

## EVALUATION OF THE ANTIRADICAL POTENTIAL OF FRUIT AND VEGETABLE SNACKS\*

Anna Gramza-Michałowska, Magdalena Człapka-Matyasik  
Poznań University of Life Sciences

**Background.** The use of plant origin polyphenols as food ingredients, supplements or antioxidants is very promising as a future trend for human health and food stability. Free radical activity, responsible for human ageing and food deterioration could be delayed by the use of antioxidants present in fruit and vegetables. The aim of the present research was to evaluate and compare the antiradical activity of selected fruits and vegetable snacks as a new promising kind of functional foods.

**Material and methods.** In the present study, seven commercial plant food snacks were analysed for the total polyphenol content and antioxidant activity evaluated according to the DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging methods.

**Results.** The highest total polyphenol content was evaluated in chokeberry and blackcurrant chips extracts, apple chips contained significantly lower amount among all snacks. Chokeberry extract exhibited the highest antiradical activity when determined by the DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging methods, while the apple and carrot chips extracts showed the lowest antiradical activity. DPPH<sup>•</sup> and ABTS<sup>•+</sup> gave comparable results and were highly correlated ( $r = 0.83$ ,  $p < 0.05$ ).

**Conclusions.** Results obtained indicate that selection of plant matrices for snack production would be very important for consumer's health, as they are potential sources of dietary antioxidants.

**Key words:** antioxidant, DPPH<sup>•</sup>, ABTS<sup>•+</sup>, fruits, polyphenols, radicals, snacks, vegetables

### INTRODUCTION

An important consideration for the food industry is the use of the antioxidant potential of naturally occurring substances in food systems instead of high potential synthetic

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antioxidants that may have unclear toxicity in many cases [Moure et al. 2001, Pokorny 2007]. Plant antioxidants provide a perfect opportunity to understand the limitations and perspectives of polyphenol use, especially as food components [Liu 2003, Hać-Szymańczuk et al. 2009]. The average diet is poor in polyphenols, which are recognized for their strong antioxidant, anticancer and antiageing potentials in the human body [Chen et al. 2005, Zhang et al. 2005, Scalbert et al. 2005, Cieślak et al. 2006]. The addition of polyphenol rich plant extracts to food will improve their nutritional quality [Gramza et al. 2007, Gramza and Reguła 2007]. The other way to improve the diet is high fruit and vegetable snacks supply. As the salty snacks and chips are a very popular part of modern youth diet then is highly demanded to replace them with other products that are sensory similar (crispness, thickness), but carry significantly higher amount of potentially bioactive compounds.

The antioxidant activity of polyphenols is very important for human health and food stability. The use of plant origin polyphenols as food ingredients, supplements or antioxidants is very promising as a future trend. Recent research is mainly focused on the study of the antioxidants in fruits extracts, particularly their characteristics and protecting effects against free radicals. Free radicals are responsible for human ageing and food deterioration. The only way to slow down or prevent such effects is to use the antioxidants, such as polyphenols which are secondary plants metabolites [Prior 2003]. Basically, polyphenols are compounds used to delay free radical accumulation and strengthen oxidative stability of food and human body [Jeszka et al. 2010]. The antioxidant activity of polyphenols is due to redox properties, which play an important role in neutralizing free radicals, quenching singlet oxygen or decomposing hydroperoxides [Rice-Evans et al. 1996, Huang et al. 2005].

Thus, discovering new combination of natural substances would be an important step in finding antioxidant with broad antiradical properties. Importance for consumers is that plants offer protection against many degenerative diseases. One of the fastest developing research fields is the study of the role of free radicals in the activity of biological systems and etiology of diseases. Food products are designed for the development of new ways of inactivating free radicals through the use of natural product constituents.

The present research aimed at the evaluation and comparison of antiradical activity of selected fruits and vegetable snacks as new promising kind of the functional foods. For the research two different radical scavenging methods based on SET mechanism (Single electron transfer) were chosen. The objective of the study was to provide useful information on antiradical potential of commercially available fruit and vegetable snacks.

## MATERIAL AND METHODS

**Chemicals and reagents.** Folin-Ciocalteu Reagent (Fluka), DPPH<sup>•</sup> (1,1-diphenyl-2-picryl-hydrazyl), ABTS<sup>•+</sup> (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and potassium persulfate were purchased from Sigma-Aldrich (Germany), and sodium carbonate was from POCH (Poland). Ethanol and methanol were of HPLC grade and other reagents were of analytical grade.

**Plant extracts.** According to fat free chips production technology eight products: apple, apple-orange, apple-blackcurrant, apple-banana, strawberries, blackcurrant, black chokeberry and carrot puffings were chosen for the research. Plant products were bought at local food store (PAULA, Poland) and were from three different consignments. Fruit chips consisted of dried fruit (apple) and vegetable (carrot), which were coated with vitamin C and concentrated juices of orange, blackcurrant, or banana pulp (0.1%). Fruit puffings contained whole freshly dried fruits of strawberry, chokeberry and blackcurrant. Plant products were extracted according to procedure of Gramza-Michałowska et al. [2008]. Chips and puffings (100 g) were freeze dried, minced and macerated with 80% ethanol (500 mL) for 24 hours at ambient temperature (procedure was repeated three times). Collected extracts were filtered and centrifuged (4500 rpm, 15 min). Ethanol was evaporated on rotary evaporator (RVO 200A, INGOS) at 60°C under reduced pressure (200 hPa). Powdered extracts were kept frozen until further use (−18°C). The range of extracts concentration was determined at level of 5, 10, 15, 20 and 25% in ethanol solutions (80%).

