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# OXYGEN RADICAL ABSORBANCE CAPACITY OF SELECTED FOOD PRODUCTS*

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**ABSTRACT**

**Background.** Recent epidemiological evidence on the growing role of food antioxidants in oxidative stress related diseases has led to a wide number of antioxidant potential evaluation assays. Main antioxidants in food are e.g. polyphenols of plant origin. Antioxidants consumption would be very promising future trend for human health and important factor in body defense system activity against reactive oxygen species (ROS), therefore in diseases prophylaxis. The aim of the present research was to evaluate the antiradical activity as $ORAC_{FL}$ value of selected food and drinks.
**Material and methods.** In the present study, twenty four products were analysed for oxygen radical absorbance capacity evaluated according to the $ORAC_{FL}$ peroxyl radical scavenging method.
**Results.** Highest $ORAC_{FL}$ values were evaluated in strawberry, dried plum and cranberry fruits, carrot and red cabbage as vegetables, orange and pomegranate juices and red tea among drinks group. Results showed significant differences between samples in selected product groups.
**Conclusions.** Research indicates that selected food products can be important peroxyl radical scavengers, and that is why information on food label, concerning the issue, would be useful for modern consumers.

**Key words:** antioxidant, oxygen radical absorbance capacity assay, $ORAC_{FL}$, fruits, vegetables, juices, tea, polyphenols, radicals

## INTRODUCTION

Latest research showed increasingly growing interest on antioxidant potential of food products and its constituents influence on diet therapy [Anwar and Przybylski 2012, Filipiak-Florkiewicz et al. 2012, Kapusta-Duch et al. 2012]. Food components are proved to be important factors in body defense system activity against reactive oxygen species (ROS), as normal cell aerobic respiration products [Lin and Tang 2007, Prior 2003, Stanner et al. 2004]. Research has provided evidence of an inverse association between plant constituents rich diet and chronic and degenerative diseases incidence, showing that high dietary intake of antioxidative compounds would help maintaining adequate antioxidant status and proper physiological functions of the body tissues [Kanazawa 2011].

In human diet vegetables and fruits are main sources of dietary antioxidants, next to supplements, easily available for the consumer [Duda-Chodak et al. 2011, Gramza and Reguła 2007, Gramza-Michałowska and Człapka-Matyasik 2011, Prior et al. 2005, Pokorny

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2007]. Antioxidants are recognized as necessary food components, however there is no standard method for its activity quantification [Frankel and Finley 2008, Perez-Jimenez et al. 2008]. There are many antioxidants in food products like vitamin E, polyphenols, carotenoids and thiol based compounds [Pellegrini et al. 2003, Scalbert et al. 2005]. According to antioxidants chemical diversity, interactions between components, their contribution to antioxidant activity and extraction methods it is very difficult to establish one proper method for antioxidant potential evaluation [Niki 2002]. Therefore, most popular method evaluating antioxidant potential of food products is oxygen radical absorption capacity assay (ORAC) [Ou et al. 2001, Wu et al. 2004]. Mechanistically ORAC method is based on the hydrogen atom transfer (HAT) reaction between an oxidant and free radical [Ou et al. 2001]. ORAC method utilizes a radical initiator (AAPH) to generate peroxyl radical, which abstracts hydrogen atom from antioxidant, therefore inhibiting further reactions. Modern, well-educated consumer needs not only basic composition information on the product's index label, but also adequate information concerning its antioxidative potential [Stockham et al. 2011].

Present research aimed at the evaluation and comparison of antiradical activity of selected fruits, vegetables and drinks expressed as oxygen radical absorbance capacity assay ($ORAC_{FL}$). The objective of the study was to provide useful information on antiradical potential of commercially available food products.

## MATERIAL AND METHODS

**Chemicals and reagents.** Phosphate buffer (75 mmol, pH 7.4): Sodium phosphate monobasic dehydrate, Potassium phosphate dibasic; Trolox (6-hydroxy--2,5,7,8-tetramethylchromane-2-carboxylic acid; Sigma-Aldrich), 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Sigma--Aldrich (Germany); Fluorescein sodium salt (Fluka).

