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THE IMPACT OF PLANT MATERIAL FREEZING AND EXTRACTANT ACIDIFYING ON THE ANTIOXIDANT POTENTIAL AND PIGMENTS CONTENT IN EXTRACTS AND RAW MATERIALS OF DIFFERENT CARROT VARIETIES

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ABSTRACT

The antioxidant activities of extracts of purple, yellow and orange carrot varieties were evaluated by *in vitro* methods i.e. DPPH, ABTS, FRAP, and CUPRAC. The total polyphenols concentration was evaluated using Folin-Ciocalteu technique. The content of anthocyanins, chlorophylls and carotenoids was evaluated in fresh and frozen carrots. The anthocyanin concentration was also determined in carrot extracts. The effect of certain parameters of ultrasound-assisted extraction, i.e. extraction time, concentration and acidifying of extractant, as well as the type of raw material (fresh or frozen) was analyzed. The antioxidant potential as well as content of polyphenols and plant pigments were the highest in purple carrot extracts. The most effective extractant seems to be 70% (v/v) ethanol. The extension of the extraction time, in majority of cases, led to an increase of antioxidant activity of extracts, whereas the freezing of raw material generally decreased the above-mentioned activities. The effect of acidification of extractant is ambiguous and depends on the method applied to evaluate antioxidant activity.

Key words: *Daucus carota* L., antioxidant activity, ultrasound-assisted extraction, plant pigments, freezing, solvent acidifying

INTRODUCTION

Carrot (*Daucus carota* L.) is a member of the *Apiaceae* family. It is characterized by white flowers, compound and dissected leaves as well as edible roots. Depending on the variety, the root color is different – from white, through yellow, orange, red to dark purple and even black [Que et al. 2019]. It is considered that the purple and yellow carrots came from Afghanistan and relatively quickly spread to the Mediterranean area as well as to Western Europe and then to China, India and Japan. The orange carrot was created as a result of an accidental mutation in The Netherlands. Due to good properties such as color, flavor, shape and smooth surface, the orange carrot is very popular around the world and is the dominant variety in terms of production and consumption. The pigments contained in the roots are responsible for their colors. The main pigments in carrot roots are carotenoids and anthocyanins. These compounds, in addition to the color properties, show antioxidant activity. Carotenoids are the lipid-soluble organic substances belonging to the tetraterpenoids group, responsible for the bright colors – from pale yellow through orange to the deep red [Singh et al. 2018]. Anthocyanins belong to the flavonoids – the biggest group of polyphenols – and are classified as secondary metabolites in majority of water-soluble plants and are responsible for the dark col-

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or of the fruit and vegetables – from purple to black. Due to their antioxidant potential, they could play a very important role in preventing of the development of so-called oxidative stress. This phenomenon could be responsible for the development of many chronic diseases, such as neoplastic, cardiovascular and metabolic diseases as well as neurodegenerative and psychiatric disorders [Muzykiewicz et al. 2018a, Singh et al. 2018]. In addition to the mentioned pigments, chlorophylls (green plant pigments) and their derivatives also show the antioxidant potential [Ferruzzi et al. 2002]. Moreover, the carrots are the sources of other pro-health biologically active compounds such as vitamins, minerals and dietary fiber [Que et al. 2019].

Due to the content of biologically active substances as well as large consumption of carrots, the aim of the study was to evaluate the antioxidant activity of extracts prepared from yellow, purple and orange carrots. The influence of ethanol concentration applied as extractant as well as acidifying of the extracting medium, and the time of ultrasound-assisted extraction were also assessed. Moreover, the influence of one of the most commonly used food processing – the freezing – on the antioxidant potential of raw material extracts was evaluated. The content of the plant pigment groups such as carotenoids, chlorophylls (a and b) as well as anthocyanins (both for carrot extracts and for fresh and frozen raw materials) was also estimated.

MATERIAL AND METHODS

Chemicals. All reagents were of analytical grade and were supplied by: Sigma-Aldrich, USA: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino--bis(3-ethylbenzothiazoline-6-sulfonic acid), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid); Merck, Germany: Folin-Ciocalteu reagent, iron(II) sulfate heptahydrate and gallic acid; Chempur, Poland: methanol, acetone, 99.5% acetic acid, potassium persulfate, sodium acetate anhydrous, 36% hydrochloric acid, iron(III) chloride hexahydrate, copper(II) chloride dihydrate, sodium carbonate anhydrous, potassium chloride, citric acid monohydrate; J&K Scientific, Germany: neocuproine; and ethanol by Linegal Chemicals, Poland.