**Total polyphenol content.** The content of total polyphenols in the extracts was determined according to method presented by Horwitz [1970]. Briefly an aliquot (10 mL) of the blank, extract or standard was placed in 50 mL volumetric flask, were 2.5 mL of Folin-Ciocalteu reagent was added and the mixture was left for 3 min reaction. Than 5 mL of sodium carbonate (20% solutions) was added, mixed and the volume was than made up for 50 mL and left for 60 min. The results of spectrophotometric evaluations at  $\lambda = 750$  nm were expressed as gallic acid equivalents, using the calibration curve over the range of 0-600  $\mu\text{g/mL}$  ( $r^2 = 0.9981$ ) and counted over a 1 gram of a product.

**Antiradical activity.** Plant extracts antiradical activity was estimated using DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging ability methods.

**DPPH<sup>•</sup> Free radical scavenging method.** The effect of product methanol extracts was estimated with DPPH<sup>•</sup> free radical scavenging method according to the procedure described by Sanchez-Moreno et al. [1998]. An aliquot of methanol (0.1 mL), solution containing experimentally established extracts concentrations (5, 10, 15, 20, 25%), was added to 3.9 mL of DPPH<sup>•</sup> 0.025  $\text{g}\cdot\text{L}^{-1}$  in methanol prepared daily. Samples absorbance decrease was measured at  $\lambda = 515$  nm after 30 min storage in the darkness (Carl Zeiss Spectrophotometer, Jena Optik). Stock solution of DPPH<sup>•</sup>, was stored at 4°C until it was used. DPPH<sup>•</sup> solution violet colour disappears in presence of antioxidant, as a result of free radical scavenging in measured medium. Faster the absorbance decreases stronger the antioxidant. The antiradical value represents percent of radical scavenging ability according to:

$$\% \text{ inhibition} = [(Ab_k - Ab_b) / Ab_k] \cdot 100$$

where:

- $Ab_b$  – sample absorbance,
- $Ab_k$  – control sample absorbance.

On the basis of the standard curve for Trolox results were also expressed as mg of Trolox per 1 g of extract's dry weight (mgT/g). The percentage of the remaining DPPH<sup>•</sup> against standard concentration was plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH<sup>•</sup> concentration by 50% and the time needed to reach the steady state to EC<sub>50</sub> concentration ( $T_{EC50}$ ). Antiradical efficiency (AE) describing

antiradical activity was also defined [Sanchez-Moreno et al. 1998]. According to AE samples were divided into four antiradical efficiency groups:

$$AE \leq 1 \cdot 10^{-3} \text{ – low antiradical activity}$$

$$1 \cdot 10^{-3} < AE \leq 5 \cdot 10^{-3} \text{ – medium antiradical activity}$$

$$5 \cdot 10^{-3} < AE \leq 10 \cdot 10^{-3} \text{ – high antiradical activity}$$

$$AE > 10 \cdot 10^{-3} \text{ – very high antiradical activity}$$

**ABTS<sup>+</sup> Free radical scavenging method.** Radical scavenging activity of extracts was measured according to the assay described by Re et al. [1999]. TEAC (Trolox Equivalent Antioxidant Capacity) evaluation was based on antioxidant stability to scavenge the blue-green colored ABTS<sup>+</sup> radical cation in comparison to scavenging ability of water soluble vitamin E analogue – Trolox. Briefly, potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) was used to generate ABTS<sup>+</sup> solutions, with stable absorbance at 734 nm for at least 2 h, than the solution was diluted with 80% methanol to an absorbance of 0.700. For the evaluation 3 ml of ABTS<sup>+</sup> solution was added to 30 μL of antioxidant and mixed well. After 6 minutes in the darkness the absorbance was measured. Antiradical activity of the examined samples was presented as percentage of radical scavenged and calculated using the same equation as in DPPH<sup>•</sup> method [Perez-Jimenez and Saura-Calixto 2008]. On the basis of standard curve for Trolox the results were also expressed as mg of Trolox per 1 g of extract's dry weight (mg T/g), the EC<sub>50</sub>, T<sub>EC50</sub> and AE were also determined.

**Statistical analysis.** The results were obtained from a minimum of three independent experiments and averaged. Data were analysed by the analysis of variance ( $p \leq 0.05$ ) to estimate the differences between values of tested extracts. The results were processed by the computer program Statistica 8.1<sup>®</sup>.

