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Analysis of antioxidant potential of fruit and vegetable juices available in Serbian markets

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Abstract

Antioxidants in fruit and vegetable juices have become increasingly popular because of their potential health benefits. Nowadays, juice mixes made from berries present frequent consumer choices, due to their nutritive value and high content of bioactive compounds. Commercial fruit and vegetable juices available in Serbian markets (n = 32) were analyzed for the physicochemical properties, chemical composition, and antioxidant activity. Relative antioxidant capacity index was used for the ranking of the juices according to antioxidant capacity, while antioxidant effectiveness of phenolic compounds contained in juice samples was investigated depending on phenolic antioxidant coefficients. Principal component analysis was applied to study the data structure. In addition, a multi-layer perceptron model was used for modeling an artificial neural network model (ANN) for prediction antioxidant activity (DPPH, reducing power, and ABTS) based on total phenolic, total pigments, and vitamin C content. The obtained ANN showed good prediction capabilities (the r^2 values during training cycle for output variables were 0.942). Phenolic, pigments, and vitamin C contents showed a positive correlation with the investigated antioxidant activity. The consumption of commercial berry fruit juices available in Serbian markets may deliver great health benefits through the supply of natural antioxidants.

Keywords

Antioxidant activity, relative antioxidant capacity index, principal component analysis, multi-layer perceptron model, artificial neural network model

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INTRODUCTION

The fruit and vegetable juice market is one of the most innovative and competitive markets in the beverage industry. Global consumption of fruit juice and nectars equated to 36.2 billion liters in 2017; EU remained the largest consumption region, with a production volume of 9.187 million liters. The largest producers are Germany, France, United Kingdom, and Spain. According to European Fruit Juice Association data for 2017, the annual production of fruit and vegetable juices in Serbia was 184.45 million liters, which is regarded as one of the respectable regional producers, prior to Greece, Hungary, Bulgaria, Croatia, and Romania. Serbia predominantly produced other fruit and vegetable juices "not elsewhere classified," which represent about 26%, followed by apple juices (19%), mixtures of fruit and vegetable juices (18%), orange juice (14%), etc. (AIJN, 2017).

In developing countries like Serbia, with rapid urbanization, less availability of time, and more concerns toward a healthier diet, consumers are relying on adopting new instant food habits. The new lifestyles have driven the food industry to develop new functional foods and beverages

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with added health benefits. Antioxidants in fruit and vegetable juices have become increasingly popular because of their potential health benefits as advertised by their manufacturers (Roy et al., 2020). Nowadays, juice mixes made from berries and red fruits present frequent consumer choices, due to their high purported nutritive value and high content of bioactive compounds (Fidelis et al., 2017).

When the quality of juices is considered, many physicochemical and chemical parameters can be served as a marker to classify juices according to their antioxidant properties and specific juice characteristics. The evaluation of these parameters can be implemented for a large number of juices, producing a complex data set. For quality control purposes and understanding the interconnections between bioactive compounds of juices and their antioxidant potential, multivariate statistical analysis is considered an essential approach (Dasenaki and Thomaidis, 2019; Tian et al., 2019).

Therefore, the aims of this study were to monitor the quality of selected commercial fruit and vegetable juices available in Serbian markets by measuring their physicochemical properties, chemical composition, and antioxidant activity. The obtained values were compared in order to explore antioxidative capacity of the fruit and vegetable juice samples, more comprehensively in order to obtain a deeper perception into antioxidant activity of analyzed juice samples.

MATERIALS AND METHODS

Juice samples

In this study, 32 commercial juices from different producers were acquired in Serbian supermarkets. These were: strawberry, apple and beetroot mix (No. 1); apple, red grape, blackberry and blueberry mix (No.2); blueberry and chokeberry mix (No. 3); orange (No. 4); apple (No. 5); pear and apple mix (No. 6); apple and beetroot mix (No. 7); apple, sour cherry, blueberry, raspberry and plum mix (No. 8); strawberry (No. 9); beetroot and apple mix (No. 10); blueberry (No. 11); tomato (No. 12); orange (No. 13); dogwood (No. 14); goji, raspberry and apple mix (No 15); apple, black grape, black currant, black elderberry, blueberry and pomegranate mix (No. 16); apple and lime mix (No. 17); pineapple (No. 18); carrot (No. 19); raspberry, apple and grape mix (No. 20); sour cherry (No. 21); black currant (No. 22); peach and apple mix (No. 23); orange (No. 24); red grapefruit (No. 25); apple (No. 26); apple, chokeberry, blueberry, and grape mix (No. 27); pomegranate, apple, black currant and grape mix (No. 28); raspberry (No. 29); apple and sour cherry mix (No. 30); chokeberry (No. 31); carbonated beverage (No. 32).

Physicochemical analysis

Titratable acidity (TA) determination was performed according to Institute for Standardization Serbia, 12147:2005 Juice pH was evaluated using lab pH Meter (AMT12 AMTAST, USA) by immersing an electrode in the sample according to Institute for Standardization Serbia,1132:2005.

Soluble solids content (SS) was measured in °Brix with a digital refractometer (ATR-BR SCHMIDT-HAENSCH, Germany) according to Institute for Standardization Serbia, 12143:2005.

Chemical analysis

Before chemical analysis and analysis of antioxidant activity, all juices were centrifuged at 4000 rpm for 10 min (model EBA 21, Hettich Zentrifugen, Tuttlingen, Germany), and filtered through 0.45 μm (pore size) membrane filters (Millipore, Bedford, MA). Total phenolic content (TPC) in juices was established using the Folin–Ciocalteau spectrophotometric method adapted to microscale (Šaponjac et al., 2016). Each well contained a mixture of 15 μL of sample, 170 μL of distilled water, 12 μL of the Folin–Ciocalteu's reagent and 30 μL of 20% (w/v) sodium carbonate. The prepared microplate was incubated for 1 h and the absorbances were measured at 750 nm. Distilled water was used as blank. The obtained results were expressed as gallic acid equivalents (GAE) per 100 mL of juice.

Total pigments (TP), i.e., the sum of total anthocyanins, carotenoids, and betalains, were analyzed according to the protocols described below.

pH Single method, adapted to 96-well microplate (Thermo Fisher Scientific Inc., Waltham, MA, USA), was used for analyzing anthocyanins in fruit juices (Lee et al., 2008). Total anthocyanins were expressed as cyanidin-3-glucoside equivalents per 100 mL of juice.