Plant material and extract preparation. The plant material consisted of the purple, yellow and orange variety of carrot. The vegetables were from crops located in The Netherlands and were purchased at a local supermarket (West Pomeranian, Poland) in April 2019. Fresh and frozen (24 hours at –20°C) raw material was used to prepare the extracts using ultrasounds at a frequency 40 kHz for 15, 30 and 60 minutes. The 96%, 70% as well as 40% (v/v) ethanol was used as extractant. To evaluate the effect of acidifying of solvent on the antioxidant activity of extracts, 96% ethanol was diluted with water to obtain 70% and 40 (v/v) alcohol (non-acidified extracts) or with a 3% (w/w) aqueous solution of citric acid (acidified extracts). The extracts were stored at +4°C until analysis.

The analysis of the antioxidant activity of extracts and pigments content. Evaluation of the antioxidant activity of extracts using the DPPH, FRAP, ABTS and F-C methods were performed as described by Muzykiewicz et al. [2019]. The CUPRAC evaluation was performed according to the technique described by Apak et al. [2004]. The anthocyanins determination in fresh and frozen raw materials as well as calculation of their concentration were performed according to the methodology of Klimek [2011], whereas the content of these pigments in carrot extracts was measured by the technique described by Lee et al. [2005]. The content of chlorophylls and carotenoids in fresh and frozen raw materials was measured by Lichtenthaler and Wellburn [1983] method using 80% acetone. The absorbance was measured in 1 cm glass cuvettes using Hitachi U-5100 spectrophotometer. Three samples were prepared from each carrot's samples and results were presented as an arithmetical mean ± standard deviation (mean ±SD). In DPPH, ABTS and CUPRAC methods, antioxidant activities have been expressed as Trolox equivalents (TEAC – mg Trolox/g raw material), whereas in FRAP technique as $FeSO_4$ equivalents (mg $FeSO_4$/g raw material). The total polyphenols content was expressed as gallic acid equivalents (GAE – mg GA/g raw material). The anthocyanin content in fresh and frozen carrot was expressed as cyanidino-3-O-glucoside chloride (%), whereas in extracts as mg cyanidino-3-O-glucoside/L. The content of chlorophylls and carotenoids were presented as µg/g fresh or frozen material.

Statistical analysis. Statistical analysis was done with Statistica 12 (Statsoft, Poland). The Wilcoxon's test was used to evaluate the statistical significance of differences between the antioxidant activity of extracts from fresh and frozen, particular types of carrot (obtained by the DPPH, ABTS, FRAP, CUPRAC and F-C technique) as well as between the anthocyanins content in the obtained extracts. The Pearson's correlations (r) between the results determined with the different methods were also evaluated. The significance level was assumed as $p < 0.05$.

RESULTS

Antioxidant activities of carrot extracts determined by the DPPH and ABTS methods are presented in Table 1 and Table 2, respectively. All the extracts evaluated by the ABTS technique showed the activity. In contrary, the most of the yellow and orange carrots extracts did not show the ability to scavenge the DPPH radical. The average antioxidant potential of extracts evaluated by the ABTS method was higher than the respective activities obtained by the DPPH technique. Table 3 presents the results of the ability of the obtained extract to reduction of ferric ions (FRAP method) whereas Table 4 reduction of cupric ions (CUPRAC method). All the extracts showed the capacity to reduce ferric ions. Similarly to the other applied methods, the highest ability was found for fresh purple carrot extracted for one hour in 70% acidified ethanol. The capacity to reduce cupric ions, evaluated with CUPRAC method, was observed only in part of the obtained extracts. The samples of fresh yellow carrot (except the extract in 70% ethanol, during one-hour extraction) as well as from the frozen orange carrot did not show any reduction abilities. Similarly, extracts from yellow carrot (both of fresh and frozen), prepared using the acidified solvents, did not show re-

Table 1. The mean (±SD) antioxidant activities of carrot extracts evaluated by the DPPH method