## RESULTS AND DISCUSSION

### Yield

Rate of production yield of different plant products varied from 4.1 to 6.2 g per 100 g of product, and can be ranked from the highest to the lowest in the following order: apple-orange (6.2%) > apple-blackcurrant = apple-banana (5.8%) > apple (5.6%) > strawberries (5.2%) > blackcurrant (4.6%) > carrot (4.6%) > chokeberry (4.1%). The yield of ethanol extract of apple-orange chips was 70% of that of chokeberry extract.

### Total polyphenol content

The total polyphenols content of the ethanol extracts were found to be affected by the plant used for chip production. Results of total polyphenol content were presented as mg of standard equivalent (gallic acid) per 1 g of the fresh product: chips and puffings (Fig. 1). The extracts were ranked from high to low as follows: chokeberry (14.8) > blackcurrant (10.6) > strawberry (4.5) > apple-banana (3.4) > carrot (2.6) > apple-

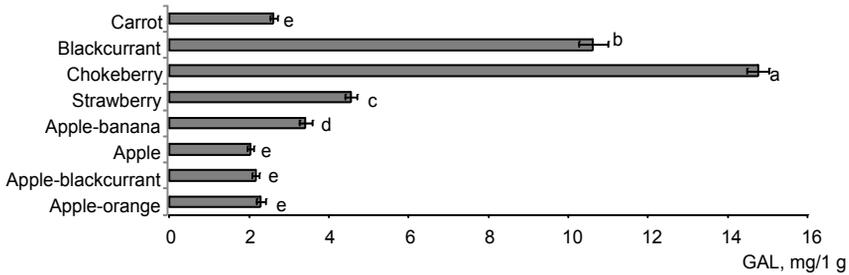


Fig. 1. Total polyphenol content in fruit and vegetable chips extracts: a, b, c, d, e – mean values with different letters differ statistically ( $P < 0.05$ )

-orange (2.3) > apple-blackcurrant (2.2) > apple (2.0). The total phenolic content of chokeberry extract was nearly six times and blackcurrant four times higher than of apple chips. Generally no statistical differences between apple-based and carrot chips were found. Among the apple-based chips only apple – banana chips contained twice higher polyphenol content as compared with other apple-based chips. Results showed that significantly higher polyphenol content was evaluated in puffings of red and dark red colour fruits. Surprisingly, low polyphenol content was found in strawberry extract, with 2.5 times higher content than in apple and carrot chips, but nearly three times lower than of chokeberry and blackcurrant extracts.

The results of Hagen et al. [2007] showed significantly higher total phenol content in sun exposed apples than of shade grown ones. Also phenolic content was higher in peel than in flesh, suggesting higher concentration under the fruits cuticle. Total phenol content in peel of sun-exposed apples was 838 mg, in flesh 215 mg of gallic acid per 100 g of fresh weight. These levels are in agreement with values found for apple chips. It was reported that apples contained high levels of flavonoids, procyanidins and epicatechin, peels however contained anthocyanins and quercetin. Its antioxidant activity expressed as Trolox equivalents was 2.8  $\mu\text{M}$  for flesh and 9.1  $\mu\text{M}$  for peel fresh weight, which is in agreement with present results. Hakkinen et al. [1999] evaluated the approximate total phenolic content per 100 g of berry seedless dry weight in chokeberry (200 mg), strawberry (90 mg) and blackcurrant (50 mg). Total phenolic of strawberries flesh were in the range of 2000-2800 mg of gallic acid per 100 g of dry weight [Aaby et al. 2005]. Strawberry achene's however contained nearly 10 times higher polyphenols content. Lin and Tang [2007] evaluated total phenolic content in strawberry as 363.7 (mg gallic acid / 100 g fresh weight). The average value obtained in present research was similar to values reported in other author's studies. Asami et al. [2003] evaluated total phenolics content in freeze dried strawberries on the level of 160 mg gallic acid per 100 g, other results showed 268.1 mg for whole fruit and approximately 5700 mg gallic acid per 100 g fresh weight of achenes [Cheel et al. 2007]. The average value of total polyphenol in carrot chips was similar to values reported in previous studies. Yen et al. [2008] examined total polyphenol content in carrots and evaluated on the similar level of 3.72 mg gallic acid per g of a product. Research of Chantaro et al. [2008] showed that processing of carrot resulted in nearly forty percent of polyphenol loss.

Resent studies reported considerable variation in the relative contents of polyphenols. Hakkinen et al. [1999] screened selected flavonoids and phenolic acids in berries.

