Antioxidant activity according to bioactive compounds content in dried pumpkin waste

SLAĐANA M. STAJČIĆ[1]*, LATO L. PEZO[2], GORDANA S. ĆETKOVIĆ[1], JASNA M. ČANADANOVIĆ-BRUNET[1], ANAMARIJA I. MANDIĆ[3], VESNA T. TUMBAS ŠAPONJAC[1], JELENA J. VULIĆ[1#], VANJA N. TRAVIČIĆ[1] and MIONA M. BELOVIĆ[3#]

[1]*University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, Novi Sad, Serbia,*
[2]*Institute of General and Physical Chemistry, Studenski trg 12/V, Belgrade, Serbia and*
[3]*University of Novi Sad, Institute for Food Technology in Novi Sad, Bulevar Cara Lazara 1, Novi Sad, Serbia*

Abstract: In this study freeze-drying and oven-drying (at 50 and 65 °C) were applied for the preparation of dried pumpkin waste. The 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 were investigated. Also, the influence of one-year storage at –20 °C on the bioactive compounds content and the 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 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.

13

INTRODUCTION

Cucurbita moschata pumpkin is one of the most commonly cultivated pumpkin species.[1,2] It contains numerous bioactive compounds (such as 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 waste (*i.e.*, thick rind, large seeds and pressed residues) is generated.[1,4] Unutilized waste can lead to potential problems for its 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 wastes as raw materials for the extraction of bioactive compounds may be their deterioration. For the 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 which remains 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 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. 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:

$$Db = m/Vb \tag{1}$$

where *m* is the mass of the sample.

For determination of tapped density (Dt), 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 (Vt) and Dt was calculated by the formula:

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

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:

$$\text{Carr index} = 100((Dt–Db)/Dt) \qquad (3)$$
$$\text{Hausner ratio} = Dt/Db \qquad (4)$$

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:

$$C^* = (a^{*2} + b^{*2})^{1/2} \qquad (5)$$
$$h° = \arctan (b^*/a^*) \qquad (6)$$

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

$$BI = 100((X – 0.31)/0.17) \qquad (7)$$

where

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

Extraction procedure

For the 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 volume ratio) in solid to solvent ratio 1:10 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 analysed 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 (dry weight, DW). The total content of phenolics was determined spectrophotometrically by Folin–Ciocalteau method,[18] adapted for 96 well microplate. The 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 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 (χ^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 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 and 65 °C) and freeze-dried (–40 °C) pumpkin waste; the results are presented as mean ± *SD*; different letters (a–c) in rows indicate that there is significant difference at p ≤ 0.05, according to Tukey-s HSD test

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, %	30.05±1.50[b]	27.31±1.24[a]	33.35±0.93[c]
Hausner ratio	1.43±0.03[b]	1.38±0.00[a]	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]
Colour parameters			
Lightness/darkness (L^*)	49.86±0.01[a]	54.11±0.03[b]	67.29±0.02[c]
Greenness/redness (a^*)	9.45±0.03[c]	7.34±0.03[b]	5.72±0.01[a]
Blueness/yellowness (b^*)	23.05±0.00[a]	25.00±0.01[b]	29.91±0.01[c]
Chroma (C^*)	24.91±0.01[a]	26.05±0.01[b]	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]

Moisture content, *i.e.*, quantity of water contained in a food system, is an important characteristic of dried powdered products, which is associated with the efficiency of drying process.[27,28] Also, the moisture content of powders has a significant influence on storage stability.[29] Similarly as in our study, the 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, the 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, the water activity has been considered as one of the most important quality parameters for long term storage of dried products.[30] In our study, the 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] Hygro-

scopicity, *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 showed 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 (mean ± *SD*) of bioactive compounds (phenolics and carotenoids, mg/100 g DW) and antioxidant activity (DPPH and RP assays, mg TE/100 g DW) of oven-dried (at 50 and 65 °C) and freeze-dried (–40 °C) pumpkin waste; different letters (a–c) in rows indicate that there is significant difference at $p \leq 0.05$, according to Tukey's HSD test; * – HPLC analysis was performed after drying of pumpkin waste; n.d. – not detected