Solvent (v/v)	Extraction time (min)	Purple		Yellow		Orange	
		fresh	frozen	fresh	frozen	fresh	frozen
		DPPH (mg Trolox/g raw material)					
96% EtOH	15	0.89 ±0.02	0.06 ±0.00	NA	NA	NA	NA
	30	0.92 ±0.00	0.32 ±0.03	NA	NA	NA	NA
	60	0.86 ±0.01	0.23 ±0.01	NA	NA	NA	NA
70% EtOH	15	0.84 ±0.02	0.45 ±0.03	NA	NA	NA	NA
	30	1.07 ±0.02	0.32 ±0.04	NA	NA	NA	NA
	60	1.33 ±0.08	0.48 ±0.01	NA	NA	NA	NA
40% EtOH	15	1.09 ±0.03	0.32 ±0.01	NA	NA	0.58 ±0.02	NA
	30	1.31 ±0.01	0.42 ±0.03	NA	NA	0.35 ±0.04	NA
	60	1.35 ±0.06	0.51 ±0.04	NA	NA	0.07 ±0.01	NA
70% EtOH acidified	15	1.19 ±0.02	0.61 ±0.02	0.42 ±0.00	0.28 ±0.01	0.45 ±0.01	0.35 ±0.02
	30	1.22 ±0.02	0.68 ±0.01	0.40 ±0.01	0.33 ±0.03	0.44 ±0.01	0.35 ±0.02
	60	1.92 ±0.00	0.79 ±0.01	0.42 ±0.02	0.35 ±0.04	0.52 ±0.03	0.36 ±0.01
40% EtOH acidified	15	1.21 ±0.01	0.78 ±0.02	0.40 ±0.00	0.30 ±0.01	NA	0.34 ±0.02
	30	1.46 ±0.04	0.82 ±0.02	0.41 ±0.01	0.32 ±0.02	0.49 ±0.01	0.34 ±0.03
	60	1.58 ±0.02	0.84 ±0.01	0.42 ±0.01	0.31 ±0.00	0.55 ±0.03	0.35 ±0.01

NA – no activity

Table 2. The mean (±SD) antioxidant activities of carrot extracts evaluated by the ABTS method

Solvent (v/v)	Extraction time (min)	Purple		Yellow		Orange	
		fresh	frozen	fresh	frozen	fresh	frozen
		ABTS (mg Trolox/g raw material)					
96% EtOH	15	1.94 ±0.03	0.53 ±0.06	0.29 ±0.01	0.27 ±0.04	0.42 ±0.02	0.10 ±0.02
	30	1.69 ±0.19	0.76 ±0.03	0.30 ±0.00	0.63 ±0.02	0.32 ±0.02	0.27 ±0.04
	60	1.79 ±0.05	0.86 ±0.05	0.51 ±0.03	0.40 ±0.01	0.43 ±0.05	0.15 ±0.01
70% EtOH	15	2.44 ±0.11	1.56 ±0.11	0.27 ±0.04	0.32 ±0.05	0.34 ±0.04	0.27 ±0.03
	30	2.66 ±0.07	1.23 ±0.02	0.35 ±0.05	0.31 ±0.07	0.31 ±0.05	0.55 ±0.06
	60	3.24 ±0.03	1.91 ±0.06	0.48 ±0.01	0.31 ±0.05	0.44 ±0.06	0.32 ±0.04
40% EtOH	15	1.97 ±0.03	1.28 ±0.03	0.23 ±0.05	0.46 ±0.05	0.46 ±0.03	0.35 ±0.05
	30	2.86 ±0.02	1.42 ±0.03	0.28 ±0.03	0.40 ±0.05	0.34 ±0.03	0.32 ±0.04
	60	3.03 ±0.02	1.55 ±0.02	0.10 ±0.01	0.37 ±0.05	0.44 ±0.03	0.26 ±0.05
70% EtOH acidified	15	1.74 ±0.01	0.79 ±0.10	0.23 ±0.03	0.25 ±0.02	0.21 ±0.03	0.27 ±0.02
	30	2.12 ±0.02	1.23 ±0.02	0.31 ±0.02	0.21 ±0.01	0.34 ±0.05	0.13 ±0.01
	60	3.31 ±0.10	1.36 ±0.09	0.29 ±0.04	0.33 ±0.02	0.44 ±0.03	0.19 ±0.03
40% EtOH acidified	15	1.85 ±0.04	1.30 ±0.03	0.13 ±0.03	0.27 ±0.02	0.65 ±0.04	0.42 ±0.01
	30	2.29 ±0.02	1.28 ±0.05	0.15 ±0.01	0.25 ±0.04	0.35 ±0.05	0.35 ±0.01
	60	2.45 ±0.04	1.26 ±0.07	0.27 ±0.02	0.38 ±0.05	0.61 ±0.03	0.41 ±0.01