Approximately percentage of the phenolic compounds ranged for blackcurrant: flavonols 50%, hydroxycinnamic acids 40%, ellagic acid 5% and other 5%; chokeberry: flavonols 37%, hydroxycinnamic acids 60%, ellagic acid 3%; strawberry: flavonols 12%, hydroxycinnamic acids 36%, ellagic acid 50% and other 2%. Phenolic profiles also varied among berries. Blackcurrant contains high amount of quercetin, *p*-coumaric acid, caffeic acid, myricetin and kaempferol; chokeberry: quercetin, ferulic acid, and caffeic acid; strawberry: *p*-coumaric acid, ellagic acid, quercetin and kaempferol. Strawberry contains also high levels of procyanidins, cinnamic acid derivatives, catechins and ellagitannins [Aaby et al. 2005]. Research of Kahkonen et al. [2001] and Wu et al. [2004] showed that anthocyanins predominate in blackcurrant, chokeberry and strawberry. Hydroxycinnamic acids dominate in apples (>250 mg/100 g dw). Evaluations of total phenolic content expressed as mg of gallic acid equivalent per g of dry weight, showed high variability of examined fruits extracts, and ranged in the following order: strawberry (8.1-10) < blackcurrant (114-208) < apple (1300) < chokeberry (4210). Total content of flavanols with a low degree of polymerization varied greatly among the examined fruits, and ranged in the following order: banana 0.26 < strawberry 55.3 < apple (Golden) 39 – (Red Delicious) 251 (mg/100 g dw) [Garcia-Alonso et al. 2004].

Results of Wu et al. [2004] suggested that total phenolic content in general paralleled hydrophilic antioxidant capacity of the berries. Also Aaby et al. [2005] found high correlation between total phenol content and antioxidant activity of strawberry.

### DPPH<sup>•</sup> radical scavenging activity

DPPH<sup>•</sup> radical discoloration degree is attributed to hydrogen donating ability of tested compound [Sanchez-Moreno et al. 1998]. Present research was conducted in two dimensions. First one was to prove the relationship between the radical inhibition percentage and antioxidant concentration, the second to evaluate the exact potential of tested sample. DPPH<sup>•</sup> radical scavenging activity was tested after 30 min of incubation. Results showed the increase of radical inhibition by higher concentrations of tested extracts, and were the highest at concentration of 25% according to reaction system (Fig. 2). The best results of radical inhibition were evaluated in samples of chokeberry and strawberry. All apple chips and blackcurrant puffing extracts possessed similar and significantly lower activity in DPPH<sup>•</sup> radical inhibition ( $p < 0.05$ ). Carrot chips extract exhibited the lowest potential in comparison to other examined extracts ( $p < 0.05$ ).

The DPPH<sup>•</sup> radical efficiency values of snack extracts analysed in this study are presented in Table 1. Results can be divided on three groups: medium, high and very high antioxidant efficiency. It was found that samples with the high polyphenol content exhibited also the high antioxidant efficiency presented as Trolox equivalents activity, but the overall correlation was low (0.62,  $p < 0.05$ ). The first group of medium activity samples of apple with additives and carrot were of  $AE\ 1.13-4.58 \cdot 10^{-3}$ . The second group which was considered to have high antioxidant efficiency ( $AE$  values  $5.17-5.34 \cdot 10^{-3}$ ), included strawberry and blackcurrant, fruits with total polyphenols differences of 50%. However results expressed as  $AE$  – the antioxidant concentration needed for scavenging the 50% of DPPH<sup>•</sup> radical ( $EC_{50}$ ) and the time needed to reach the steady state ( $T_{EC50}$ ) showed different correlation with total polyphenol contents (0.85,  $p < 0.05$ ). Such results suggest that polyphenol content is not the only reason for antioxidant potential, but also its quality and interactions between components matter. Similar observations were

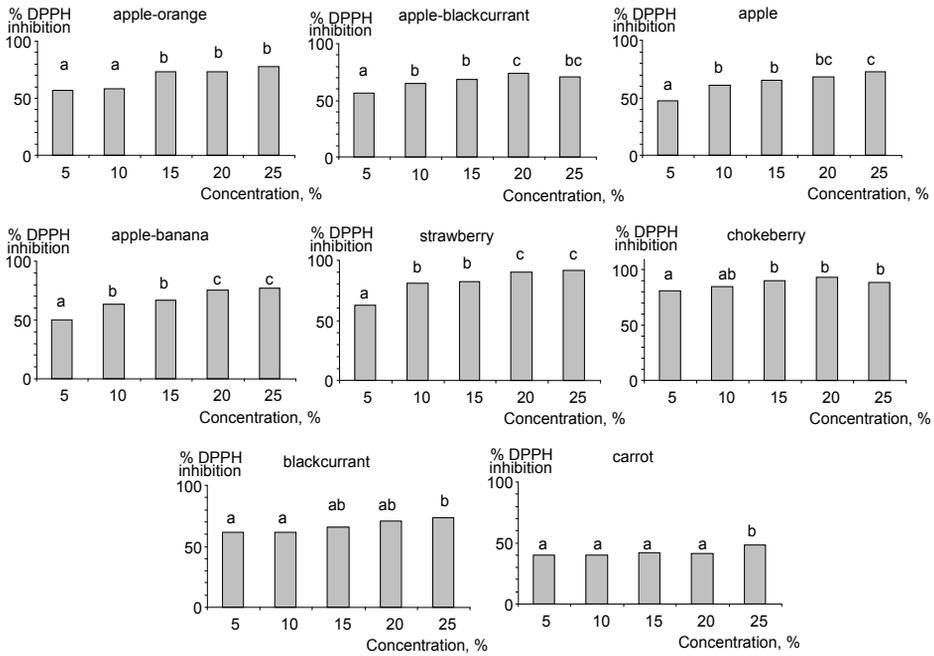


Fig. 2. DPPH' inhibition of fruit and vegetable chips extracts

made by Vasco et al. [2008], who suggested that phenolic compounds might be bound to other molecules, which considerably influence its activity. The best antioxidant efficiency was evaluated in sample with highest total polyphenol content – chokeberry. Results showed that AE value of  $10.70 \cdot 10^{-3}$  indicated also high antioxidant efficiency, where the time needed to reach the scavenging effect of 50% was shortest among the examined samples.