Total carotenoid content was analyzed spectrophotometrically by the method of Nagata and Yamashita adapted for 96-well microplate (Nagata and Yamashita, 1992). Distilled water was used as blank. The total carotenoids were expressed as mg of β-carotene or lycopene per 100 mL of juice. The content of carotenoids was calculated using the following equation:

$$C(\mathrm{mg}\,\beta-\mathrm{carotene}\,100\,\mathrm{mL}^{-1}) = 0.216\mathrm{x}A_{663\,\mathrm{nm}} - 1.22\,\mathrm{x}A_{645\,\mathrm{nm}} - 0.304\mathrm{x}A_{505\,\mathrm{nm}} + 0.452\mathrm{x}A_{453\,\mathrm{nm}}$$

$$C(\mathrm{mg\,lycopene}\,100\,\mathrm{mL}^{-1}) = -0.0458\mathrm{x}A_{663\,\mathrm{nm}} + 0.204\mathrm{x}A_{645\,\mathrm{nm}} + 0.372\mathrm{x}A_{505\,\mathrm{nm}} - 0.0806\mathrm{x}A_{453\,\mathrm{nm}}$$

where A is the absorbance measured at 663, 645, 505, and 453 nm.

Total betalain content in the juices was determined according to the method previously described modified for 96 well microplate (Wrolstad et al., 2005). Briefly, in the microplate well, the sample was mixed with 0.05 M phosphate buffer pH 6.5 to the final volume of 250 μL.

Phosphate buffer was used as the blank sample. Betacyanins and betaxanthins were measured at the wavelengths of 538 and 476 nm, respectively, and 600 nm was used for correction. Absorbances of red and yellow pigments were calculated by the following equations:

$$X = 1.095\,x\,(a\text{-}c)$$
$$Y = b\text{-}Z\text{-}X/3.1$$
$$Z = a\text{-}X$$

where a is the absorbance at 538 nm, b is the absorbance at 476 nm, c is the absorbance at 600 nm, X is the absorbance of betanin corrected for colored impurities, Y is the absorbance of vulgaxanthin-I corrected for colored impurities and Z is the absorbance of impurities. Concentration (C) of red and yellow pigments in juices were calculated using the following equation:

$$C(\text{mg}\,100\,\text{mL}^{-1}) = X(Y) \times F \times 1000 / A^{1\%}$$

where F is the dilution factor (25 or 12.5) and $A^{1\%}$ is the absorbance coefficient (1120 for betanin, 750 for vulgaxanthin).

Ascorbic acid (vitC) content was determined spectrophotometrically, according to the method by Jagota & Dani adapted for 96-well microplate (Jagota and Dani, 1982). Absorbance was measured after 10 min at 765 nm, and the content of L-ascorbic acid was expressed using calibration curve.

In vitro antioxidant activity

The DPPH radical scavenging assay was performed spectrophotometrically according to (Gironés-Vilaplana et al., 2012). DPPH radical scavenging activity values were calculated using the following equation:

$$DPPH = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the blank and A_{sample} is the absorbance of juice sample. The results were expressed in μmol Trolox equivalent (TE) per 100 mL of juice.

Reducing power (RP) was determined by the method of Oyaizu adapted for 96-well microplate (Oyaizu, 1986). Absorbances were measured at 700 nm. The results were expressed in μmol Trolox equivalent (TE) per 100 mL of juice.

The ABTS radical scavenging assay was evaluated employing modified method according to Tumbas Šaponjac et al., 2014. The absorbances of 250 μL activated $ABTS^{+\bullet}$ (with MnO_2), before and 35 min (incubated at 25 °C) after the addition of 2 μL of juice were measured at 414 nm. Water was used as blank. The results were expressed as μmol Trolox equivalent (TE) per 100 mL of juice.

According to the experimental results of obtained antioxidant assays, relative antioxidant capacity index (RACI) was evaluated for each juice sample (Da Pozzo et al., 2018). Antioxidant effectiveness of phenolics contained in examined juice samples was investigated depending on phenolic antioxidant coefficients (PACs), calculated as a ratio among the specific antioxidant assay and total phenolic content (TPC) (Petrovic et al., 2016).

Artificial neural network modeling (ANN)

A multi-layer perceptron model (MLP), which consisted of three layers (input, hidden, and output), was used for modeling an artificial neural network model (ANN) for prediction of DPPH, RP, and ABTS based on TCP, TP, and VitC. In the known literature, the ANN model was proven as quite capable of approximating nonlinear functions (Kleijnen, 2015; Yun et al., 2013). Before the calculation, both input and output data were normalized to improve the behavior of the ANN. During this iterative process, input data were repeatedly presented to the network (Kollo, 2005). Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was used as an iterative method for solving unconstrained nonlinear optimization during the ANN modeling. In addition, an artificial neural network model, based on the Broyden-Fletcher-Goldfarb-Shanno iterative algorithm, for the prediction of antioxidant activity (DPPH, reducing power, and ABTS) based on total phenolics, total pigments, and vitamin C content showed good prediction capabilities (the r^2 values during training cycle for output variables were 0.942).

The experimental database for ANN was randomly divided into training, cross-validation, and testing data (with 60%, 20%, and 20% of experimental data, respectively). A series of different topologies were used, in which the number of hidden neurons varied from 5 to 20, and the training process of the network was run 100,000 times with random initial values of weights and biases (Ochoa-Martínez and Ayala-Aponte, 2007).

Global sensitivity analysis

The Yoon's global sensitivity equation was used to calculate the relative impact of the input parameters on output variables, according to weight coefficients of the developed ANN models (Yoon et al., 1993):

$$RI_{ij}(\%) = \frac{\sum_{k=0}^{n} (w_{ik} \cdot w_{kj})}{\sum_{i=0}^{m} \left| \sum_{k=0}^{n} (w_{ik} \cdot w_{kj}) \right|} \cdot 100\%$$

where: w—weight coefficient in ANN model, i—input variable, j—output variable, k—hidden neuron, n—number of hidden neurons, m—number of inputs.

Statistical analysis

The principal component analysis (PCA), as a pattern recognition method, was applied effectively to classify and

separate the different samples. The assessment of PCA of the acquired experimental data was performed using Statistica software version 12 (Statistica, StatSoft Inc. 2012, USA)®. Results are presented as means ± SD (n = 3).

RESULTS AND DISCUSSION

Experimental data analysis

Table 1 summarizes the results of physicochemical and chemical properties of 32 juices commonly consumed in the Serbian diet. According to the fruit content, analyzed samples could be classified in the following categories: fruit juice, fruit nectar, and carbonated beverage. Sixteen samples were 100% fruit juice, fifteen samples were classified as fruit nectar with fruit content between 25 and 60%, and one carbonated beverage.