**Samples.** Vegetables and fruits were collected from various marketplaces and groceries in the Wielkopolska region in late summer. The examined samples included fruits: strawberry, fresh and dried peach, fresh and dried cranberries, apples and dried plums; among vegetables: carrot, red cabbage, beetroot, black and red bean, tomato, potato, broccoli; drinks: apple, orange, pomegranate and tomato juices, white, green, red and black teas.

**Sample preparation.** Lyophilized samples were accurately weighed and macerated with ethanol/water (80:20, v/v) solvent, then the mixture was shaken for 15 minutes on an orbital shaker at 40°C and centrifuged at 4500 rpm for 15 minutes. Collected supernatants were ready for the analysis, concentrated samples were diluted with 75 mmol potassium phosphate buffer solution (pH 7.4). Prepared extracts were analysed on the same day, to minimize the product's degradation.

**Oxygen Radical Absorbance Capacity Assay ($ORAC_{FL}$).** The procedure was based on a report by Ou and et al. [2001] with slight modifications. Water-soluble analogue of vitamin E (Trolox), was used as a control standard. Fluorescein solution (42 nmol) prepared freshly each day, was preincubated in water bath at 37°C for at least 15 min before measurements. AAPH (153 nmol) was placed in an ice bath through the measurements (IsoTherm system, Sigma-Aldrich). Readings were taken on fluorescence spectrophotometer (Hitachi F-2700) at 493 nm excitation wavelength and 515 nm emission wavelength, both slits at 2.5. Equipment was auto-zeroed with 75 mmol phosphate buffer at pH 7.4 (blank), and Trolox solutions were used as standards (12.5; 25; 50 and 100 µmol). Trolox was used also as the quality control (25 µmol). Each cuvette was properly labelled as blank, control, standard or sample. The 2.25 ml of fluorescein working solution (48 nmol) was placed in each cuvette, then 375 µl of buffer in blank cuvette and 375 µl of standards or samples solutions in other cuvettes. There was no need for time control. Each mixture should be vigorously agitated and then incubated for 30 s, afterwards the fluorescence reading (time zero $F_0$) should be taken. After time zero reading 375 µl of AAPH (153 nmol) should be added into each cuvette with time control. Fluorescence readings were taken at 15 s and then every five minutes thereafter (f1, f2, ...) for duration of 45 min. During assay all samples were incubated at 37°C to avoid readings errors. The ORAC final calculations were made applying a regression equation between standard samples and the net area under fluorescence decay curve, obtained by subtracting the AUC of the blank from

that of sample. The area under the curve (AUC) was calculated as follows:

$$AUC = (5 + f5/f0 + f10/f0 + f15/f0 + f20/f0 + ... + f40/f0 + f45/f0) \cdot 5$$

where:
f0 – initial fluorescence before AAPH addition at time 0, f5, f10, ..., f45 – fluorescence at time 5, 10, ..., 45 minutes after addition of AAPH.

The relative oxygen radical absorbance capacity ($ORAC_{FL}$) value was calculated as follows: ORAC value = (AUC sample – AUC blank)/(AUC standard – AUC blank) · (molarity of Trolox/molarity of sample).

Results were expressed as μM of Trolox equivalents (TE) per 100 gram or 100 ml of tested product.

**Statistical analysis.** Results are expressed as mean values ± standard deviation of at least three independent measurements. Mean values were compared by one-way analysis of variance (ANOVA) at significance level of $p \leq 0.05$, using computer system Statistica 9.0.

## RESULTS AND DISCUSSION

Oxygen Radical Absorbance Capacity Assay ($ORAC_{FL}$) of tested samples were found to be affected by the form used for the extraction procedure. The evaluation of antiradical activity was divided into three groups of products: fruits, vegetables and drinks. The results showed that among the examined fruits the highest activity was evaluated for strawberries and dried fruits. Fresh fruits exhibited lower antiradical activity (Fig. 1). The fruits were ranked from highest to lowest potential as follows: plum dried > strawberry = cranberry dried > peach dried > apple fresh = cranberry fresh > peach fresh. The antiradical activity of strawberry was two times higher than fresh cranberry and almost five times higher than of fresh peach. The results showed that significantly higher $ORAC_{FL}$ values were evaluated in dark red colour fruits.

