Potravinarstvo, vol. 7, 2013, no. 1, p. 151-155
doi:10.5219/307
Received: 11 June 2013. Accepted: 12 June 2013.
Available online: 11 November 2013 at www.potravinarstvo.com

ISSN 1337-0960 (online)

STUDY OF ANTIOXIDANT ACTIVITY OF NATURAL FOOD SUPPLEMENTS

Tetyana Lozova

ABSTRACT

This article describes the results of a study of antioxidant activity of natural food supplements suggested for use in flour confectionery production. Oxidation rate of the model substance - cumene - was measured using a volumetric unit. Diagram of absorbed oxygen amount as a function of time (ΔH_{O2} over t) was built by measuring time in minutes and absorbed oxygen volume in cm^3. This diagram was subsequently used to graphically determine the oxidation rate as the slope ratio of the line in specified coordinates. Afterwards, the oxidation rate was measured at a different initiation rate (different azobisisobutyronitrile solution volume), while all other parameters of the experiment remained unaltered. On the basis of the resulting data, diagrams of oxidation rate as a function of initiation rate were built for all investigated substances (both extracts and powders). The study revealed that apian products, including pollen and propolis, as well as kidney bean powder and phytosupplements (leaves of leather bergenia, lime blossom, heartsease, wild chamomile, pepper mint, bog rosemary, and elderflowers), possessed high antioxidant activity. According to the research data, the highest activity was detected in propolis 0.482·20 pollen 0.802 and powdered forms of pepper mint 1.066 leather bergenia leaves 0.937 heartsease 0.385 lime blossom 0.331 and kidney beans 0.323. Relatively lower antioxidant activity was found in powdered bog rosemary 0.242 elderflowers 0.238 and wild chamomile 0.212. (Introduction of the investigated supplements will allow inhibiting oxidation processes in the lipide fraction of foodstuffs, including flour confectionery, to ensure stability of their qualitative characteristics over a longer period).

Keywords: antioxidant activity; chain free-radical oxidation; alternative raw materials; natural food supplements; apian products

INTRODUCTION

Nutrition is one of the dominant factors that have a significant impact on human health. This said, the healthy nutrition industry was established with the aim of producing prophylactic (functional) food products. The main trend in the development of such products is to reduce artificial supplement content by introducing natural polynutrient complexes of plant or animal origin. Use of apian products and medical/technical raw materials proves to be a promising approach to the development of new types of functional foodstuffs. Such natural supplements will enrich food, including flower confectionery, with biologically active compounds while inhibiting oxidation processes. The role of free radicals in triggering pathological changes in the human body has been known for a long time. Free radical particles, generated by biochemical processes occurring in the body, initiate oxidation processes that eventually result in damage to genetic material T-cells and cause various deceases. The free radical oxidation process is considered to be one of the reasons behind aging **(Ivanova & Karyakina, 2011)**.

Theoretical and practical aspects of the identification of antioxidant activity in certain supplements have been covered in the works published by several researchers. In this study, data on antioxidant activity of drupaceous fruit (cherry, plum, peach apricot, nectarine) obtained using the DPPH, ABTS, FRAP and TBARS methods were summarized. Further presented were the results of studies of antioxidant activity in drupaceous fruit as compared to other fruit (raspberry, apple, mango etc.), which were followed by a discussion of the examples of practical application of drupaceous fruit as oxidation inhibitors in meat products **(Makarova & Zuzina, 2011)**.

It was determined that extracts from raw unshelled sesame seeds were most effective in terms of protection of oils against oxidation. They can be used as a source of natural antioxidants in vegetable oil production **(Konsoula & Liakopoulou - Kyriakides, 2010)**.