Table 3. The ability (mean ±SD) of extracts to reduce the ferric ions, evaluated by the FRAP method

Solvent (v/v)	Extraction time (min)	Purple		Yellow		Orange	
		fresh	frozen	fresh	frozen	fresh	frozen
		FRAP (mg $FeSO_4$/g raw material)					
96% EtOH	15	2.30 ±0.05	0.73 ±0.01	0.51 ±0.00	0.37 ±0.03	0.53 ±0.00	0.46 ±0.04
	30	2.27 ±0.04	1.17 ±0.01	0.41 ±0.02	0.40 ±0.03	0.49 ±0.01	0.41 ±0.00
	60	2.29 ±0.02	1.10 ±0.02	0.47 ±0.03	0.40 ±0.02	0.56 ±0.01	0.45 ±0.03
70% EtOH	15	2.56 ±0.03	2.00 ±0.00	0.48 ±0.00	0.44 ±0.00	0.51 ±0.01	0.40 ±0.01
	30	3.01 ±0.04	1.77 ±0.03	0.49 ±0.01	0.40 ±0.02	0.53 ±0.00	0.47 ±0.04
	60	3.51 ±0.05	2.30 ±0.02	0.48 ±0.01	0.54 ±0.01	0.55 ±0.00	0.43 ±0.02
40% EtOH	15	3.26 ±0.02	1.66 ±0.03	0.42 ±0.00	0.41 ±0.02	1.70 ±0.05	0.36 ±0.03
	30	3.22 ±0.00	1.90 ±0.01	0.49 ±0.00	0.42 ±0.02	0.60 ±0.02	0.44 ±0.00
	60	2.46 ±0.02	2.15 ±0.01	0.47 ±0.01	0.50 ±0.02	0.57 ±0.01	0.42 ±0.01
70% EtOH acidified	15	3.71 ±0.02	2.44 ±0.01	1.45 ±0.04	1.49 ±0.00	1.40 ±0.02	1.52 ±0.01
	30	2.70 ±0.04	2.93 ±0.01	1.42 ±0.02	1.55 ±0.03	1.43 ±0.01	1.53 ±0.02
	60	5.32 ±0.02	3.43 ±0.01	1.64 ±0.01	1.59 ±0.01	1.64 ±0.02	0.35 ±0.00
40% EtOH acidified	15	2.80 ±0.02	2.71 ±0.02	1.50 ±0.02	1.68 ±0.01	0.53 ±0.00	1.25 ±0.00
	30	3.42 ±0.06	2.82 ±0.03	1.80 ±0.04	1.71 ±0.00	1.81 ±0.06	1.24 ±0.03
	60	3.57 ±0.02	2.95 ±0.01	1.98 ±0.06	1.79 ±0.06	2.12 ±0.00	1.33 ±0.01

Table 4. The ability (mean ±SD) of extracts to reduce the cupric ions, evaluated by the CUPRAC method

Solvent (v/v)	Extraction time (min)	Purple		Yellow		Orange	
		fresh	frozen	fresh	frozen	fresh	frozen
		CUPRAC (mg Trolox/g raw material)					
96% EtOH	15	3.51 ±0.05	0.15 ±0.02	NA	NA	0.32 ±0.03	NA
	30	3.39 ±0.14	1.31 ±0.01	NA	NA	NA	NA
	60	3.38 ±0.08	0.68 ±0.06	NA	NA	0.34 ±0.02	NA
70% EtOH	15	3.77 ±0.01	1.91 ±0.04	NA	NA	0.08 ±0.01	NA
	30	4.63 ±0.22	2.08 ±0.16	NA	0.18 ±0.01	0.17 ±0.03	NA
	60	4.34 ±0.03	2.86 ±0.12	0.46 ±0.02	0.13 ±0.03	0.45 ±0.00	NA
40% EtOH	15	3.97 ±0.11	1.99 ±0.10	NA	0.19 ±0.02	NA	NA
	30	4.45 ±0.11	2.56 ±0.19	NA	0.13 ±0.01	0.32 ±0.00	NA
	60	4.10 ±0.26	3.05 ±0.10	NA	0.29 ±0.01	0.49 ±0.01	NA
70% EtOH acidified	15	1.90 ±0.08	0.63 ±0.10	NA	NA	NA	NA
	30	1.82 ±0.10	1.03 ±0.09	NA	NA	NA	NA
	60	3.42 ±0.11	1.33 ±0.00	NA	NA	NA	NA
40% EtOH acidified	15	1.66 ±0.08	NA	NA	NA	NA	NA
	30	2.09 ±0.14	NA	NA	NA	NA	NA
	60	2.22 ±0.18	0.66 ±0.01	NA	NA	NA	NA