The values of the studied physicochemical parameters varied significantly within each group of juices. The pH values of juices ranged from 2.5 (No. 32; carbonated beverage) to 4.45 (No. 19; carrot juice). Titratable acidity ranged from 0.18 mg 100 mL^{-1} (No. 32; carbonated beverage) to 1.19 mg 100 mL^{-1} (No. 29; raspberry juice), while total soluble solids content ranged from 5.74 °Brix (No. 12; tomato juice) to 16.66 °Brix (No. 31; chokeberry juice). The differences in the values of presented physicochemical parameters could be related to the variety of fruit material, as well as the variation in seasons of harvest and levels in fruit ripeness. In general, all investigated commercial juices are strongly acidic, which implies their less susceptibility to bacterial actions. In terms of titratable acidity, FAO reported that the juices containing more than 1.2% acid are declared as sour (FAO, 2005). The obtained results in this study pointed that all commercial juices are not soured but sweet and within the acidity range value. Thereby, the obtained results pointed that all commercial juices are sweet instead of sour. Total soluble solids represent a good indicator of sweetness and indirectly of fruit content in the fruit juices. Soluble solids shown in Table 1 meet the requirements of the domestic Regulation which is in accordance with the European Regulation concerning the quality of fruit juices and nectars (Council Directive 2012/112; Rulebook, 2018). High levels of this parameter indicate that fruits have more simple sugars. According to FAO, fruit juices with less than 7 °Brix are categorized as weak and watery juices.

Total phenolics (TPC) were found in the highest concentrations in chokeberry juice (No. 31), dogwood juice (No. 14), and beetroot and apple juice mix (No. 10); TPC for these juices amounts 879.82 mg L^{-1}, 222.86 mg L^{-1}, and 198.02 mg L^{-1}, respectively. High concentrations of phenolics were also found in cherry and apple juice mix (No. 30), as well as in blueberry and chokeberry juice mix (No. 3) with levels of 144.65 mg L^{-1} and 136.75 mg L^{-1}. On the other side, besides the carbonated beverage, the lowest values of TPC exhibited the samples of apple juice (No. 5 and 26) and orange juice (No. 4) as well. (Nowak et al., 2017) compared the total phenolics content of elderberry, pomegranate, cranberry, and chokeberry organic juices; the highest value was observed for chokeberry juice, where predominant phenols were anthocyanins and phenolic acids. The highest concentrations of total phenolic compounds (TPC) were found in in chokeberry juice (No. 31), dogwood juice (No. 14), and beetroot and apple juice mix, with concentrations of 879.82 mg L^{-1}, 222.86 mg L^{-1}, and 198.02 mg L^{-1}, respectively. In the study of (Granato et al., 2015), 20 organic fruit juices from the local market in the Netherlands were assessed for total phenolics; the pomegranate and elderberry juices exhibited the highest concentrations, while apple and orange juices showed the lowest values. In the study of Jakobek et al. (2007), the highest concentrations of polyphenols among red juices were found in chokeberry and elderberry, followed by black currant, sour cherry, blackberry, sweet cherry, strawberry, and red raspberry, respectively. Red fruit juices (sour cherry, black currant, and red grape) produced in Serbia and purchased from local supermarkets, were examined for total phenolics as well. The highest value was recorded for black currant juices, followed by sour cherry and black grape juices. The same order was found in this study, but differences in the quantities can be explained by the cultivation method, and especially the processing method (milling, pressing, pasteurization, filtration, clarification, concentration), which causes more or less degradation which could decrease the content of these compounds in different ways.

Among 32 commercial juices, anthocyanins were detected in 16 samples, which ranged from 0.99 mg 100 mL^{-1} to (No. 8, fruit mix) to 30.58 mg 100 mL^{-1} (No. 31, chokeberry juice).

In the study of anthocyanins in blackberry, redcurrant, and pomegranate commercial juices, it was reported that these juices were a good source of anthocyanins together with purees (from strawberry, cherry, and raspberry) and concentrates (from blueberry, grape, and elderberry) (Garcia-Herrera et al., 2016). (Casedas et al., 2017) performed a comparative study where they analyzed anthocyanin profile in blueberry and cranberry juices and their bioactivity. In bioactivity studies, the authors analyzed antioxidant activity and inhibition of several physiologically important enzymes (α-glucosidase, tyrosinase, monoamine oxidase A, acetylcholinesterase, and dipeptidyl peptidase-4). It was established that blueberry juice is a richer source of anthocyanins and had a higher antioxidant activity, in comparison to cranberry juice. Both juices also inhibited enzymatic activity in a dose dependent manner.

Carotenoids were detected in 11 samples, where the highest concentration was found for carrot juice (21.53 mg 100 mL^{-1}; No. 19), while the lowest contents (0.01 mg 100 mL^{-1}) were found in apple juice (No. 26),

Table 1. Results for the physicochemical and chemical properties of 32 juices commonly consumed in the Serbian diet.