Subsequently, the antioxidant activity of methanonic extract of Kotschyi var. Persica, as well as its fatty acid profile, was examined. Antioxidant activity of methanonic extract was evaluated using gas chromatography methods. The IC30 value was 37.09 μg/mL. It has been shown that the extract inhibits oxidation with linoleic acid by 65.22% in a β-carotene/linoleic acid system. The total phenol content and total antioxidant activity amounted to 36.52 mg of gallic acid equivalent and 74.93 mg of ascorbic acid equivalent, respectively. The primary fatty acid in the studied sample was C 18:3 ω3 (α-linolenoic

acid). It was demonstrated that this material could be used as a natural food supplement **(Zenginet al., 2011)**.

Furthermore, it was shown that fruit and vegetables rich in polyphenols possessed antioxidant properties and were an important source of bioactive compounds and dietary fibers **(Hervert - Hernandezetal., 2011, Múller et al., 2010)**. Natural antioxidants found in fruit, vegetables and legumes help maintain the vascular system in healthy condition **(Wanget al., 2011)**.

Antioxidant activity studies showed that extracts with a high content of anthocyanidins obtained from Bactrisguineensis fruit, as well as anthocyanins from Camarosa strawberry, dried cranberry, rosemary leaves (DRL) and thyme leaves (DTL), could be used in the food industry as an effective antioxidant material **(Osorio et al., 2011, Cerezoet al., 2010, Nieto et al., 2011, Ramalho & Jorge, 2008)**. Additionally demonstrated was the antiradical action of phenol components and anthocyans in Myrtaceae fruit **(Reunertsonet al., 2008)**.

It was also proven that methanonic extracts obtained from Sicilian red grape pomace with removed stems possessed high antioxidant activity. The highest activity was discovered in the sample with the highest content of anthocyanins that had a free catechol group in their structure **(Giuseppeet al., 2007)**. Very high antioxidant activity was also identified in mashed unpeeled apples with an addition of 5% rhubarb juice due to large phenol compound content **(Oszmiański & Wojdyło, 2008)**. However, scientific data show that the problem of identifying and exploring antioxidant activity of natural plant raw materials has remained generally under-investigated, giving relevance to our studies, the results of which are described in this article.

MATERIAL A METHODOLOGY

Oxidation rate of the model substance - cumene - was measured using a volumetric unit. 3 ml of cumene (isopropylbenzene) were introduced into a 10 ml reactor using a pipette. After that, either the investigated extract (0.2 ml) or powdered inhibitor substance (100 mg) was placed in the reactor. 0.1-0.5 cm^3 of the initiator solution (azobisisobutyronitrile) in o-xylene with the concentration of 0.1 mol/dm^3 were introduced into the same reactor using a 0.5 cm^3 measuring pipette. Since the mixture volume in the reactor (according to the accepted methodology) should always be 5 cm^3, a 5 cm^3 pipette was used to draw the necessary amount of o-xylene (solvent) so that the volume of all substances in the reactor would be 5 cm^3, and transfer it to the reactor. Temperature during all measurements reached 74 ˚C. An oxygen absorption measurement unit was employed (Fig. 1).

Temperature-controlled burette (6) was filled with oxygen. To do that, valve (3) was opened to connect the system to the atmosphere while valve (5) was closed, and leveling vessel (7) was lifted until the liquid level in burette (6) reached the upper mark. After that valve (3) was closed, and the burette was filled with oxygen through the valve (5), while vessel (7) was lowered until the liquid level in vessel (7) reached the lower mark of the burette. Reactor (1) and burette (6) were connected to each other through three-way valve (4) and to the atmosphere through valve (3).

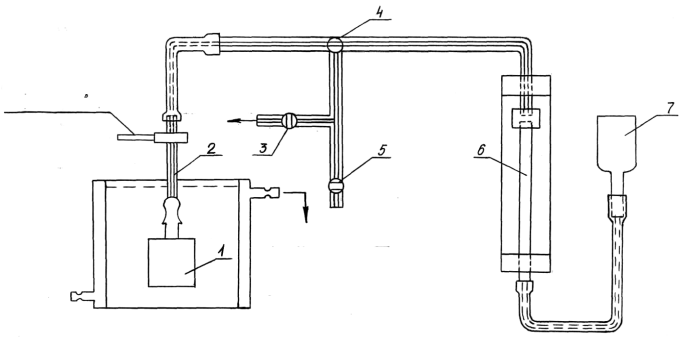

Fig. 1 Oxygen absorption measurement unit: 1 - reactor; 2 - glass capillary tube; 3, 5 - one-way valves; 4 - three-way valve; 6 - measuring burette; 7 - leveling vessel