NA – no activity

Table 5. The correlation coefficients between the results obtained with different methods

	ABTS	FRAP	F-C	CUPRAC
DPPH	0.866*	0.945*	0.828*	0.738*
ABTS		0.811*	0.966*	0.923*
FRAP			0.753*	0.687*
F-C				0.957*

* $p < 0.001$

duction activity. The evaluation of total polyphenols content in the carrot extracts showed, that this group of compounds were found in purple carrot extracts (Fig. 1). Anthocyanin contents in fresh and frozen carrots, as well as in obtained extracts, are presented in Figure 2. The markedly higher content of this group of compounds was found for purple carrot as compared to the yellow and orange variety. The concentration of these pigments in the yellow and orange carrots (both in a fresh and frozen raw material) were low. The extracts of the other raw materials contained less amount of anthocyanins. Moreover, in several extracts from the yellow and orange variety (both fresh and frozen) no anthocyanins were found. Figure 3 presents the content of the individual chlorophylls as well as the carotenoids in the fresh and frozen carrots. The tested raw materials were characterized by a higher content of chlorophyll b than a. The highest content of chlorophyll b, as well as carotenoids, was found for frozen purple carrot. The slightly lower concentration of

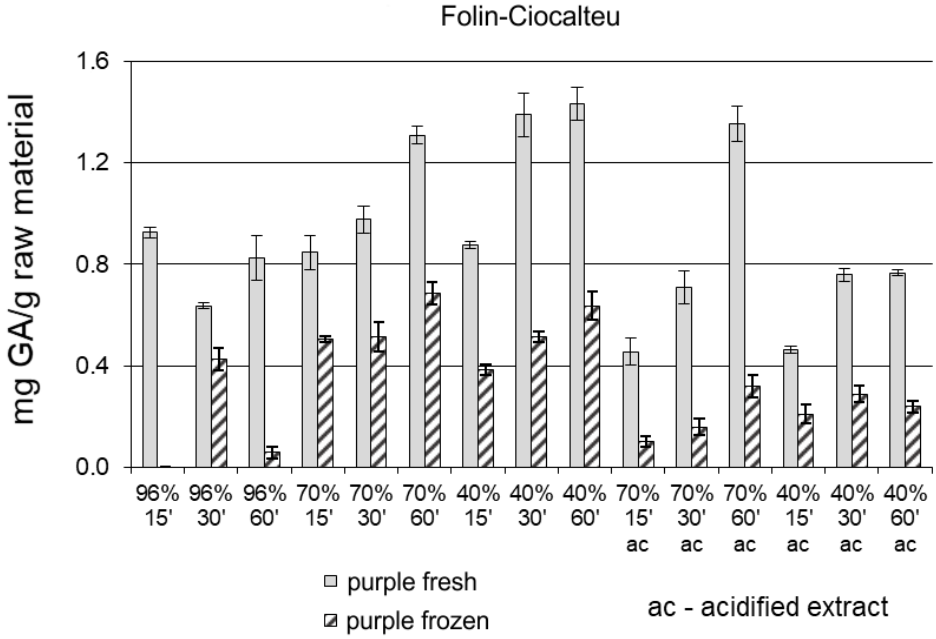

Fig. 1. The mean (±SD) total polyphenols content in purple carrot extracts, evaluated by the Folin-Ciocalteu technique. Vertical lines represent standard deviation (SD)