Table 1. DPPH' radical scavenging potential of fruit and vegetable chip extracts

Product	mg Trolox/g	EC <sub>50</sub> mg/mL	T <sub>EC50</sub>	AE, ·10 <sup>-3</sup>	Antioxidant efficiency
Apple	5.02 ±0.14 <sup>c</sup>	14.72 <sup>b</sup>	19.1 <sup>b</sup>	3.55 <sup>d</sup>	medium
Apple-banana	5.29 ±0.11 <sup>c</sup>	13.11 <sup>c</sup>	16.4 <sup>d</sup>	4.65 <sup>c</sup>	medium
Apple-blackcurrant	5.31 ±0.08 <sup>c</sup>	14.28 <sup>b</sup>	17.3 <sup>c</sup>	4.04 <sup>c</sup>	medium
Apple-orange	5.38 ±0.11 <sup>c</sup>	12.67 <sup>c</sup>	17.2 <sup>c</sup>	4.58 <sup>c</sup>	medium
Carrot	3.47 ±0.06 <sup>d</sup>	23.19 <sup>a</sup>	38.1 <sup>a</sup>	1.13 <sup>e</sup>	low/medium
Strawberry	6.40 ±0.11 <sup>b</sup>	11.23 <sup>d</sup>	17.2 <sup>c</sup>	5.17 <sup>b</sup>	high
Chokeberry	6.82 ±0.08 <sup>a</sup>	10.04 <sup>e</sup>	9.3 <sup>f</sup>	10.70 <sup>a</sup>	very high
Blackcurrant	5.28 ±0.07 <sup>c</sup>	12.71 <sup>c</sup>	14.71 <sup>c</sup>	5.34 <sup>b</sup>	high

a, b, c, d, e, f – mean values with different letters in a column differ statistically (p < 0.05).

### ABTS<sup>++</sup> radical scavenging activity

Similarly to evaluation of DPPH<sup>•</sup> radical scavenging activity also the ABTS<sup>++</sup> radical scavenging activity was evaluated in two dimensions. Results of ABTS<sup>++</sup> radicals scavenging activity was tested after 6 min of incubation. Results showed the increase of radical inhibition with higher concentrations of tested extracts (Fig. 3). The best results of radical inhibition were evaluated in samples of chokeberry, where its activity was twice higher than of strawberry and blackcurrant puffing extracts. Apple chips possessed similar and significantly lower activity in ABTS<sup>++</sup> radical inhibition ( $p < 0.05$ ). Similarly to DPPH<sup>•</sup> radical inhibition results carrot chips extract exhibited the lowest potential among examined extracts ( $p < 0.05$ ).