The reaction mixture was ventilated with oxygen for 1.0 minute through a capillary tube inserted into reactor (1). Reactor (1) was immersed into a thermostat, where temperature set by the experimenter was maintained with the accuracy of ± 0.1 ˚C, and shaken with the frequency of approximately 50 cycles per second to ensure oxygenation of the reaction mixture in the process of mixing. A stopwatch was activated at the same time. The reactor was thermostatically controlled for approximately 12-15 minutes (but always for the same period). After warming up the reactor, burette (6) and reactor (1) were disconnected from the atmosphere using three-way valve (4), but left connected to each other, and measurement of oxygen absorption by cumene started by periodically measuring liquid levels in burette (6) and leveling vessel (7) and recording the values on minute-by-minute basis. The liquid meniscus progression speed in the measuring burette was proportional to the rate of oxygen absorption by cumene. Diagram of absorbed oxygen amount as a function of time (ΔH_{O2} over t) was built by measuring time in minutes and absorbed oxygen volume in cm^3. This diagram was then used to graphically determine the oxidation rate as the slope ratio of the line in the specified coordinates. After that the oxidation rate was measured at a different initiation rate (different azobisisobutyronitrile solution volume), while all other parameters of the experiment remained unaltered. On the basis of the resulting data, diagrams of oxidation rate as a function of initiation rate were built for all investigated substances (extracts or powders).

In the unit shown in Figure 1, the amount of absorbed oxygen is proportional to the sealing liquid column height measured in mm. The initiation rate is proportional to the volume of introduced initiator in ml. To convert the reaction rate expressed in mm/minute into mol/l×sec, the result is multiplied by the coefficient of the unit used to conduct the study: 1 cat. mark = 2.323×10^{-7}mol O_2, unit coefficient = K=7.667×10^{-7}mol/L×sec. To convert the ml for 0.1 M azobisisobutyronitrile initiator solution into initiation rate, the X-axis readings on the diagram of oxidation rate as a function of initiation rate should be multiplied by the coefficient: 1.647×10^{-7}mol/L×s.

RESULTS AND DISCUSSION

Experimental results are shown in the Fig. 2 - Fig. 11.