No.	Commercial juice sample	Fruit content	TA	Ph	SS	TPC	TP	VitC	DPPH	RP	ABTS	Price
1	Strawberry, apple and beetroot mix	50	0.41	3.71	12.52	64.32 ± 3.76	1.06 ± 0.04	0.06 ± 0.00	146.02 ± 0.68	307.06 ± 16.61	1523.33 ± 24.98	1.40
2	Apple, red grape, blackberry and blueberry mix	100	0.37	3.83	12.66	79.78 ± 3.11	2.14 ± 0.05	0.05 ± 0.00	182.12 ± 16.77	379.30 ± 13.28	972.12 ± 22.49	1.36
3	Blueberry and chokeberry mix	50	0.34	3.31	11.10	136.75 ± 8.27	5.97 ± 0.17	0.08 ± 0.00	277.70 ± 17.00	550.99 ± 15.51	3996.47 ± 26.27	1.65
4	Orange	50	0.64	3.61	11.48	20.63 ± 0.60	0.01 ± 0.00	0.02 ± 0.00	26.32 ± 4.64	103.53 ± 3.44	283.11 ± 4.35	1.14
5	Apple	100	0.50	3.63	11.46	18.84 ± 1.04	0.06 ± 0.00	0.01 ± 0.00	35.45 ± 2.27	140.18 ± 0.71	304.09 ± 0.31	0.85
6	Pear and apple mix	100	0.36	3.78	11.04	55.55 ± 2.05	0.01 ± 0.00	0.06 ± 0.00	100.44 ± 7.61	290.91 ± 10.58	1148.79 ± 28.73	1.53
7	Apple and beetroot mix	100	0.36	3.98	11.72	49.54 ± 1.17	11.85 ± 0.39	0.04 ± 0.00	140.87 ± 6.49	275.56 ± 10.89	1566.17 ± 7.49	2.20
8	Apple, sour cherry, blueberry, raspberry and plum mix	50	0.44	3.49	11.60	35.22 ± 2.29	0.99 ± 0.05	0.03 ± 0.00	104.45 ± 5.55	217.62 ± 10.20	1186.11 ± 45.28	0.89
9	Strawberry	55	0.45	3.55	12.98	73.60 ± 4.48	2.14 ± 0.11	0.05 ± 0.00	172.62 ± 0.99	259.60 ± 8.60	2000.55 ± 43.29	1.57
10	Beetroot and apple mix	100	0.49	3.89	11.88	198.02 ± 14.81	36.06 ± 0.54	0.08 ± 0.00	339.27 ± 7.04	531.14 ± 65.17	3791.53 ± 41.88	2.25
11	Blueberry	25	0.56	3.15	12.06	51.52 ± 2.45	3.18 ± 0.03	0.05 ± 0.00	29.58 ± 1.15	231.04 ± 1.74	868.77 ± 21.35	1.10
12	Tomato	100	0.36	4.25	5.74	27.49 ± 1.69	1.56 ± 0.01	0.02 ± 0.00	7.48 ± 0.66	176.74 ± 5.94	427.10 ± 4.97	1.14
13	Orange	50	0.44	3.92	11.12	25.55 ± 1.99	0.01 ± 0.00	0.02 ± 0.00	11.29 ± 3.60	129.82 ± 2.79	147.30 ± 4.68	1.19
14	Dogwood	50	0.75	3.20	14.80	222.86 ± 7.44	2.25 ± 0.11	0.16 ± 0.00	518.14 ± 5.32	620.32 ± 8.02	5201.35 ± 22.17	5.13
15	Goji, raspberry and apple mix	100	0.54	3.62	12.00	60.72 ± 2.50	2.22 ± 0.06	0.05 ± 0.00	89.35 ± 0.44	284.05 ± 12.41	2425.88 ± 25.28	5.08
16	Apple, black grape, black currant, black elderberry, blueberry and pomegranate mix	50	0.63	3.57	11.02	32.63 ± 0.38	1.53 ± 0.06	0.03 ± 0.00	60.18 ± 3.02	188.43 ± 7.20	984.71 ± 13.69	0.85
17	Apple and lime mix	100	0.64	3.51	12.20	90.37 ± 5.02	0.03 ± 0.00	0.09 ± 0.00	123.43 ± 5.75	417.24 ± 23.22	2285.43 ± 19.61	1.69
18	Pineapple	100	0.61	3.82	12.90	42.97 ± 3.60	0.03 ± 0.00	0.05 ± 0.00	49.05 ± 2.45	168.08 ± 2.36	917.35 ± 15.47	1.61
19	Carrot	100	0.38	4.45	9.08	40.02 ± 0.18	21.53 ± 0.98	0.04 ± 0.00	44.03 ± 1.40	197.36 ± 6.95	704.91 ± 21.24	7.46
20	Raspberry, apple and grape mix	50	0.62	3.56	12.78	76.84 ± 2.05	5.99 ± 0.14	0.06 ± 0.00	164.12 ± 7.81	370.68 ± 11.54	2730.41 ± 14.11	1.36
21	Sour cherry	35	0.62	3.23	12.26	66.80 ± 4.18	4.37 ± 0.19	0.04 ± 0.00	104.52 ± 7.06	238.23 ± 5.55	863.25 ± 4.06	1.36
22	Black currant	25	0.59	3.47	12.18	85.57 ± 2.72	4.63 ± 0.17	0.08 ± 0.00	154.15 ± 19.18	376.96 ± 9.10	2708.55 ± 10.77	1.53
23	Peach and apple mix	100	0.40	4.08	10.22	35.82 ± 3.32	0.13 ± 0.00	0.03 ± 0.00	14.09 ± 1.24	211.74 ± 3.27	778.89 ± 5.31	0.85
24	Orange	100	0.76	3.99	11.72	37.41 ± 3.99	0.02 ± 0.00	0.04 ± 0.00	51.63 ± 0.83	141.61 ± 3.55	465.74 ± 27.17	1.44
25	Red grapefruit	100	0.95	3.37	10.40	37.99 ± 1.69	1.31 ± 0.01	0.04 ± 0.00	47.81 ± 0.34	152.20 ± 6.94	646.17 ± 20.61	1.27

(continued)

Table 1. Continued.

No.	Commercial juice sample	Fruit content	TA	Ph	SS	TPC	TP	VitC	DPPH	RP	ABTS	Price
26	Apple	50	0.43	3.25	11.06	10.95 ± 0.69	0.01 ± 0.00	0.01 ± 0.00	28.33 ± 0.50	80.90 ± 1.93	199.86 ± 25.92	0.81
27	Apple, chokeberry, blueberry, and grape mix	50	0.54	3.31	11.20	75.83 ± 3.67	5.00 ± 0.05	0.06 ± 0.00	133.29 ± 1.88	352.13 ± 1.36	2869.32 ± 39.66	1.19
28	Pomegranate, apple, black currant and grape mix	100	0.86	3.41	12.34	91.62 ± 3.95	1.57 ± 0.01	0.05 ± 0.00	198.99 ± 17.19	478.77 ± 4.34	3806.77 ± 56.22	2.20
29	Raspberry	60	1.19	3.11	12.28	74.86 ± 4.17	3.07 ± 0.14	0.06 ± 0.00	155.59 ± 6.95	377.47 ± 12.10	2762.22 ± 13.06	3.39
30	Apple and sour cherry mix	100	0.34	3.46	14.04	144.65 ± 10.30	2.27 ± 0.14	0.10 ± 0.00	200.80 ± 10.84	552.58 ± 24.26	2229.34 ± 32.17	1.74
31	Chokeberry	100	0.68	3.68	16.66	879.82 ± 40.67	30.58 ± 1.32	0.32 ± 0.00	2062.94 ± 16.07	2419.84 ± 39.73	9863.42 ± 67.21	9.75
32	Carbonated drink	0	0.18	2.50	10.60	10.61 ± 0.75	0	0	16.10 ± 0.94	28.67 ± 0.43	14.40 ± 3.25	0.68

Fruit content (%); TA: Titratable acidity (% of citric acid); SS: Soluble solids (°Brix); TPC: Total phenolic content (mg GAE 100 mL^{-1}); TP: Total pigments (mg 100 mL^{-1}); VitC: Vitamin C content (mg L-ascorbic acid 100 mL^{-1}); DPPH: DPPH radical scavenging assay (μmol TE 100 mL^{-1}); RP: Reducing power (μmol TE 100 mL^{-1}), ABTS: ABTS radical scavenging assay (μmol TE 100 mL^{-1}); Price (Eur L^{-1}).

orange juice (No. 4 and 13), and pear and apple juice mix (No. 6). (Bunea et al., 2019) observed significantly higher concentrations of total carotenoids in carrot juice samples as compared to apple juices, as well. Surprisingly, no carotenoid compound was identified in commercial apple juice as compared to pasteurized and unpasteurized fresh juices. Pasteurization is a commonly used way to remove bacteria or other microorganisms; however, it usually leads to the degradation of bioactive compounds. Thus, this step should be monitored with caution in order to preserve the quality of products. Several authors reported that pasteurization process induces isomerization reactions of carotenoids in carrot juices and emulsion (Bunea et al., 2019; Knockaert et al., 2013).