Second group of the examined products were vegetables, which were ranked from highest to lowest $ORAC_{FL}$ values as follows: carrot = red cabbage > beetroot = black bean ≥ tomato = red bean > potatoes > broccoli. The antiradical activity of carrot was nearly four times higher than broccoli and two times higher than that of potatoes (Fig. 2).

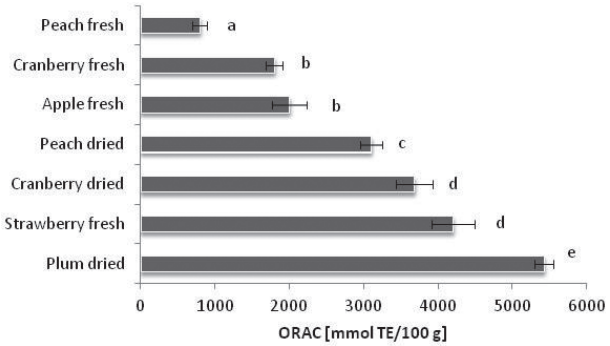


**Fig. 1.** Rank order for common fruits $ORAC_{FL}$ values: a-e – mean values with different letters differ statistically ($p \leq 0.05$)

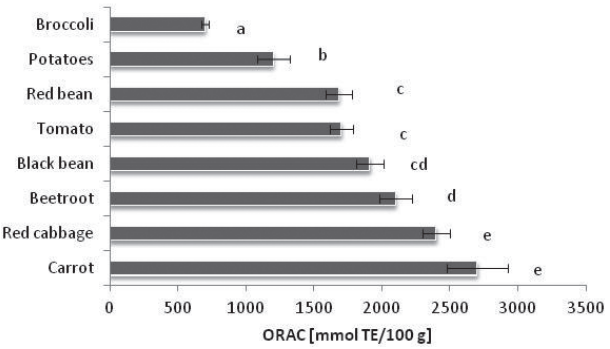


**Fig. 2.** Rank order for common fresh vegetables $ORAC_{FL}$ values: a-e – mean values with different letters differ statistically ($p \leq 0.05$)

The evaluation of antiradical activity of commonly consumed drinks and juices showed high differences among the samples. The results showed that highest activity of the examined drinks was evaluated for orange and pomegranate juices and red tea. Lowest activity was evaluated for herbal tea and apple juice (Fig. 3). Drinks were ranked from highest to lowest potential as follows: orange juice > red tea = pomegranate juice = white tea ≥ green tea = black tea = tomato juice = apple juice > herbal tea.

ORAC value was successfully applied for a wide range of samples, especially to a complex nature samples like food [Zulueta et al. 2009]. It is increasingly applied in the area of nutraceuticals, first to establish the antioxidative activity of single ingredients, than

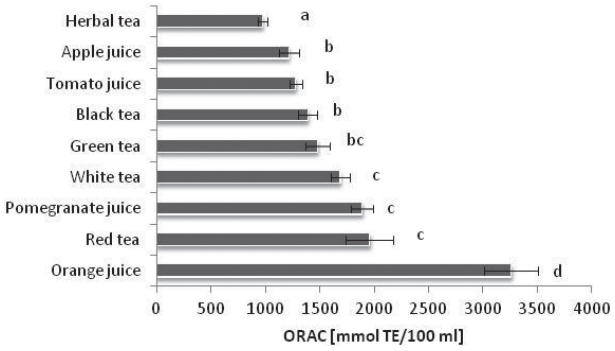


**Fig. 3.** Rank order for common drinks and juices $ORAC_{FL}$ values: a-d – mean values with different letters differ statistically ($p \leq 0.05$)