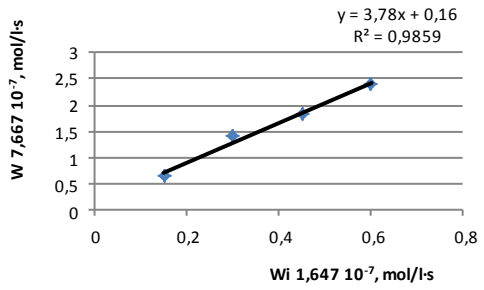

Fig. 2 Oxidation rate as a function of initiation rate of Propolis

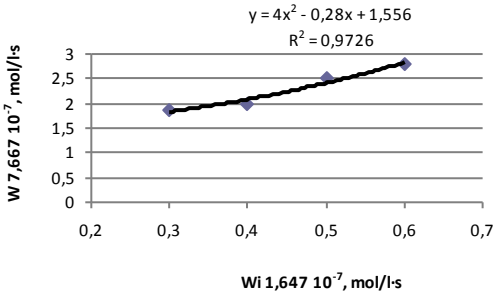

Fig. 3 Oxidation rate as a function of initiation rate of Mint powder

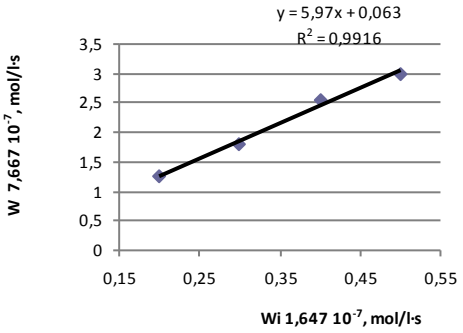

Fig. 4 Oxidation rate as a function of initiation rate of Kidney bean powder

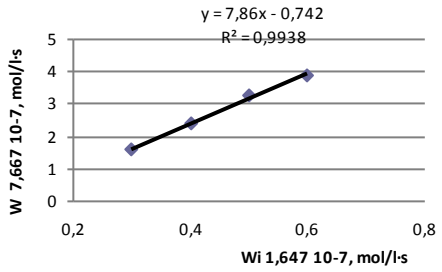

Fig. 5 Oxidation rate as a function of initiation rate of Rosemary powder

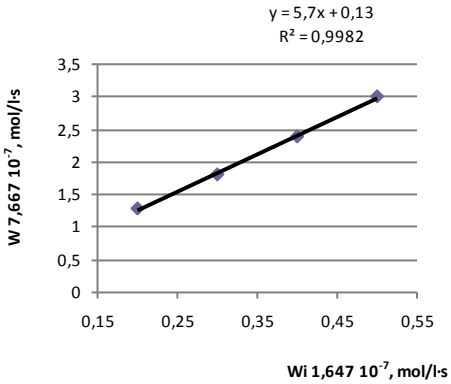

Fig. 6 Oxidation rate as a function of initiation rate of Lime blossom powder

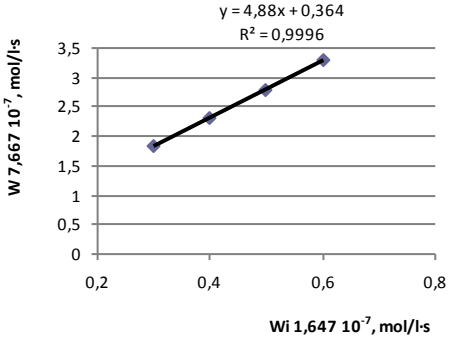

Fig. 7 Oxidation rate as a function of initiation rate of Heartsease powder

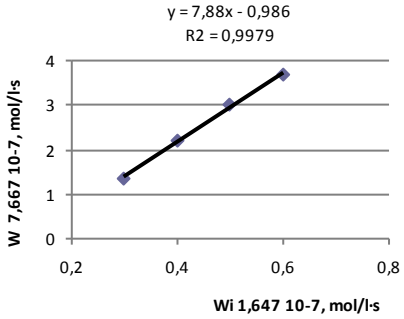

Fig. 8 Oxidation rate as a function of initiation rate of Elderflower powder

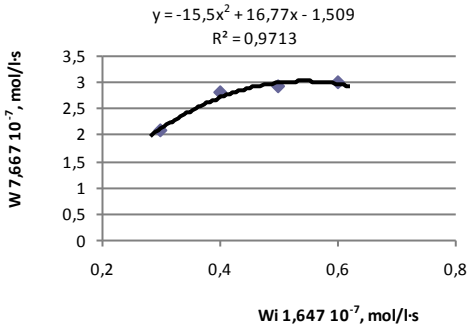

Fig. 9 Oxidation rate as a function of initiation rate of Wild chamomile flower powder

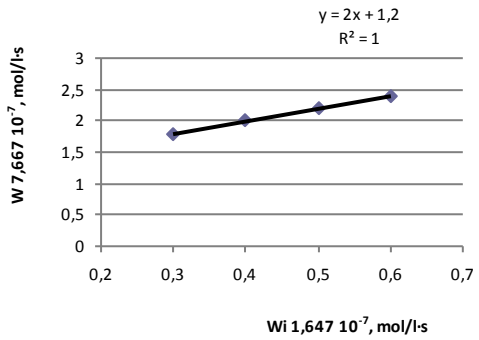

Fig. 10 Oxidation rate as a function of initiation rate of Bergenia leaves powder