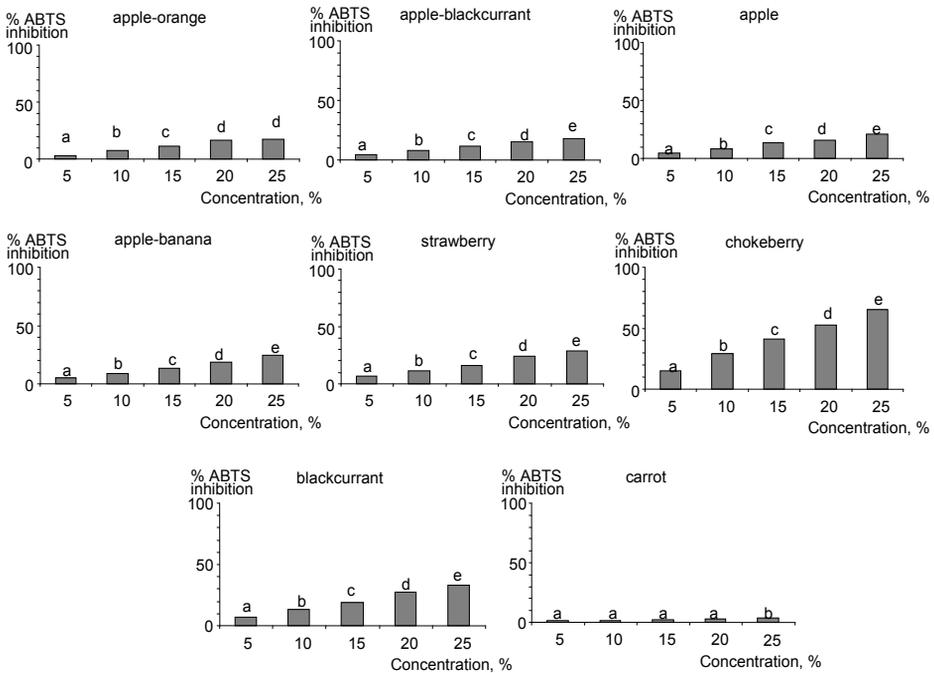


Fig. 3. ABTS<sup>++</sup> inhibition of fruit and vegetable chips extracts

The ABTS<sup>++</sup> radical efficiency values of snacks extracts are presented in Table 2. Similarly to DPPH<sup>•</sup> radical scavenging activity results can be distinguished as two groups. First group are the samples with low and second group with medium antioxidant efficiency. It was found that chokeberry sample with the highest polyphenol content exhibited also the highest antioxidant efficiency ( $AE 1.81 \cdot 10^{-3}$ ), and was ranked as medium antioxidant efficiency sample. Statistical analysis showed that the overall correlation between polyphenol content and ABTS<sup>++</sup> radical scavenging activity presented as Trolox equivalents was low ( $0.49, p < 0.05$ ), however expressed as AE significantly higher ( $0.92, p < 0.05$ ). The group of low efficiency included apple, carrot, strawberry and blackcurrant snacks, where AE ranged from  $0.18-0.80 \cdot 10^{-3}$ .

Table 2. ABTS<sup>++</sup> radical scavenging potential of fruit and vegetable chip extracts

Product	mg Trolox/g	EC <sub>50</sub> mg/mL	T <sub>EC50</sub> min	AE, ·10 <sup>-3</sup>	Antioxidant efficiency
Apple	11.76 ±0.12 <sup>de</sup>	64.79 <sup>c</sup>	29.2 <sup>d</sup>	0.53 <sup>d</sup>	low
Apple-banana	13.16 ±0.12 <sup>d</sup>	55.62 <sup>d</sup>	28.7 <sup>d</sup>	0.63 <sup>c</sup>	low
Apple-blackcurrant	10.74 ±0.16 <sup>e</sup>	71.92 <sup>b</sup>	30.2 <sup>c</sup>	0.46 <sup>e</sup>	low
Apple-orange	10.59 ±0.14 <sup>e</sup>	69.95 <sup>c</sup>	30.3 <sup>c</sup>	0.47 <sup>e</sup>	low
Carrot	2.23 ±0.08 <sup>f</sup>	437.12 <sup>a</sup>	12.4 <sup>e</sup>	0.18 <sup>f</sup>	low
Strawberry	16.16 ±0.31 <sup>c</sup>	46.28 <sup>e</sup>	36.7 <sup>a</sup>	0.59 <sup>d</sup>	low
Chokeberry	37.44 ±0.38 <sup>a</sup>	20.11 <sup>g</sup>	27.4 <sup>d</sup>	1.81 <sup>a</sup>	medium
Blackcurrant	18.61 ±0.29 <sup>b</sup>	38.92 <sup>f</sup>	32.1 <sup>b</sup>	0.80 <sup>b</sup>	low

a, b, c, d, e, f, g – mean values with different letters in a column differ statistically ( $p < 0.05$ ).

Comparison between the DPPH<sup>·</sup> and ABTS<sup>++</sup> radical scavenging methods would show the differences between antioxidants behaviour. When the radical scavenging activity of extracts was presented as mg of Trolox equivalents it was found that ABTS<sup>++</sup> values were significantly higher than those of DPPH<sup>·</sup>. Two facts should be taken into consideration. First one is that reaction times are different according to the method used (DPPH<sup>·</sup> – 30 min and ABTS<sup>++</sup> 6 min), but counted on the basis of standard curve for Trolox. Second fact is that both reactions were conducted until the reaction reached the plateau, so the EC<sub>50</sub>, T<sub>EC50</sub> and AE could be evaluated. Results of many investigations are very difficult to compare as the methods used are different. Arnao [2000] suggested that the differences between DPPH<sup>·</sup> and ABTS<sup>++</sup> might be the result of that the DPPH<sup>·</sup> is measured at a wavelength closer to the visible than the ABTS<sup>++</sup>. Occurrence of such situation may result in an underestimation of the antioxidant capacity of the sample because of the interferences presence. Perez-Jimenez and Saura-Calixto [2008] applied the same kinetic methodology for both methods used. It was found that T<sub>EC50</sub> is much lower for the ABTS<sup>++</sup> than for the DPPH<sup>·</sup> assay. The present results showed that DPPH<sup>·</sup> T<sub>EC50</sub> values of apple extracts were approximately 30% lower than those obtained with ABTS<sup>++</sup>. Similar results were observed in other samples except carrot, where the T<sub>EC50</sub> value of DPPH<sup>·</sup> (38.1) was three times higher than measured with the ABTS<sup>++</sup> assay (12.4). Regarding the AE coefficient of DPPH<sup>·</sup> assay it was shown that chips extracts exhibited rather medium (apple and carrot), high (strawberry and blackcurrant) or very high (chokeberry) antioxidant efficiency. As compared to ABTS<sup>++</sup> assay results, AE was from six times (apple) up to ten times lower (other extracts). Chokeberry extract was the only sample possessing medium antioxidant efficiency. It was evaluated that among all samples chokeberry extract was the most potent antioxidant, which efficiently scavenged both radicals used for the research. Briefly, it was stated that antioxidant efficiency relative order would depend on the radical in reaction. Such suggestions were confirmed by other authors [Prior et al. 2005]. Perez-Jimenez and Saura-Calixto [2008] explained such behaviour with the steric hindrance. It was found that caffeic acid, with a methoxy group that increases the steric hindrance, shows much higher antioxidant potential with ABTS<sup>++</sup> radicals than with DPPH<sup>·</sup> radicals. Ferulic acid however, with hy-

droxyl instead of methoxy group is better antioxidant in the presence of DPPH<sup>•</sup> than of ABTS<sup>•+</sup> radicals.