Bioactive compounds/antioxidant activity	Oven-dried (50 °C)	Oven-dried (65 °C)	Freeze-dried (–40 °C)
Total phenolics – after drying	201.31±7.27[a]	213.61±7.60[b]	294.69±14.13[c]
Total phenolics – after storage	183.47±7.03[b]	180.34±5.39[b]	175.60±6.11[a]
β-Carotene – after drying	6.51±0.23[b]	5.56±0.00[a]	14.26±0.27[c]
β-Carotene – after storage	2.85±0.12[a]	4.57±0.00[b]	7.89±0.22[c]
Gallic acid*	1.658±0.080[a]	1.757±0.078[b]	3.802±0.17[c]
Protocatechuic acid*	7.644±0.322[b]	4.555±0.221[a]	9.217±0.43[c]
Catechin*	0.490±0.021[a]	1.588±0.071[b]	1.672±0.067[c]
Caffeic acid*	0.128±0.005[c]	0.096±0.003[a]	0.116±0.003[b]
Syringic acid*	0.470±0.021[b]	0.288±0.011[a]	1.835±0.076[c]
Coumaric acid*	0.103±0.004[b]	0.097±0.003[a]	0.108±0.002[b]
Chlorogenic acid*	0.049±0.002[b]	0.038±0.001[a]	0.224±0.008[c]
p-Hydroxybenzoic acid*	2.642±0.131[b]	1.726±0.076[a]	12.606±0.598[c]
Vanillic acid*	0.171±0.006[c]	0.117±0.004[a]	1.385±0.060[b]
Myricetin*	0.010±0.001[b]	0.007±0.001[a]	1.385±0.056[c]
Rutin*	0.127±0.004[b]	0.083±0.002[a]	0.313±0.011[c]
Total phenolics*	13.492	10.352	31.365
Lutein*	0.033±0.001[a]	0.037±0.001[b]	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. 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 (*C. 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 (*C. 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 the profile of bioactive compounds in pumpkin (*C. moschata*) is considerably diversified in its cultivars. In agreement with our study, the content of identified flavonoids were lower than the 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 our study, higher total carotenoid content (between 5 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 °C (59.43, –5.27 and 66.53, respectively), oven-dried at 65 °C (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 the 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, 6.87 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 the 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 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 to 5.26 µmol TE g^{-1}), and by reducing power test (from 0.69 to 5.63 µmol TE g^{-1}) in comparison to the antioxidant activity of dried pumpkin flours (from 5.57 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, the highest reducing power was observed for waste dried at 65 °C, while the 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 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	χ^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.

<div align="center">CONCLUSION</div>

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 the freeze-dried pumpkin waste was higher than of the oven-dried one. The freeze-dried pumpkin waste exhibited higher antioxidant activity by DPPH test, while higher antioxidant activity in reducing power assay was determined for the 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 the oven-dried (50 °C and 65 °C) and the 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
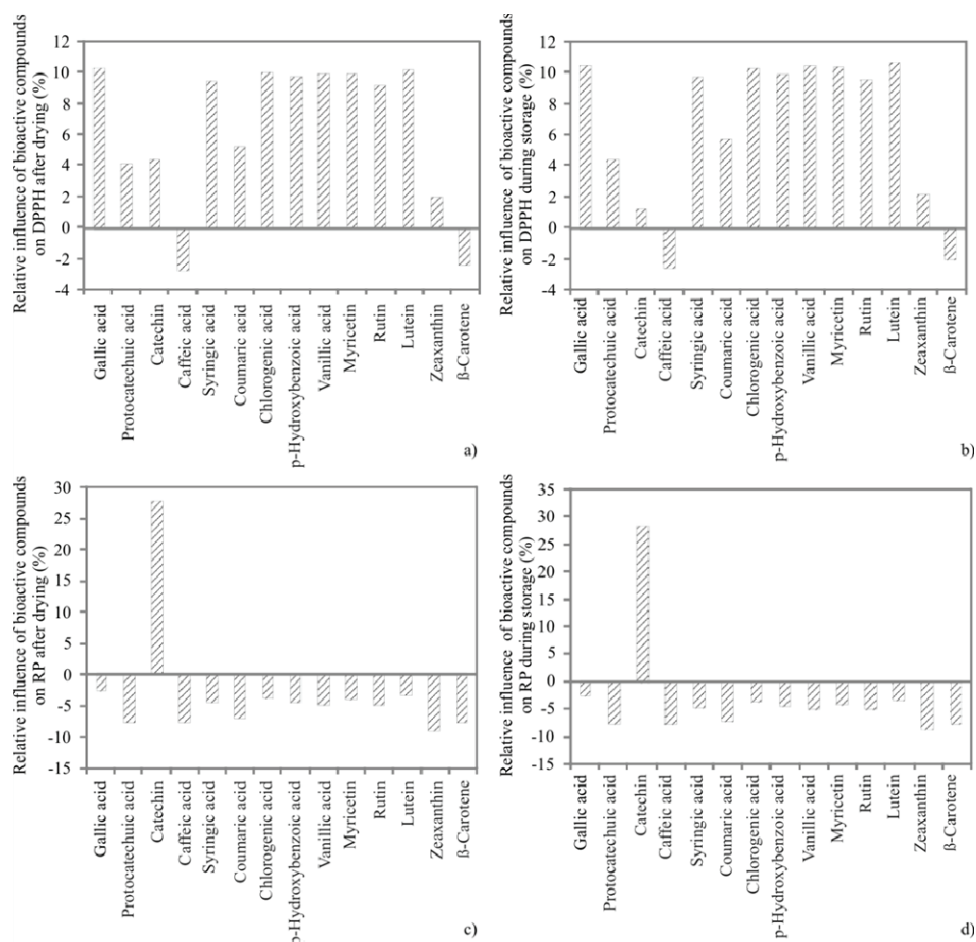
Fig. 1. The relative importance of the bioactive compounds content on antioxidant activity determined by DPPH assay after drying (a) and storage (b), and by RP assay after drying (c) and storage (d) of pumpkin waste samples.