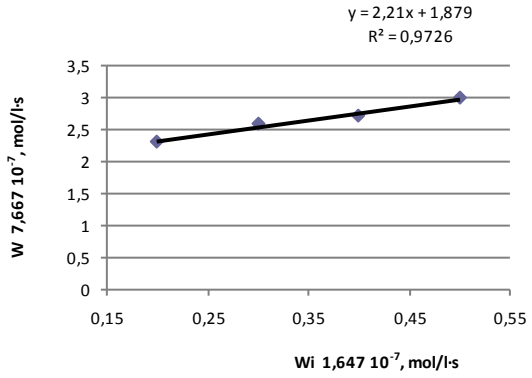

Fig. 11 Oxidation rate as a function of initiation rate of Pollen

It is known that the nature of substance oxidation, i.e. presence (or absence) of inhibitors in the system, can be determined on the basis of the dependency between the rate of chain free-radical oxidation of organic substances (in our case, this is model substance - cumene or fat) and the initiation rate. For instance, the following dependency applies to systems with no inhibitors:
$V_{O2} = (k_2/k_6^{0.5}) \cdot [RH] \cdot V_i^{0.5} = \alpha \cdot V_i^{0.5}$, that is, if we oxidize the model substance with addition of a certain amount of an extract or powder-like substance at different initiation rates, determine the oxidation rates, and then build a diagram in the V_{O2} -$V_i^{0.5}$ coordinate system, and the experimental points form a straight line as a result, this will mean that there are no inhibitors in the system. The inclination of the resulting straight line is:
$\alpha = (k_2/k_6^{0.5}) \cdot [RH]$. In the presence of inhibitors (i.e., if our extract or powder contain substances with antioxidant properties), the dependency between the oxidation rate and the initiation rate obeys the following equation:
$V_{O2} = (k_2 \cdot [RH] / k_7 \cdot f \cdot n \cdot [InH]) \cdot V_i = \alpha \cdot V_i$. In this case, if we oxidize the substance at different initiation rates and then build a diagram in the V_{O2} - V_i coordinate system, and the experimental points form a straight line as a result, this will indicate the presence of inhibitors in the system.

The results of the experimental study were processed on a personal computer using the Microsoft Excel environment. The following steps were performed to build the model: initial data collection and preprocessing; generation of a list of factors and their logical analysis; regression function specification; regression function evaluation; model adequacy verification; model parameter interpretation; forecasting of unknown values of the dependent variable. The simplest equation that can be used to characterize the dependency between the two variables is a straight line equation that describes a relation between the variables, where any change of the independent variable by a constant value results in a change of the dependent variable by another constant value. A parabolic equation was also employed. The model accuracy was evaluated using determination coefficient R^2 which established consistency between the resulting regression equation and the empirical data. This coefficient ranges from 0 to 1, and the closer it is to 1, the more accurate the model is. The regression models that we built can be considered adequate. For instance, determination coefficient value $R^2 = 0.97$ for the "Pollen" antioxidant shows that 97% of the total variation is due to changes in the factorial attribute, i.e. the initiation rate. The resulting regression models allow forecasting the oxidation rate at a specified initiation rate. For example, the equation for the "Pollen" antioxidant is y = 2.21x + 1.879. To forecast the oxidation rate at the initiation rate of, for instance, $0.36 \cdot 10^7$mol/l·s, let us insert the value of $x=0.36 \cdot 10^7 \cdot 1.647 \cdot 10^{-7}=0.6$ into the equation. The resulting value is y=3.205. Taking into account the transformation coefficient of $7.667 \cdot 10^{-7}$, we now determine the oxidation rate: $W=3.205/7.667 \cdot 10^{-7}=0.418 \cdot 10^7$mol/l·s.