The presence of betalains was determined in three samples, which include beetroot in the mixture (No. 1, 7, and 10). The highest value was noted for juice mix containing beetroot and apple, and the lowest for strawberry, apple, and beetroot juice mix. Betalains mainly found in beetroot are betacyanins and betaxanthins (Skalicky et al., 2020). (Wruss et al., 2015) investigated the betalain content of juices prepared from seven beetroot varieties. The authors reported that the differences between individual beets of the same variety were lower (%CV = 16.5) than those of different varietes (%CV = 23.0), therefore, betalain content appeared to be variety specific.

Vitamin C levels in products varied from 0.01 mg 100 mL^{-1} (apple juices, No. 5 and 26) to 0.32 mg 100 mL^{-1} (chokeberry juice, no. 31). Among 32 samples, 10 juices were fortified with Vitamin C by producers, however, values still low for this kind of product. It is well known that vitamin C content declines during juice processing, such as pasteurization (Tchuenchieu et al., 2018). In general, it is very difficult to compare the content of this vitamin between juices obtained by different producers, due to completely different processing methods.

All juices showed different antioxidant activities in relation to the applied method. The measurement of antioxidant activity of biological samples largely depends upon the free radical or the oxidants used in the assays and the degree and type of antioxidants. Hence, it is important to use different antioxidant assays, instead of relying on a single assay to assess and compare the antioxidant activity in fruits and vegetable juices. Synergistic effects and concentration may also change the results that are not observed when individual constituents are tested (Liu, 2013). Overall, chokeberry (No. 31), dogwood (No. 14), beetroot and apple mix (No. 10), blueberry and chokeberry mix (No. 3), as well as other juices that contain red pigments showed the highest antioxidant activity. These results are in agreement with other studies on commercial juices (Cardeñosa et al., 2016; Fidelis et al., 2017; Gardner et al., 2000; Nowak et al., 2017). In all cases, except for the mixture containing beetroot, red color was originated by anthocyanins. Anthocyanins are known to be one of the most powerful natural antioxidants. Berries and their products are very often recognized as "super foods." Besides anthocyanins, they contain high concentrations of other phenolic compounds, such as flavon-3-ols, tannins and ellagitannins, phenolic acids, especially ellagic, chlorogenic, and gallic acid. Numerous epidemiological studies found high correlation between consumption of berries and diseases associated with oxidative stress, determined by the reduction of oxidative stress biomarkers, which was attributed to the high content of phenolic antioxidants, such as anthocyanins and phenolic acids (Olas, 2018). Beetroot is ranked among the 10 most powerful vegetables with respect to antioxidant capacity. Betalains possess other desirable biological activities as well, including antiinflammatory, hepatoprotective, and antitumor properties (Vulić et al., 2013). Beetroot contains also phenolic acids such as *p*-coumaric, protocatechuic, ferulic, vanillic, *p*-hydroxybenzoic, and syringic (Bangar et al., 2022).

Chemometrics approaches

Evaluation of relative antioxidant capacity index (RACI) and phenolic antioxidant coefficients (PAC). The results expressed by the values of the antioxidant activity assays (DPPH, RP, and ABTS) could be compared to the values of the total phenol content (TPC), and it can be concluded that the antioxidant activity of the samples is correlated with the amount of phenolics present therein. This confirms that the content of total phenolic compounds contributes to the total antioxidant potential, which coincides to the results of Yao and Ren, 2011 and Zheng and Wang, 2001.

In order to create a complete picture of the ranking of the fruit juices antioxidant capacities, a relative antioxidant capacity index (RACI) was calculated by merging the antioxidant capacity values obtained from the different antioxidant methods. RACI is the mean value of standard scores transformed from the initial data generated by different tests without unit limitation and no variance between methods (Sun and Tanumihardjo, 2007). According to the results of the classification of samples (Figure 1), the superiority of the chokeberry juice (No. 31) is demonstrated in the totality of tests, giving the highest RACI value of +4.798. The lowest RACI value (−0.738) was obtained for carbonated beverage (No. 32).

In order to evaluate the efficiency of the extracted phenolics, the phenol antioxidant coefficient (PAC) was used. PAC of the samples was evaluated for quick comparison of antioxidant efficiency of total phenolics using different antioxidant capacity assays. PAC values also showed the influence of TPC on each antioxidant assay, for each juice sample. According to calculation, the highest PAC_{DPPH} was observed for samples No. 7 (apple and beet juice mix) and No. 8 (fruit mix), due to exceptionally high DPPH values and also the relatively low TPC values.