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 the 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 the 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.

ИЗВОД

АНТИОКСИДАТИВНА АКТИВНОСТ НА ОСНОВУ САДРЖАЈА БИОАКТИВНИХ ЈЕДИЊЕЊА У СУШЕНОМ ОТПАДУ ТИКВЕ

СЛАЂАНА М. СТАЈЧИЋ[1], ЛАТО Л. ПЕЗО[2], ГОРДАНА С. ЋЕТКОВИЋ[1], ЈАСНА М. ЧАНАДАНОВИЋ-БРУНЕТ[1], АНАМАРИЈА И. МАНДИЋ[3], ВЕСНА Т. ТУМБАС ШАПОЊАЦ[1], ЈЕЛЕНА Ј. ВУЛИЋ[1], ВАЊА Н. ТРАВИЧИЋ[1] и МИОНА М. БЕЛОВИЋ[3]

[1]*Универзишеш у Новом Саду, Технолошки факулшеш, Булевар цара Лазара 1, Нови Сад,* [2]*Инсшишуш за ойшшу и физичку хемију, Сшуденшски шрг 12/В, Београд и* [3]*Универзишеш у Новом Саду, Научни инсшишуш за йрехрамбене шехнологије у Новом Саду, Булевар цара Лазара 1, Нови Сад*

У овој студији за припрему сушеног отпада тикве примењено је сушење лиофилизацијом и у сушници (на 50 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 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.

(Примљено 17. маја, ревидирано 11. јуна, прихваћено 21. јула 2023)

REFERENCES

1. B. Kulczyński, A. Gramza-Michałowska, *Molecules* **24** (2019) 2945 (https://doi.org/10.3390/molecules24162945)
2. A. Hussain, T. Kausar, A. Din, A. Murtaza, M. A. Jamil, S. Noreen, H. Rehman, H. Shabbir, M. A. Ramzan, *J. Food Process. Preserv.* **45** (2021) e15542 (https://doi.org/10.1111/jfpp.15542)
3. F. Que, L. Mao, X. Fang, T. Wu, *Int. J. Food Sci. Technol.* **43** (2008) 1195 (https://doi.org/10.1111/j.1365-2621.2007.01590.x)
4. A. A. Shetty, R. Rana, T. Buckseth, S. P. Preetham, *Waste and Biomass Valor.* **3** (2012) 363 (https://doi.org/10.1007/s12649-012-9114-x)
5. A. Hussain, T. Kausar, S. Sehar, A. Sarwar, A. H. Ashraf, M. A. Jamil, S. Noreen, A. Rafique, K. Iftikhar, M. Y. Quddoos, J. Aslam, M. A. Majeed, *Food Chem. Adv.* **1** (2022) 100067 (https://doi.org/10.1016/j.focha.2022.100067)