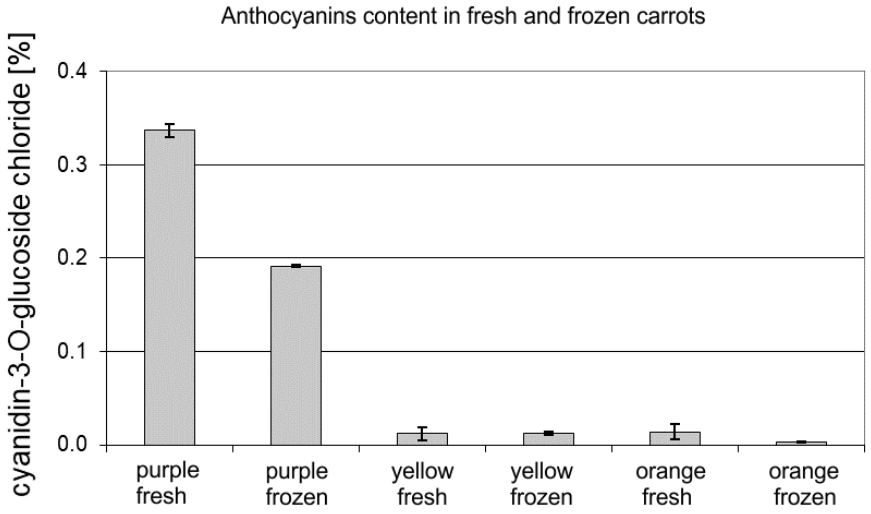

Fig. 2. The anthocyanins content in a) fresh and frozen raw material of carrots; b) in extracts of fresh and frozen carrots. Vertical lines represent standard deviation (SD)

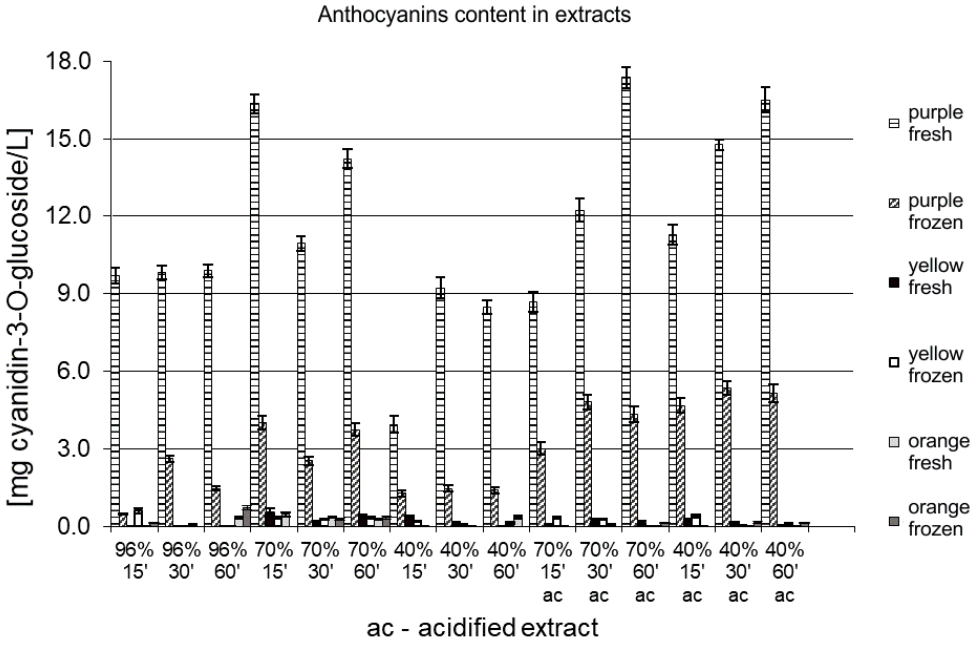

Fig. 3. The chlorophylls and carotenoids content in fresh and frozen raw material of carrots. Vertical lines represent standard deviation (SD)

these pigments was observed for fresh purple carrots. However, it should be emphasized, that the fresh purple carrot was characterized by the highest content of the total chlorophyll (a + b) as compared to the frozen raw material. The yellow and orange varieties were characterized by a markedly lower content of chlorophylls and carotenoids.