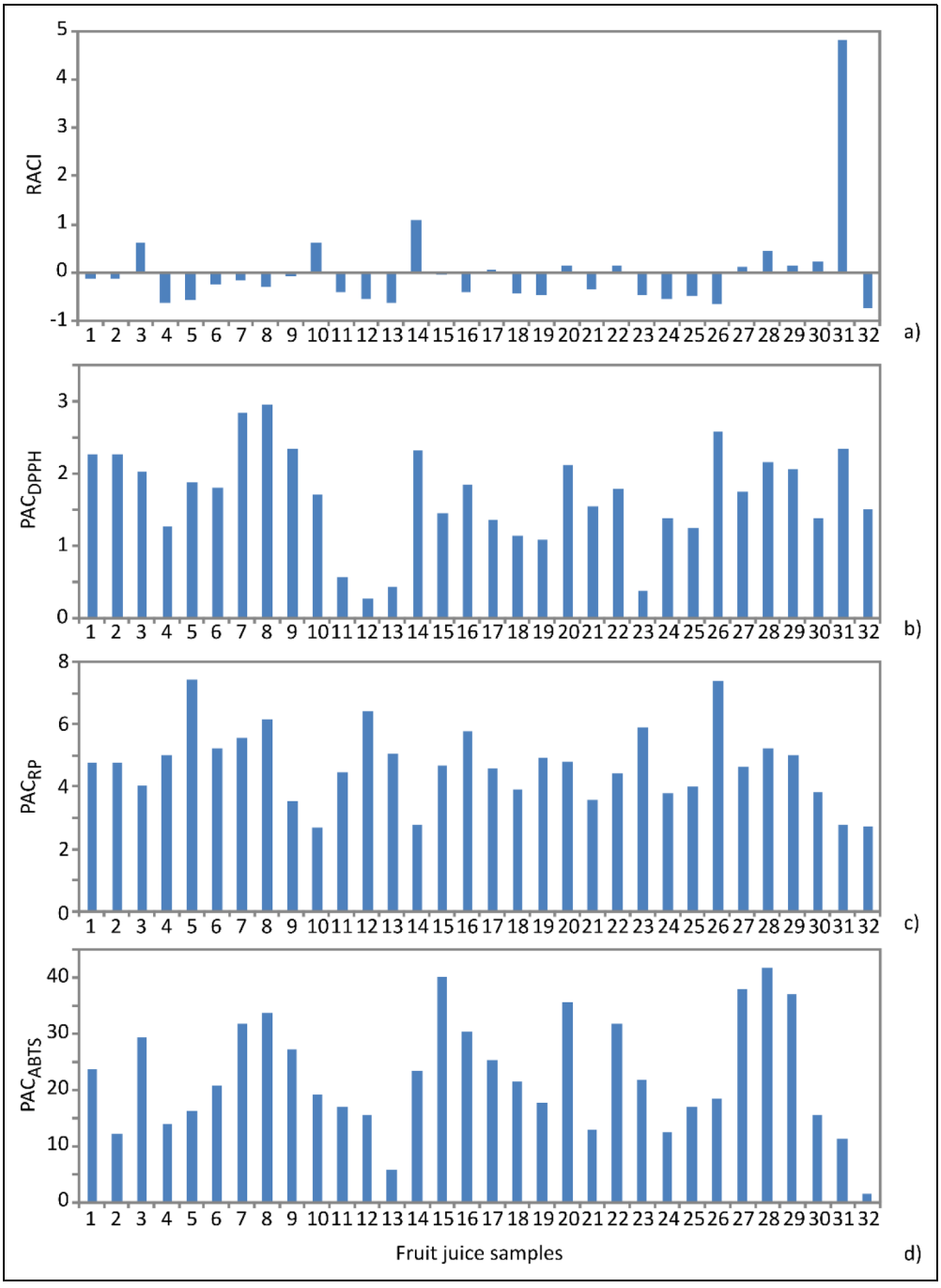

Figure 1. Relative antioxidant capacity index (RACI) and phenolic antioxidant coefficients for fruit juice samples (PAC_{DPPH}, PAC_{RP}, and PAC_{ABTS}) RACI—relative antioxidant capacity index, PAC_{DPPH}—phenol antioxidant coefficient for DPPH, PAC_{RP}—phenol antioxidant coefficient for RP, PAC_{ABTS}—phenol antioxidant coefficient for ABTS.

An attempt to correlate the relative antioxidant capacity index and the price of juice was presented in Figure 2. According to this calculation, the most prominent positive values were obtained for samples: No. 3 (blueberry and chokeberry juice mix), No. 10 (apple and beet juice mix), No. 14 (cornel juice) and No. 31 (chokeberry juice). The prices for these juices were high (1.65, 2.25, 5.13, and 9.75 EUR L^{-1}, respectively), but the RACI values obtained were higher, compared to other samples (0.611, 0.617, 1.096, and 4.797, accordingly).

Correlation analysis. According to results presented in Table 2, the intensive positive statistically significant correlations between antioxidant assays were observed, while

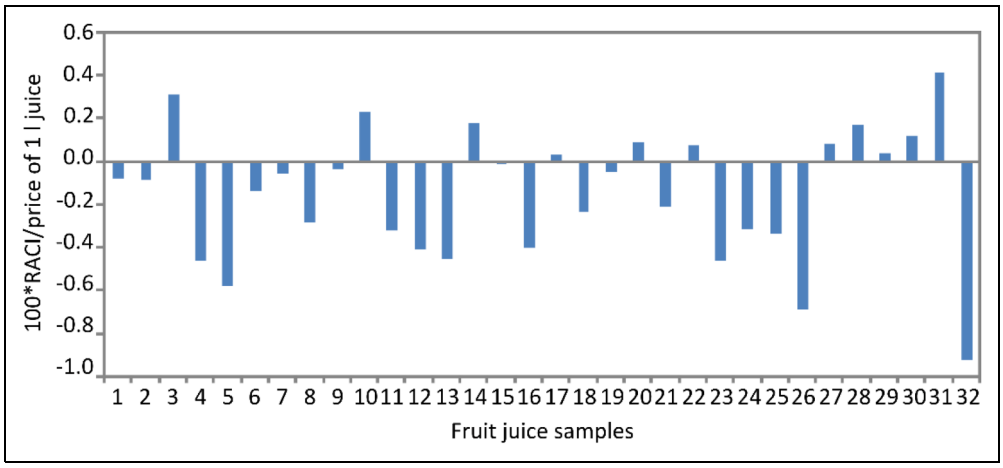

Figure 2. The ratio between RACI (relative antioxidant capacity index) and price of juice.

Table 2. Correlation matrix.

	TP	VitC	DPPH	RP	ABTS	RACI
TPC	0.637	0.953	0.995	0.990	0.893	0.983
TP		0.549*	0.611	0.608	0.586	0.617
VitC			0.941	0.956	0.924	0.964
DPPH				0.984	0.888	0.981
RP					0.915	0.990
ABTS						0.957

All correlations are statistically significant at $p < 0.001$ level, except for correlations marked with asterisk, which were significant at $p < 0.01$ level. TPC: Total phenolic content (mg GAE 100 mL^{-1}); TP: Total pigments (mg 100 mL^{-1}); VitC: Vitamin C content (mg L-ascorbic acid 100 mL^{-1}); DPPH: DPPH radical scavenging assay (μmol TE 100 mL^{-1}); RP: Reducing power (μmol TE 100 mL^{-1}), ABTS: ABTS radical scavenging assay (μmol TE 100 mL^{-1}); RACI: Relative antioxidant capacity index.

RACI (as the relative antioxidant index) was mostly positive correlated to TPC, as well as antioxidant assays, such as DPPH, RP, or ABTS. Vitamin C content was also correlated to RACI. TP was positively correlated to TPC and antioxidant assays, statistically significant at $p < 0.001$ level, and also to Vitamin C content, statistically significant at $p < 0.01$ level.

Principal component analysis (PCA). PCA is a multivariate analysis procedure used to transform the coordinates of the samples into orthogonal components which are appropriate for data examination. It allows the recognition of patterns in a data series and expressing them in such manner that the similarities and differences can be observed, changing the dimensionality without losing too much information (Abdi and Williams, 2010). This technique is used to achieve maximum diversity among clusters of parameters and it is characterized by a high degree of flexibility (de Souza et al., 2016). This procedure is set in such a manner that the highest possible variance has the first component. PCA is giving confirmation to the spatial relation among processing parameters, empowering a separation among samples, as well.