6. A. Hussain, T. Kausar, S. Sehar, A. Sarwar, A. H. Ashraf, M. A. Jamil, S. Noreen, A. Rafique, K. Iftikhar, J. Aslam, M. Y. Quddoos, M. A. Majeed, M. Zerlasht, *Appl. Food Res.* **2** (2022) 100241 (https://doi.org/10.1016/j.afres.2022.100241)

7. R. Li, H. Shang, H. Wu, M. Wang, M. Duan, J. Yang, *Sci Rep.* **8** (2018) 9529 (https://doi.org/10.1038/s41598-018-27874-4)

8. A. Nawirska, A. Figiel, A. Z. Kucharska, A. Sokoł-Łętowska, A. Biesiada, *J. Food Eng.* **94** (2009) 14 (http://dx.doi.org/10.1016/j.jfoodeng.2009.02.025)

9. M. U. H. Suzihaque, S. A. Hashib, U. K. Ibrahim, *Proc. Soc. Behav. Sci.* **195** (2015) 2829 (https://doi.org/10.1016/j.sbspro.2015.06.401)

10. V. N. Šeregelj, G. S. Ćetković, J. M. Čanadanović-Brunet, V. T. Tumbas Šaponjac, J. J. Vulić, S. M. Stajčić, *Acta Period. Technol.* **48** (2017) 261 (https://doi.org/10.2298/APT1748261S)

11. Z. Fang, B. Bhandari, *Trends Food Sci. Technol.* **21** (2010) 510 (https://doi.org/10.1016/j.tifs.2010.08.003)

12. A. Hussain, T. Kausar, S. Sehar, A. Sarwar, M. Y. Quddoos, J. Aslam, A. Liaqat, T. Siddique, Q. U. An, S. Kauser, A. Rehman, R. Nisar, *Food Prod. Process. Nutr.* **5** (2023) 1 (https://doi.org/10.1186/s43014-023-00138-z)

13. V. Šeregelj, V. Tumbas Šaponjac, S. Lević, A. Kalušević, G. Ćetković, J. Čanadanović-Brunet, V. Nedović, S. Stajčić, J. Vulić, A. Vidaković, *J. Microencapsul.* **36** (2019) 704 (https://doi.org/10.1080/02652048.2019.1668488)

14. V. Phuon, I. N. Ramos, T. R. S. Brandão, C. L. M. Silva, *J. Food Process Eng.* **45** (2021) e13794 (https://doi.org/10.1111/jfpe.13794)

15. M. R. I. Shishir, F. S. Taip, N. A. Aziz, R. A. Talib, *Agric. Sci. Proc.* **2** (2014) 74 (https://doi.org/10.1016/j.aaspro.2014.11.011)

16. C. Yamashita, M. M. Song Chung, C. dos Santos, C. Malacrida, I. C. F. Moraes, I. Branco, *LWT – Food Sci. Technol.* **84** (2017) 256 (https://doi.org/10.1016/j.lwt.2017.05.063)

17. M. Nagata, I. Yamashita, *J. Jpn. Soc. Food Sci. Technol.* **39** (1992) 925 (https://doi.org/10.3136/nskkk1962.39.925)

18. V. L. Singleton, J. A. Rossi, *Am. J. Enol. Vitic.* **16** (1965) 144 (https://doi.org/10.5344/ajev.1965.16.3.144)

19. V. Tumbas Šaponjac, G. Ćetković, J. Čanadanović-Brunet, A. Mandić, V. Šeregelj, J. Vulić, S. Stajčić, *J. Chem.* **2019** (2019) 7184293 (https://doi.org/10.1155/2019/7184293)

20. A. Girones-Vilaplana, P. Mena, D. A. Moreno, C. Garcia-Viguera, *J. Sci. Food Agric.* **94** (2014) 1090 (https://doi.org/10.1002/jsfa.6370)

21. M. Oyaizu, *Jpn. J. Nutr. Diet.* **44** (1986) 307 (https://doi.org/10.5264/eiyogakuzashi.44.307)

22. T. Kollo, D. von Rosen, *Advanced Multivariate Statistics with Matrices*, Springer Dordrecht, 2005 (https://doi.org/10.1007/1-4020-3419-9)

23. C.I. Ochoa-Martínez, A.A. Ayala-Aponte, *LWT – Food Sci. Technol.* **40** (2007) 638 (https://doi.org/10.1016/j.lwt.2006.03.013)

24. M. Aćimović, L. Pezo, V. Tešević, I. Čabarkapa, M. Todosijević, *Ind. Crops Prod.* **154** (2020) 112752 (https://doi.org/10.1016/j.indcrop.2020.112752)