the formulation preparation impact and also in the consumer interest in products marketing [Stockham et al. 2011]. Wu et al. [2004] measured $ORAC_{FL}$ values for acetone/water extract of strawberry at level of 35.41 (µmol TE/g). Higher $ORAC_{FL}$ values were evaluated for dark coloured fruits like raspberry, blueberry and plum, lowest for cantaloupe and watermelon, which corresponds with present results. Other group were vegetables where highest potential was evaluated for broccoli (14.18 µmol TE/g), carrot (11.56 µmol TE/g) and potato (12.72 µmol TE/g), lowest for tomato (3.13 µmol TE/g). Ou et al. [2002] evaluated oxygen radical absorbance capacity for selected vegetables on the level of 60 (µmol TE/g) for carrot, 67 (µmol TE/g) for tomato and 126 (µmol TE/g) for broccoli, which are not in agreement with reported values in present research. Present evaluations showed $ORAC_{FL}$ values nearly three times lower for carrot and tomato, and eighteen times lower than that of broccoli. Comparison of present results with those of Stockham et al. [2011] showed similarities for peach, plum, black tea, tomato and apple juices. Also Ou et al. [2001] evaluated $ORAC_{FL}$ for black tea on the level of 17 267.00 (µmol TE/liter), which corresponds with present results (13 900 µmol TE/liter).

The results of Wu et al. [2004] and Liu [2003] provided a number of factors, like season of sampling, storage, processing and cooking or additive and synergistic activity combinations of phytochemicals, that significantly impact the level of antioxidants in plants. Perez-Jimenez and Saura-Calixto [2006] concluded that there is a need for ORAC assay unification, which would allow placing comparable results on the food label. Summarizing, it should be noticed that, there are many factors introducing variations as a result of antioxidative activity of foods.

## CONCLUSIONS

The application of oxygen radical absorbance capacity assay is regarded as the most adequate and popular method for fast and reliable food samples antiradical activity evaluations. The method can be used as the first step of antioxidant potential quantification since consumers need a tool for right selection of new products, which consumption would benefit body tissues oxidative stability enhancement.

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## ZDOLNOŚĆ WIĄZANIA RODNIKÓW TLENOWYCH (ORAC) PRZEZ SKŁADNIKI WYBRANYCH PRODUKTÓW SPOŻYWCZYCH

### STRESZCZENIE

**Wstęp.** Ostatnie badania epidemiologiczne wskazują na wzrastającą rolę przeciwutleniaczy z żywności w występowaniu chorób wynikających ze stresu oksydacyjnego oraz znaczne zwiększenie liczby metod oznaczania potencjału przeciwutleniającego tych składników. Główne przeciwutleniacze z żywności to związki pochodzenia roślinnego takie, jak polifenole. Spożycie przeciwutleniaczy może obiecująco wpływać na układ ochronny organizmu przeciwko reaktywnym formom tlenu i być wykorzystane w profilaktyce chorób z tym związanych. Celem badań było określenie aktywności przeciwrodnikowej wyrażonej jako $ORAC_{FL}$ dla wybranych produktów spożywczych.

**Materiał i metody.** Dwadzieścia cztery produkty spożywcze poddano ekstrakcji, a następnie badaniom w kierunku zdolności zmiatania rodników tlenowych metodą $ORAC_{FL}$.

**Wyniki.** Największymi wartościami $ORAC_{FL}$ wyróżniały się: wśród owoców – truskawki, suszone śliwki i żurawina; w grupie warzyw – marchew i czerwona kapusta; soki – pomarańczowy i z granatów, a także

napar z czerwonej herbaty. Wyniki badań wskazują na istotne różnice pomiędzy produktami w obrębie danej grupy asortymentowej.
**Podsumowanie.** Stwierdzono, że badane produkty spożywcze mogą być dobrymi zmiataczami rodnika nadtlenkowego a informacja na etykiecie produktu dotycząca potencjału przeciwutleniającego może być bodźcem do jego zakupu przez nowoczesnego konsumenta.

**Słowa kluczowe:** przeciwutleniacz, zdolność wiązania rodników tlenowych, $ORAC_{FL}$, owoce, warzywa, soki, herbata, polifenole, rodniki

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