The PCA, applied to the given data set, has revealed a clear separation between RACI, TPC, VitC, and TP. There is also a quite good discrimination between the observed samples, according to used assays. Quality results demonstrate that the first two principal components, accounting for 84.68% and 13.81% of the total variability, can be taken into consideration as sufficient for data representation. The map of the PCA showed that TPC (which contributed 28.51% of total variance, based on correlations), TP (15.99%), VitC (27.08%), and RACI (28.42%) have demonstrated a negative score value according to first principal component (Figure 3). VitC (which contributed 10.0% of total variance, based on correlations) presented the positive influence toward the second principal component calculation, while the influence of TP was negative (82.89%).

According to the results of the PCA analysis, the main differentiation between samples was noticed according to differences in RACI, TPC, and VitC values (which was explained by principal component PC1) and the differences in TP (which was clarified according by the PC2 component). Samples located at the left side of the PCA biplot showed higher RACI, TPC, and VitC values, while samples located at the bottom of the graph were characterized by increased TP values. Sample 31 (chokeberry juice) showed the most pronounced RACI, TPC and VitC values. Samples No. 10 (apple and beet juice mix), No. 14 (cornel juice), and No. 3 (blueberry and chokeberry juice mix) also obtained the increased RACI, TPC, and Vit C values. The lowest RACI, TPC, and VitC values were recorded for carbonated beverage (No. 32). Samples No. 7, No. 10 (apple and beet juice mix) and No. 19 (carrot juice) showed the highest TP values.

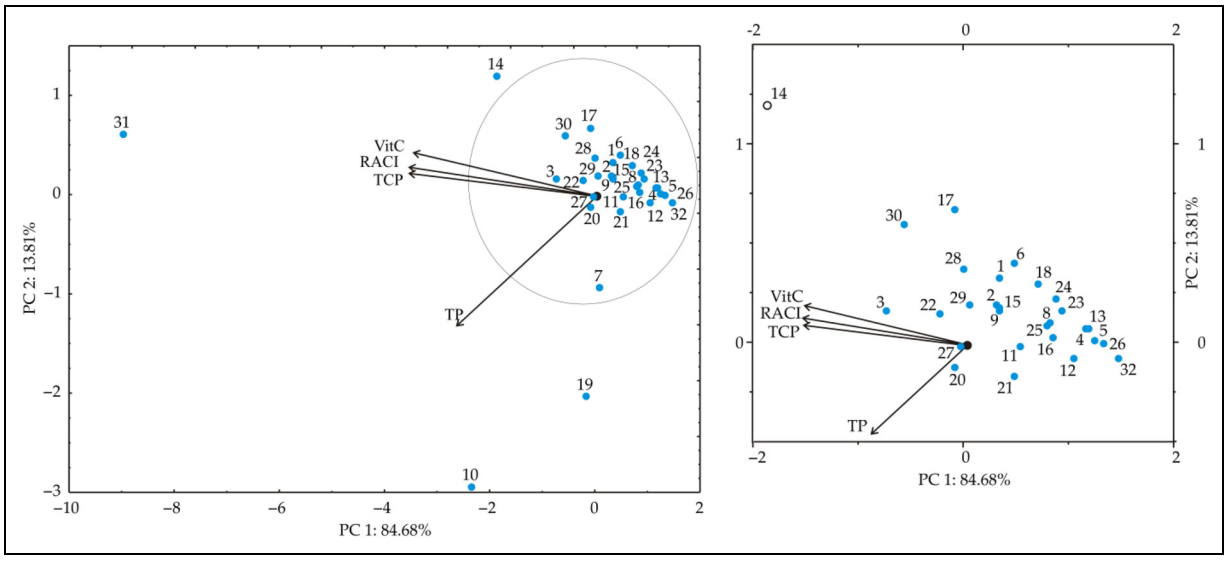

Figure 3. Graphical presentation of samples based on PCA analysis. RACI—relative antioxidant capacity index, TPC—total phenol content, TP—total pigments content, VitC—vitamin C content.

Table 3. Artificial neural network model summary (performance and errors), for training, testing, and validation cycles for the MLP 3-8-3 network.

Performance*			Error*			Training algorithm	Error function	Hidden activation	Output activation
Train.	Test.	Valid.	Train.	Test.	Valid.				
0.942	0.985	0.999	179,227.2	24,315.73	624,099.6	BFGS 54	SOS	Exponential	Exponential

*Performance term represents the coefficients of determination, while error terms indicate lack of data for the ANN model.

ANN model. The acquired optimal neural network model No. 8 (network MLP 3-8-3) obtain the highest values of r^2 (during the training cycle r^2 for output variables was: 0.942) showed a good generalization capability for the experimental data, and could be used to accurately predict the DPPH, RP, and ABTS based on TPC, TP, and VitC (Table 3). The obtained ANN model for the prediction of output variables was complex (59 weights-biases), due to the high non-linearity of the observed system.

The accuracy of the ANN model could be visually assessed by the dispersion of points from the diagonal line in the graphics presented in Figure 4. For the ANN model, the predicted values were very close to the measured values in most cases, in terms of r^2 values. SOS obtained with ANN model were of the same order of magnitude as experimental errors for DPPH, RP, and ABTS.

Table 4 presents the elements of matrix W_1 and vector B_1 (presented in the bias column), and Table 5 presents the elements of matrix W_2 and vector B_2 (bias) for the hidden layer, used for calculation in Equation 2.

The goodness of fit between experimental measurements and model-calculated outputs, represented as ANN performance (sum of r^2 between measured and calculated output variables), during training, testing and validation steps, are shown in Table 6.

The ANN model predicted experimental variables reasonably well for a broad range of the process variables. For the ANN model, the predicted values were very close to the measured values in most cases, in terms of r^2 values. SOS values obtained with the ANN model was of the same order of magnitude as experimental errors for output variables reported in the literature (Doumpos and Zopounidis, 2011; Kollo, 2005).

The ANN model had insignificant lack of fit tests, which means the model satisfactorily predicted output variables. A high r^2 is indicative that the variation was accounted for and that the data fitted the proposed model satisfactorily (Erbay and Icier, 2009; Turányi and Tomlin, 2014).

Global sensitivity analysis—Yoon's interpretation method. In this section, the influence of input variables on TPC, TP, and VitC on DPPH, RP, and ABTS, was studied, based on the Yoon's interpretation method of a developed ANN model. The graphical presentation of the ANN model results was shown in Figure 5.