The line slope ratio is $\alpha = (k_2 \cdot [RH] / k_7 \cdot f \cdot n \cdot [InH])$, where: k_2 is chain extension rate constant; k_7 - chain interruption rate constant; [InH] - molar concentration of the inhibitor (Table 1). Based on the experimental data (tg α) and with known [RH], one can determine the value of $k_2 / k_7 \cdot f \cdot n \cdot [InH]$, and then, based on known k_2 (from reference data), determine $k_7 \cdot f \cdot n \cdot [InH]$. After that, k_7 can be evaluated. This value serves as a measure of the substance's antioxidant activity. The larger it is, the higher the antioxidant properties of the supplement are. Dimension of quantity $K_7 \times f \times n \times [InH]$ = [l/mol×s] [mol/l] = [1/s]. As shown by the results of the study, antioxidant activity of the used types of natural food supplements is relatively pronounced. However, this property is most strongly exhibited by apian products (propolis - 0.482 · 20; pollen - 0.802). High antioxidant activity was found in pepper mint powder - 1.656, bergenia leaves powder - 0.937, heartsease flower powder - 0.385; small-leaved lime powder - 0.331; and kidney bean powder - 0.323. Relatively lower antioxidant activity was demonstrated by powdered forms of bog rosemary - 0.242, elderflowers - 0.238, and wild chamomile - 0.212.

The results of the study were widely discussed at international research-to-practice conference *Commodity Science and Commercial Business: Professional Development, Research and Innovation* held at Kyiv National University of Trade and Economics **(Lozova, 2009)**, as well as international research-to-practice conference *Environment and Human Health* held at Uzhhorod National University **(Lozova, 2008)**. In the course of discussion, it was shown that this method for determination of antioxidant activity was equivalent to a range of other methods usually applied to analyze the antioxidant properties of supplements based on the peroxide, benzidine or thiobarbituric number.

Table 1 Results of the antioxidant activity study

Antioxidant	tgα; (mm/min)/ml	tg α = (k_2· [RH] / k_7·f·n· [InH]).	K_7× f×n×[InH]
Pollen	2.335	10.977	0.802
Elderflower powder	7.867	36.985	0.238
Bergenia leaves powder	2.000	9.403	0.937
Heartsease flower powder	4.867	22.881	0.385
Small-leaved lime blossom powder	5.667	26.642	0.331
Bog rosemary powder	7.733	36.355	0.242
Kidney bean powder	5.800	27.267	0.323
Pepper mint powder	1.132	5.321	1.656
Wild chamomile flower powder	8.833	41.526	0.212
Propolis 5% in xylene	3.889	18.282	0.482×20

CONCLUSION

The results of the study show that introduction of the investigated supplements will allow inhibiting oxidation processes in the lipids fraction of foodstuffs, including flour confectionery, to ensure stability of their qualitative characteristics over a longer period. The best effect can be achieved by using propolis, pollen, powdered forms of pepper mint, leather bergenia, heartsease flowers, small-leaved lime blossom, and kidney beans. Application of the investigated natural food supplements in cake baking is protected under Patent of Ukraine No. 71041 (composition of cake with filling) **(Lozova, 2012).**

REFERENCES

Cerezo, A., Cuevas, E. 2010. Isolation, identification, and antioxidant activity of anthocyanin compounds in Camarosa strawberry. *Food Chem.*, vol. 123, no. 3, p. 574-582. http://dx.doi.org/10.1016/j.foodchem.2010.04.073

Giuseppe, R., Agatino, R., Carmello D. 2007. Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape curtivars. *Food Chem.,* vol. 100, no. 1, p. 203-210. http://dx.doi.org/10.1016/ j.foodchem.2005.09.041

Hervert-Hernandez, D., Garsia, O., Rosado, J. 2011. The contribution of fruit to dietary intake of polyphenols and antioxidant capacity in Mexican rural diet: Importance of fruit and vegetable variety. *Food Res. Int*., vol. 44, no. 5, p. 1182-1189. http://dx.doi.org/10.1016/j.foodres.2010.09.021

Ivanova, V. D., Karyakina, N. M. 2011. Study of the effect of extracts from alternative plant raw materials on qualitative parameters of icecream. *Food Science and Technology,* no. 2, p. 55-59.