Methods used for the assessment of phenolic compounds and antioxidant capacity are based on redox properties, that is why there should be found some correlation between content of total phenolic compounds and antioxidant capacity as measured by the DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging methods. Both methods are classified as SET, but the actual mechanism depends on the other factors like the kind of component or pH of the reaction. The following conditions might result in SET and HAT (Hydrogen Atom Transfer) mixing, mainly in ABTS<sup>•+</sup> method. The values of total polyphenol content and the antioxidant efficiency measured using DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging methods differ. The results presented as Trolox equivalents, showed weaker correlation than presented as the AE coefficient. However, the results of DPPH<sup>•</sup> method showed high correlation with the ABTS<sup>•+</sup> method results presented as AE (0.97,  $p < 0.05$ ), and presented as mg of Trolox (0.83,  $p < 0.05$ ). In the literature there is missing data concerning the correlations between total polyphenol content and antiradical efficiency of fruit and vegetable snacks. That is why the present research is the basis for further specified research.

## CONCLUSIONS

In conclusion, the extracted matrices significantly affected the yield, total polyphenol content and antiradical activity of fruit and vegetable crispy snacks. The highest total polyphenol content was evaluated in chokeberry and blackcurrant extracts, apple chips contained significantly lower amount of polyphenols among all snacks. Chokeberry extract had the highest total polyphenol recovery, as well as the highest antiradical activity when determined by the DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging methods, while the apple and carrot chips extracts showed the lowest antiradical activity. The results showed that antioxidant potential evaluated with methods based on the same kind of reaction would not always react with the same manner. That is why there is a necessity of using more than one method to determine antioxidant or antiradical capacity of the component.

Thus results of the present research indicate that selection of plant matrices for snack production would be very important for consumer's health. Dietary guidelines could be favourably enriched with the results of the present research, since contemporary consumers look for the new products, which would enhance oxidative stability of the body tissues, its wellness and longevity.

## REFERENCES

- Aaby K., Skrede G., Wrolstad R.E., 2005. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). *J Agric. Food Chem.* 53, 4032-4040.
- Arnao M.B., 2000. Some methodological problems in the determination of antioxidant using chromogen radicals: a practical case. *Trends Food Sci. Technol.* 11, 419-421.
- Asami D.K., Hong Y., Barrett D.M., Mitchell A.E., 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown

- using conventional, organic, and sustainable agricultural practices. *J. Agric. Food Chem.* 51, 1237-1241.
- Chantaro P., Devahastin S., Chiewchan N., 2008. Production of antioxidant high dietary fiber powder from carrot peels. *LWT Food Sci. Technol.* 41, 1987-1994.
- Cheel J., Theoduloz C., Rodríguez J.A., Caligari P.D.S., Schmeda-Hirschmann G., 2007. Free radical scavenging activity and phenolic content in achenes and thalamus from *Fragaria chiloensis* ssp. *chiloensis*, *F. vesca* and *F. x ananassa* cv. Chandler. *Food Chem.* 102 (4), 36-44.
- Chen C., Liu K., Hsu J., Huang H., Yang M., Wang C., 2005. Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food Chem.* 91, 601-607.
- Cieślik E., Greda A., Adamus W., 2006. Contents of polyphenols in fruit and vegetables. *Food Chem.* 94, 135-142.
- Garcia-Alonso M., Pascual-Teresa S., Santos-Buelga C., Rivas-Gonzalo J.C., 2004. Evaluation of the antioxidant properties of fruits. *Food Chem.* 84, 13-18.
- Gramza A., Regula J., 2007. Use of tea extracts (*Camelia sinensis*) as polyphenols sources in human diet. *Asia Pac. J. Clin. Nutr.* 16, 43-46.
- Gramza A., Korczak J., Regula J., 2007. Use of plant extracts in summer and winter season butter oxidative stability improvement. *Asia Pac. J. Clin. Nutr.* 16, 85-88.
- Gramza-Michałowska A., Hes M., Korczak J., 2008. Tea extracts antioxidative potential in emulsified lipid systems. *Acta Sci. Pol., Technol. Aliment.* 7 (3), 29-34.
- Hagen S.F., Borge G.I.A., Bengtsson G.B., Bilger W., Berge A., Haffner K., Solhaug K.A., 2007. Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh., cv. Aroma): Effect of postharvest UV-B irradiation. *Postharvest Biol. Techn.* 45, 1-10.
- Hać-Szymańczuk E., Roman J., Bednarczyk K., 2009. Badanie aktywności przeciwbakteryjnej rozmarynu lekarskiego (*Rosmarinus officinalis*) [A study of the antibacterial activity of rosemary (*Rosmarinus officinalis*)]. *Nauka Przyr. Technol.* 3, 4, #128 [in Polish].
- Hakkinen S., Heinonen M., Karenlampi S., Mykkanen H., Ruuskanen J., Torronen R., 1999. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* 32, 345-353.
- Horwitz W., 1970. Official methods of analysis of the AOAC. Washington; 15.049-15.055.
- Huang D., Ou B., Prior R.L., 2005. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53, 1841-1856.
- Jeszka M., Flaczyk E., Kobus-Cisowska J., Dziedzic K., 2010. Związki fenolowe – charakterystyka i znaczenie w technologii żywności [Phenolics – characteristic and significance in food technology]. *Nauka Przyr. Technol.* 4, 2, #19 [in Polish].
- Kahkonen M.P., Hopia A.I., Heinonen M., 2001. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* 49, 4076-4082.
- Lin J., Tang C., 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* 101, 140-147.
- Liu R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* 78, 517S-520S.
- Moure A., Cruz M.J., Franco D., Dominguez J.M., Sineiro J., Nuniez M.J., Parajo J.C., 2001. Natural antioxidants from residual sources. *Food Chem.* 72, 145-171.
- Perez-Jimenez J., Saura-Calixto F., 2008. Antioxidant capacity of dietary polyphenols determined by ABTS assay: a kinetic expression of the results. *Int. J. Food Sci. Tech.* 43 (1), 185-191.
- Pokorny J., 2007. Are the natural antioxidants better – and safer – than synthetic antioxidants? *Eur. J. Lipid Sci. Tech.* 109, 629-642.
- Prior R., 2003. Fruits and vegetables in the prevention of cellular oxidative damage. *Am. J. Clin. Nutr.* 78, 570S-578S.
- Prior R., Wu X., Schaich K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 53, 4290-4302.
- Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 26, 1231-1237.