Correlations between the activities of the extracts evaluated by the applied methods were statistically significant. The respective correlation coefficients (r) between the obtained values are summarized in Table 5. The differences between the activity of extracts prepared from all of fresh and frozen carrots, carried out with Wilcoxon's test, were statistically significant ($p < 0.001$). Only the antioxidant activities of fresh and frozen yellow carrots extract did not differ significantly. The differences between anthocyanins content in extracts prepared from purple carrot (fresh vs. frozen) were statistically significant ($p < 0.001$), while in the case of extracts from yellow and orange carrots (also fresh vs. frozen) were insignificant.

DISCUSSION

The obtained results suggest that among three studied carrots the purple variety extracts were characterized by the highest antioxidant activity. The extracts of this variety showed the highest activity evaluated by the DPPH, ABTS, FRAP and CUPRAC methods Moreover, the highest content of anthocyanins and assimilation pigments, such as chlorophylls and carotenoids, as well as the highest content of total polyphenols was observed in contrary to the samples of yellow and orange carrots. The comparison of antioxidant potential of yellow and orange carrot extracts showed, that higher activities were obtained for majority of orange variety samples. Exception was the anthocyanins content – higher results were found for yellow carrot extract. A similar observation was done by Singh et al. [2018]. They compared the antioxidant activity of samples prepared from different carrot varieties, e.g. orange, yellow and black. In their study, the black carrot showed the highest content of phe-

nolic compounds, such as flavonoids, as well as the activity evaluated by the FRAP, CUPRAC and ABTS methods. They found that the antioxidant potential of purple carrot samples was even 6 to 36 times higher than of other varieties. Only in the case of carotenoids, higher concentration was observed for orange carrot. In our study, the purple carrot showed higher content of carotenoids and chlorophylls than the orange and yellow variety. Lee et al. [2011] in the studies of the various breeding lines also observed higher carotenoids content in the purple carrot than in orange ones. They suggested that this phenomenon may be a result of using the seed batches, in which the selective breeding for higher carotene was performed, which resulted in a considerable increase in the content of these compounds in the purple carrots. Moreover, Grassmann et al. [2007] emphasized, that many factors such as growing seasons, growing conditions as well as soil and genetic factors could affect carotenoids content in carrots. Comparison of the activities of yellow and orange varieties showed, in most cases, higher content of phenolic compounds (including flavonoids) as well as the cupric ion reduction activity evaluated by the CUPRAC method in the orange carrot. However, the yellow carrot was characterized by the higher activity obtained by the FRAP and ABTS techniques. The above-mentioned results were confirmed in our study. It should be noted, that the correlations between the results obtained by the individual methods are statistically significant. Yang et al. [2017] evaluated the antioxidant activity of *Morus atropurpurea* Roxb. extracts and also obtained high correlation coefficients between the results of the DPPH, F-C, ABTS and FRAP techniques (from 0.864 to 0.968). They suggested that the high values of these coefficients, between the antioxidant activity estimated by DPPH, ABTS and FRAP methods and the total polyphenols content assessed by the F-C technique, indicated that phenolic compounds could markedly contribute to the total antioxidant activity.

The evaluation of the effect of ethanol concentration in extracts led to the conclusion, that in most cases, regardless of the applied method, the highest activities were obtained for extracts prepared in 70% (v/v) ethanol. However, the lowest results, in most cases, were found for samples preparing in concentrated ethanol. Hossain and Shah [2015] evaluated the antioxidant activity of *Merremia borneensis* leave extracts and confirmed the effectiveness of 70% (v/v) ethanol, as compared to other solvents of different polarity such as hexane, ethyl acetate, chloroform, or butanol. Qadir et al. [2017] also emphasized the effectiveness of ethanol as extractant to obtain extracts with high antioxidant activity. They compared the antioxidant potential of garlic, onion, thyme, ginger, oak, mint and aloe vera extracts prepared in 80% ethanol, methanol, acetone and in distilled water. In most cases, the ethanolic extracts showed higher activity, evaluated by the different methods, including the content of total phenolic compounds as well as flavonoids. The effectiveness of 96% and 70% ethanol to prepare extracts with an antioxidant potential were also compared in studies of Muzykiewicz et al. [2017]. Based on results obtained for extracts of different parts of *Sorbus aucuparia*, they suggested that 70% ethanol was more effective than 96% to prepare extracts with antioxidant properties.

