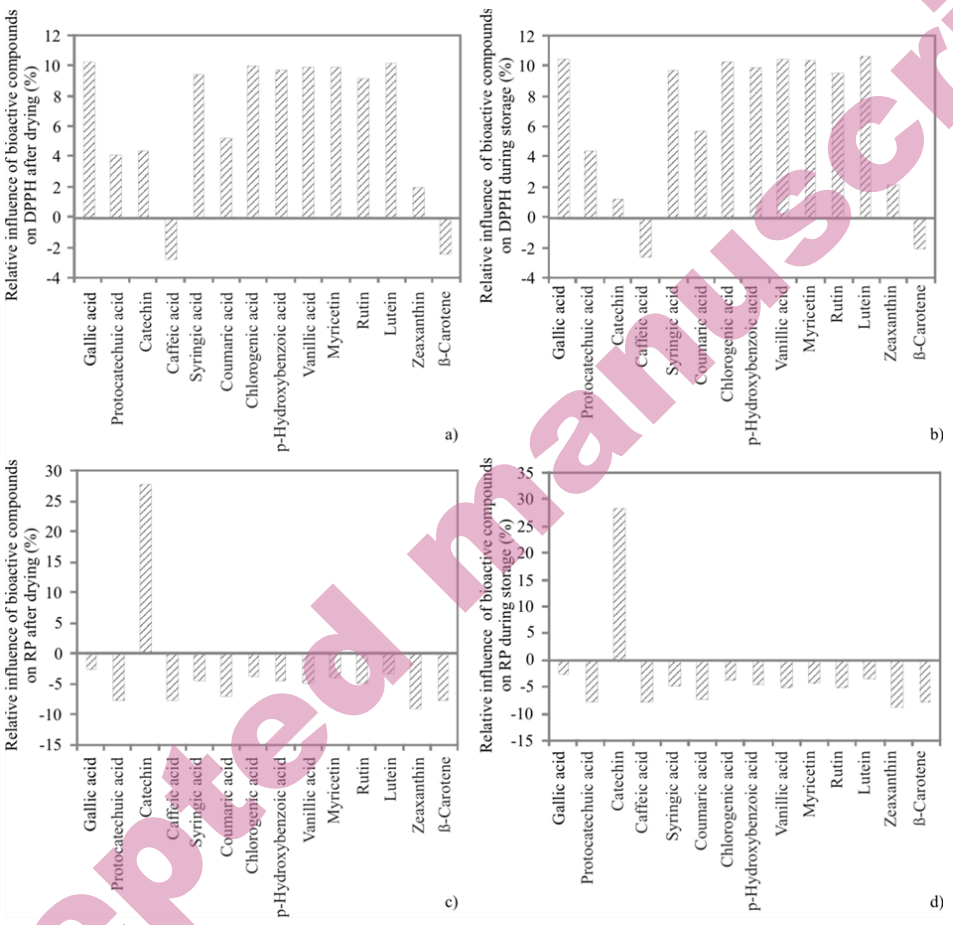


Fig. 1. The relative importance of the bioactive compounds content on antioxidant activity determined by DPPH assay after drying (a) and storage (b), and by RP assay after drying (c) and storage (d) of pumpkin waste samples.

## CONCLUSION

Different drying methods, including freeze-drying and oven-drying (at 50 °C and 65 °C) caused significant impact on physical properties, bioactive compounds content and antioxidant activity of dried pumpkin waste. In general, carotenoids and phenolics content of freeze-dried was higher than in oven-dried pumpkin waste. Freeze-dried pumpkin waste exhibited higher antioxidant activity by DPPH test, while higher antioxidant activity in reducing power assay was determined by oven-dried pumpkin waste. After storage at –20 °C during one year, higher content of β-carotene and antioxidant activity by DPPH test, while lower content of phenolics and reducing power were achieved by freeze-dried than by oven-dried pumpkin waste samples.

Also, findings of this study indicate that antioxidant activity of oven-dried (50 °C and 65 °C) and freeze-dried (–40 °C) pumpkin waste samples after one year storage could be anticipated, based on the phenolics and carotenoids content. The artificial neural network model showed to be adequate for the prediction of output variables (the $r^2$ values during training cycle for these variables were: 0.999).

It was demonstrated that pumpkin waste is potentially an important source of bioactive compounds with significant antioxidant properties. In the future, to overcome a main drawback for application of natural bioactive compounds, namely their instability, different protection systems including encapsulates could be formulated. Therefore, our results support the need for preparation of encapsulates of bioactive compounds extracted from dried pumpkin waste and evaluation of their physicochemical and stability characteristics, in order of development functional ingredients for value added food products.

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## И З В О Д

## АНТИОКСИДАТИВНА АКТИВНОСТ НА ОСНОВУ САДРЖАЈА БИОАКТИВНИХ ЈЕДИЊЕЊА У СУШЕНОМ ОТПАДУ ТИКВЕ

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У овој студији за припрему сушеног отпада тикве примењено је сушење лиофилизацијом и у сушници (на 50 °C и 65 °C). Испитан је утицај метода сушења на физичка својства (садржај влаге, активност воде, хигроскопност, растворљивост у води, насипну и тапкану густину, проточност и боју), садржај биоактивних једињења (каротеноида и полифенолних једињења) и антиоксидативну активност. Такође, испитан је утицај складиштења на –20 °C у периоду од годину дана на садржај биоактивних једињења и антиоксидативну активност сушеног отпада тикве. Резултати су показали да метода сушења значајно утиче на испитане карактеристике. Садржај биоактивних једињења и антиоксидативна активност DPPH тестом лиофилизованог отпада били су већи у односу на отпад тикве сушен у сушници, док је већа антиоксидативна активност тестом редукционе способности одређена у отпаду тикве сушеном у сушници. Развијен је модел вештачке неуронске мреже, за предвиђање антиоксидативне активности према садржају биоактивних једињења (полифенолна једињења и каротеноиди), у отпаду тикве сушеном лиофилизацијом и у сушници (на 50 °C и 65 °C) након годину дана складиштења. Ови модели су показали добра својства предвиђања (вредност $r^2$ током циклуса тренинга за излазне варијабле била је 0,999). Показано је да је отпад тикве потенцијално важан извор

биоактивних једињења, која се након екстракције могу користити у одговарајућим облицима у развоју функционалних прехрамбених производа.

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