25. Y. Yoon, G. Swales, T. M. Margavio, *J. Oper. Res. Soc.* **44** (2017) 51 (https://doi.org/10.1057/jors.1993.6)

26. S. Roshanak, M. Rahimmalek, S. A. H. Goli, *J. Food Sci. Technol.* **53** (2016) 721 (https://doi.org/10.1007/s13197-015-2030-x)

27. N. M. Nawi, I. I. Muhamad, A. M. Marsin, *Food Sci. Nutr.* **3** (2015) 91 (https://doi.org/10.1002/fsn3.132)
28. S. J. Cynthia, J. D. Bosco, S. Bhol, *Int. J. Food Prop.* **18** (2015) 1793 (https://doi.org/10.1080/10942912.2014.940536)
29. K. Papoutsis, J. B. Golding, Q. Vuong, P. Pristijono, C. E. Stathopoulos, C. J. Scarlett, M. Bowyer, *Foods.* **7** (2018) 115 (https://doi.org/10.3390/foods7070115)
30. G. Caliskan, S. N. Dirim, *Heat Mass Transf.* **53** (2017) 2129 (https://doi.org/10.1007/s00231-017-1967-x)
31. E. Aydin, D. Gocmen, *LWT – Food Sci. Technol.* **60** (2015) 385 (http://dx.doi.org/10.1016/j.lwt.2014.08.025)
32. W. Roongruangsri, J. E. Bronlund, *Int. Food Res. J.* **23** (2016) 962 (http://www.ifrj.upm.edu.my/volume-23-2016.html)
33. R. V. Tonon, S. S. Freitas, M. D. Hubinger, *J. Food Process. Preserv.* **35** (2011) 691 (https://doi.org/10.1111/j.1745-4549.2011.00518.x)
34. A. Nafiunisa, N. Aryanti, D. H. Wardhani, A. C. Kumoro, *J. Phys. Conf. Ser.* **909** (2017) 012084 (https://doi.org/10.1088/1742-6596/909/1/012084)
35. A. R. Oliveira, A. E. C. Ribeiro, É. R. Oliveira, M. C. Garcia, M. S. Soares Júnior, M. Caliari, *Food Sci. Technol.* **40** (2019) 282 (https://doi.org/10.1590/fst.34818)
36. P. B. Pathare, U. L. Opara, F. A. J. Al-Said, *Food Bioprocess Tech.* **6** (2013) 36 (https://doi.org/10.1007/s11947-012-0867-9)
37. N. Bahloul, N. Boudhrioua, M. Kouhila, N. Kechaou, *Int. J. Food Sci. Technol.* **44** (2009) 2561 (https://doi.org/10.1111/j.1365-2621.2009.02084.x)
38. M. Henríquez, S. Almonacid, M. Lutz, R. Simpson, M. Valdenegro, *CyTA – J Food*. **11** (2013) 127 (http://dx.doi.org/10.1080/19476337.2012.703693)
39. D. Mohapatra, Z. M. Bira, J. P. Kerry, J. M. Frías, F. A. Rodrigues, *J. Food Sci.* **75** (2010) 146 (https://doi.org/10.1111/j.1750-3841.2010.01518.x)
40. S. N. Dirim, G. Çalışkan, *Gida J. Food* **37** (2012) 203 (https://dergipark.org.tr/en/pub/gida/issue/6933/92554)
41. A. Nawirska-Olszanska, B. Stępien, A. Biesiada, *LWT – Food Sci. Technol*. **77** (2017) 276 (https://doi.org/10.1016/j.lwt.2016.11.067)
42. D. C. Potosi-Calvache, P. Vanegas-Maheceha, H. A. Martinez-Correa, *DYNA* **84** (2017) 112 (http://dx.doi.org/10.15446/dyna.v84n202.63904)
43. Z. Erbay, F. Icier, *J. Food Eng.* **91** (2009) 533 (https://doi.org/10.1016/j.jfoodeng.2008.10.004)
44. T. Turanyi, A. S. Tomlin, *Analysis of kinetics reaction mechanisms*, Springer, Berlin, 2014 (https://doi.org/10.1007/978-3-662-44562-4)
45. M. Doumpos, C. Zopounidis, *Eur. J. Oper. Res.* **209** (2011) 203 (https://doi.org/10.1016/j.ejor.2010.05.029).