- Rice-Evans C., Miller N.J., Paganga G., 1996. Structure – antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad. Biol. Med.* 20, 933-956.
- Sanchez-Moreno C., Larrauri J.A., Saura-Calixto F., 1998. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* 76, 270-276.
- Scalbert A., Johnson I.T., Saltmarsh M., 2005. Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr.* 81, 215S-217S.
- Vasco C., Ruales J., Kamal-Eldin A., 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem.* 111, 816-823.
- Wu X., Gu L., Prior R.L., McKay S., 2004. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. *J. Agric. Food Chem.* 52, 7846-7856.
- Yen Y., Shih C., Chang C., 2008. Effect of adding ascorbic acid and glucose on the antioxidative properties during storage of dried carrot. *Food Chem.* 107, 265-272.
- Zhang Y., Vareed S.K., Nair M.G., 2005. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life Sci.* 76, 1465-1472.

## POTENCJAŁ PRZECIWRODNIKOWY PRZEKĄSEK Z OWOCÓW I WARZYW

**Wstęp.** Wykorzystanie związków polifenolowych jako składników żywności, suplementów lub przeciwutleniaczy to obiecujący trend w żywieniu człowieka oraz produkcji żywności. Aktywność wolnych rodników, odpowiedzialnych za starzenie się organizmu człowieka oraz psucie żywności, można zmniejszyć dzięki zastosowaniu przeciwutleniaczy znajdujących się w owocach i warzywach. Celem badań było oznaczenie oraz porównanie aktywności przeciwrodnikowej wybranych przekąsek owocowych i warzywnych jako źródła żywności funkcjonalnej.

**Materiał i metody.** Do analiz wykorzystano siedem przekąsek roślinnych dostępnych na rynku EU, w których oznaczono zawartość polifenoli ogółem oraz aktywność przeciwrodnikową w układach z wykorzystaniem rodników DPPH<sup>•</sup> i ABTS<sup>•+</sup>.

**Wyniki.** Największą zawartość polifenoli ogółem oznaczono w ekstraktach z wyrobów przekąskowych z aronii i czarnej porzeczki, natomiast najmniej z jabłek. Aronia wykazywała największą aktywność w układzie z rodnikiem DPPH<sup>•</sup> i ABTS<sup>•+</sup>, natomiast jabłka i marchew charakteryzowały się najmniejszą aktywnością przeciwrodnikową. Stwierdzono korelację pomiędzy wynikami uzyskanymi w oznaczeniach z rodnikami DPPH<sup>•</sup>, ABTS<sup>•+</sup> ( $r = 0,83$ ,  $p < 0,05$ ).

**Wnioski.** Wyniki analiz wskazują, że odpowiedni dobór matrycy roślinnej do produkcji przekąsek może być niezwykle istotny dla zdrowia konsumenta, będąc potencjalnym źródłem przeciwutleniaczy w diecie.

**Słowa kluczowe:** przeciwutleniacz, DPPH<sup>•</sup>, ABTS<sup>•+</sup>, owoce, plifenole, rodniki, przekąski, warzywa

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