Konsoula, Z., LiIakopoulou-Kyriakides, M. 2010. Effect of endogenous antioxidants of sesame seeds and sesame oil to the thermal stability of edible vegetable oils. LWT - *Food Sci. and Technol.,* vol. 43, no. 9, p. 1379-1386. http://dx.doi.org/10.1016/j.lwt.2013.08.010

Leusink, G., Kitts, D., Yaghmaee, P. 2010. Retention of antioxidant capacity of vacuum microwave dried cranberry. *Food Sci.,* vol. 75, no. 3, p. 311-316. PMid:20492285

Lozova, T. M. 2008. Application of biologically active supplements in fat stabilization. Environment and Human Health: Proceedings of the International Research-to-Practice Conference, Uzhhorod, Uzhhorod National University, Slovak University of Agriculture in Nitra, p. 276-279.

Lozova, T. M. 2009. Application of a spectrophotometric analysis method for investigation of oxidation processes in fats. Commodity Science and Commercial Business: Professional Development, Research and Innovation, International Research-to-Practice Conference, Kyiv, p. 127-129.

Makarova, N. V., Zuzina, A. V. 2011. Flavonoid content and antioxidant activity of apples. News of Institutes of Higher Education. *Food Technology*, no. 2-3, p. 27-29.

Müller, L., Gnoyke S., Popken, A., Böhm V. 2010. Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Sci. And Technol.,* vol. 43, no. 6, p. 992-999. http://dx.doi.org/10.1016/j.lwt.2010.02.004

Nieto, G., Huvaere, K., Skibsted, L. 2011. Antioxidant activity of rosemary and thyme by-products and synergism with added antioxidant in a liposome system. *Food Res. and Technol.*, vol. 233, no. 1, p. 11-18. http://dx.doi.org/10.1007/ s00217-011-1486-9

Osorio, C., Carriazo, Josè G., Almanza, O. 2011. Antioxidant activity of corozo (*Bactris guneensis*) fruit by electron paramagnetic resonance (EPR) spectroscopy. *Eur. Food Res. and Technol*, vol. 233, no. 1, p. 103-108. http://dx.doi.org/10.1007/s00217-011-1499-4

Oszmiański, J., Wojduło A. 2008. Polyphenol content and antioxidative activity in apple purées with rhubarb juice supplement. *Int. J. Food Sci. and Technol.,* vol. 43, no. 3, p. 501-509. http://dx.doi.org/10.1111/j.1365-2621.2006.01481.x

Ramalho, V., Jorge, N. 2008. Antioxidant action of Rosemary extract in soybean oil submitted to thermoxidation. *Grasas y aceites,* vol. 59, no. 2, p. 128-131. http://dx.doi.org/10.3989/gya.2008.v59.i2.500

Reynertson, K., Yang, H., Jiang, B. 2008. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruit. *Food Chem.*, vol. 109, no. 4, p. 883-890. http://dx.doi.org/10.1016/j.foodchem.2008.01.021 PMid: 21340048

Wang, S., Melnyk, J. P., Rong, T. 2011. How natural dietary antioxidants in fruit, vegetables and legumes promote vascular health. *Food Res. Int.,* vol. 44, no. 1, p. 14-22. http://dx.doi.org/10.1016/j.foodres.2010.09.028

Zengin, G., Guler, G., Cakmar, Y. 2011. Antioxidant capacity and fatty acid profile of Centaurea kotschyi (Boiss. & Heldr.) Hayek var. Persica (Boiss.). Wagenitz from Turkey. *Grasas y aceites,* vol. 62, no. 1, p. 90-95. http://dx.doi.org/10.3989/gya.056010

Contact address:

Tetyana Lozova, Lviv Academy of Commerce, Commodity and Commercial Sciences Faculty, Department of commodity food products, St. T.-Baranovskoho, 10, 79005, Lviv, Ukraine, E-mail: lozovatm@gmail.com