The impact of solvent acidification on antioxidant activity of obtained extracts was also evaluated. This process led to increased activities of extracts, in the case of the DPPH and FRAP methods as well as in the anthocyanins content, whereas in the case of the ABTS, CUPRAC and F-C techniques, the higher potential was observed for samples prepared using non-acidified solvents. An impact of acidification of the solvent on the increase of antioxidant activity of tea extracts was also recognized by Kopjar et al. [2015]. They found that the acidified methanol enhanced the antioxidant potential of extracts evaluated by DPPH, ABTS and F-C method. In the previously mentioned studies, Yang et al. [2017] also analyzed the effect of acidified with acetic acid methanol and acetone on the antioxidant properties of prepared extracts. They observed a higher content of phenols, particularly flavonols, in extracts prepared using the acidified solvents diluted with water than in samples prepared in concentrated solvents and without acid addition. The highest antioxidant potential, as well as the highest phenols concentration, was observed in extracts prepared using 50% aqueous solution of methanol and acetone with the addition of 0.5% acetic acid. Water was the least effective solvent in extraction of the phenolic compounds from plant material. The authors suggested that using to the extraction the water-based organic solvents is more efficient than the pure organic solvents or water. Kaniewska et al. [2013] evaluated the

influence of ethanol acidification on the anthocyanins contents in extracts of *Lonicera caerulea*. The application of such extractants, led to increase the activity similarly as in our study.

The impact of extraction time on antioxidant potential of extracts was also observed. In most cases, the prolongation of extraction time increased the above-mentioned activity. A similar dependence was observed in studies of Muzykiewicz et al. [2018a,b]. The one-hour ultrasound-assisted extraction of leaves, ripe and unripe fruits of *Hippophae rhamnoides* L. as well as *Cydonia oblonga* Mill., was more effective than 15 and 30 minutes process. The extracts of both plants, prepared during 15 minutes, only in a few cases showed the highest antioxidant activity evaluated by the DPPH, ABTS, FRAP techniques, as well as the total polyphenols content estimated by F-C method.

The assessment of the effect of the plant material freezing on the antioxidant activity of extracts, suggested that in most cases this process decreased this activity. Rickman et al. [2007] in their review paper emphasized, that freezing can reduce carotenoids content in the fruits and vegetables even to 48%. In our study, a different observation was noticed in the case of carotenoids content in the purple carrot, where the frozen samples showed higher content of these compounds than fresh. Scott and Eldridge [2005] in the studies about corn also found higher content of carotenoids in a frozen sample than in fresh. Authors suggested that the increase of carotenoid concentrations found in frozen corn may be a result of water loss from the kernels in the freezing process. It should be emphasized, that in our study the carotenoid content in purple carrots was high and the differences between the content of these compounds in fresh and frozen carrot was not too big. Danesi and Bordoni [2008] evaluated the antioxidant potential of different fresh and frozen vegetables. In case of red and yellow vegetables as carrot, red tomato and yellow pepper, the activity of fresh plant samples was higher than that of frozen vegetables. The freezing led to increase of the activity of green vegetables such as green beans, zucchini, and peas. Moreover, the authors assessed the impact of the different heating processes such as using of microwave, steaming and boiling on the antioxidant activity of fresh and frozen vegetables. In case of carrot, a higher potential after heating was observed in the fresh samples, whereas for zucchini and green beans – in the frozen vegetables. The observation of Danesi and Bordoni [2008] and Azwanida [2015], as well as our own, suggested, that the selection of appropriate parameters of the extraction process, such as time, concentration and solvent composition, is rather complicated and these parameters, as well as the scheme of plant material preparation, should be optimized before extraction for each plant to be studied.

CONCLUSION

The results of our studies suggest that the purple variety of carrot shows the highest antioxidant activity as well as the content of polyphenols and plant pigments, evaluated by the *in vitro* techniques. The evaluation of solvent concentration revealed, that the most effective seemed to be using of 70% (v/v) ethanol. The effect of acidification of extractant on antioxidant potential of the extracts is ambiguous. The extension of the extraction time, in most cases, increased the potential of extracts. However, the freezing of carrots before the ultrasound-assisted extraction generally decreased the above-mentioned activity as well as the content of polyphenols, carotenoids, anthocyanins, and total chlorophylls.

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