According to the Figure 5, TCP content was the most positive influential parameter on DPPH value, with approximately relative importance of +76.7%, while the influence of TP content was also positive, reaching +22.5%. The most important positive impact on RP value

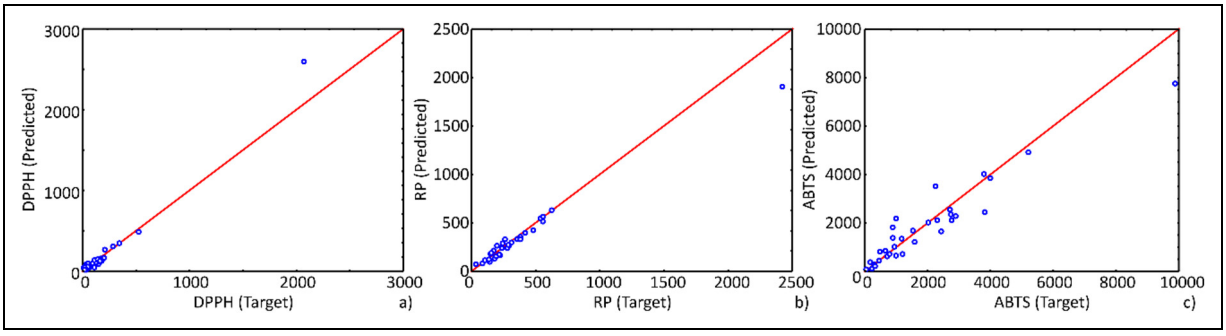

Figure 4. Comparison between experimental and calculated values for DPPH, RP, and ABTS.

Table 4. Elements of matrix W_1 and vector B_1 (presented in the bias column).

	1	2	3	4	5	6	7	8
TPC	−4.920	−4.295	−1.822	−1.677	−1.733	1.349	−0.661	−2.153
TP	−1.860	0.518	−0.896	−1.847	−1.096	−2.566	0.707	−0.338
VitC	−1.092	−2.531	−1.233	0.965	−2.146	−1.204	−0.965	−1.248
Bias	0.603	0.710	0.163	0.578	0.186	−0.526	0.722	0.955

Table 5. Elements of matrix W_2 and vector B_2 (presented in the bias column).

	1	2	3	4	5	6	7	8	Bias
DPPH	−1.280	−0.506	0.248	−1.499	0.500	0.118	−1.345	1.003	1.505
RP	−0.018	−0.152	−0.835	−0.002	−0.070	1.329	0.574	−0.867	−0.876
ABTS	−0.589	−2.111	0.527	−0.880	0.893	−0.573	−1.090	0.876	1.312

Table 6. The "goodness of fit" tests for the developed ANN model.

Output variable	χ^2	RMSE	MBE	MPE	SSE	AARD	r^2
DPPH	1.1E + 04	99.910	−19.358	58.607	3.1E + 05	1318.983	0.989
RP	1.0E + 04	96.393	17.510	17.658	2.9E + 05	1711.257	0.983
ABTS	4.5E + 05	641.616	62.236	49.236	1.3E + 07	16,206.917	0.905

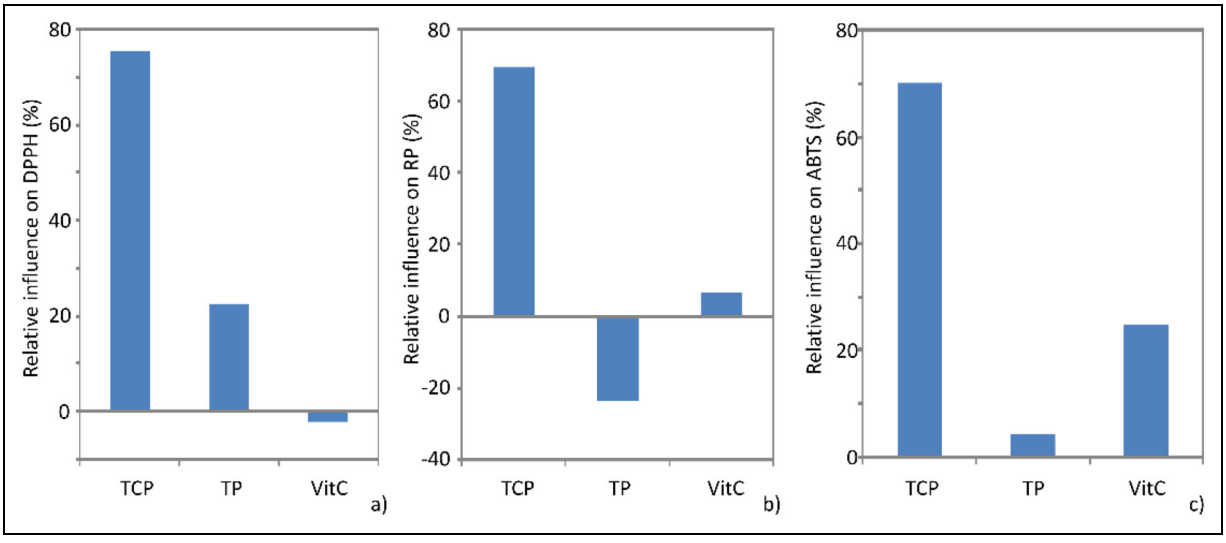

Figure 5. Graphical presentation of the ANN model results.

was noticed for the content of TCP (with relative importance of +69.8%), while the most pronounced negative influence was obtained for TP content (relative influence was equal to −22.8%). The highest positive influence on ABTS value was gained for the content of TCP (with relative importance of +70.2%), while the influence of vitamin C content reached the relative influence was equal to +24.8%.

CONCLUSION

The findings from the present study reveal that the consumption of commercial red fruit juices available in Serbian markets may deliver great health benefits through the supply of natural antioxidants. Phenolics, pigments, and vitamin C contents showed a positive correlation with the investigated antioxidant activity. The use of simple physicochemical characteristics of juices and rapid spectrophotometric measurements supplemented with chemometrics was a suitable approach to study the quality traits of commonly consumed juices in Serbia. Furthermore, an artificial neural network model in the form of multi-layer perceptron was developed for anticipation of antioxidant activity of samples (DPPH, reducing power, and ABTS) according to total phenolic, total pigments, and vitamin C content. The developed neural network model displayed adequate forecasting abilities (the coefficient of determination reached 0.942, during training cycle). Overall, this study will be useful for different populations: for consumers that plan rich antioxidant diets; for nutritionists in estimating the daily intakes of phenolic antioxidants and their impact on health; for juice producers, to consider the antioxidant and chemical perspectives of commonly used juices in the daily diet and direct efforts for creation of new, healthier, and economically acceptable products.

AUTHOR CONTRIBUTIONS

Vanja Šeregelj—Writing—original draft, Formal analysis; Vesna Tumbas Šaponjac—Writing—review & editing; Lato Pezo—Data curation, Writing—original draft; Jovana Kojić—Investigation, Methodology; Biljana Cvetković—Formal analysis; Nebojsa Ilić—Conceptualization, Supervision, Visualization.

DECLARATION OF CONFLICTING INTERESTS

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