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KINETICS OF NON-ISOTHERMAL OXIDATION OF RASPBERRY AND BLACKBERRY SEED OILS BY DSC

NEIZOTERMNA KINETIKA OKSIDACIJE ULJA IZ SEMENA MALINE I KUPINE POMOĆU DSC-a

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ABSTRACT

Studies were conducted to evaluate and compare the kinetic parameters related to the oxidation process of blackberry and raspberry seed oils in non-isothermal conditions by DSC. For that purpose, the oil samples were heated at six different heating rates, β (2, 5, 7.5, 10, 15, 20 °C/min), starting from 40 ^{o}C to the beginning of the oxidation process,under an oxygen flow of 50 ml/min.Experiments were performed using TA Instruments DSC Q1000, Differential Scanning Calorimeter (Delaware, USA). Kinetic parameters (activation energy, E_a (J/mol) andpre-exponential factor, A (min^{-1})) were calculated using isoconversional Ozawa-Flynn-Wall (OFW) method. Using the values of E_a and A the oxidation rate constant was calculated at 25 °C and 120 °C by Arrhenius equation. The oxidation rate constants of raspberry seed oil was about 2 times higher than that of blackberry seed oil, which indicates that blackberry seed oil is more stable in terms of oxidation.

Key words: *blackberry seed oil, raspberry seed oil, oxidation kinetic, DSC.*

REZIME

U ovom radu određeni su kinetički parametri procesa oksidacije ulja iz semena maline i kupine pomoću DSC-a u neizotermnim uslovima. U tu svrhu, uzorci ulja su grejani na šest različitih brzina, β (2, 5, 7.5, 10, 15, 20°C/min) od 40°C do početka procesa oksidacije, pri protoku kiseonika od 50 ml/min. Eksperimenti su sprovedeni na TA Instruments DSCQ 1000, diferencijalnom skenirajućem kalorimetru (Delaware, USA). Kinetički parametri (aktivaciona energija, E_a (J/mol) i preeksponencijalni factor A (min^{-1})) su izračunati pomoću izokonverzione Ozawa - Flynn - Wall (OFW) metode. Korišćenjem vrednosti E_a i A izračunate su konstante brzina procesa oksidacije na 25°C i 120°C pomoću Arenijusove jednačine. Konstante brzina procesa oksidacije ulja iz semena maline bile su oko 2 puta veće u odnosu na konstante brzina kod ulja iz semena kupine, što ukazuje na to da je ulje iz semena kupine stabilnije u pogledu oksidacije.

Ključne reči: *ulje iz semena kupine, ulje iz semena maline, kinetika oksidacije, DSC.*

INTRODUCTION

Fats and oils are important ingredients of the human diet, both in terms of nutritional value, and in terms of organoleptic properties (smell, taste). Several phytochemicals that have been detected in edible seed oils may include tocopherols, carotenoids, phenolic and polyphenolic compounds,and especially fatty acids such as linolenic acid (18:3, *n*-3). Linolenic acid is an essential *n*-3 fatty acid that cannot be synthesized by the human body and must be obtained through the diet.*N*-3 fatty acids are reported to provide potential health benefits in terms of reducing the risk of heart disease, cancer, hypertension and autoimmune disorders (*Burdge*, *2006*; *Connor*, *2000*; *Tapiero et al.*, *2002*).

All berry seed oils have in common a high content of polyunsaturated fatty acids, providing essential fatty acids (*Van Hoed et al.*, *2009*). Recently, the properties of some berry seed oils have been reported in literature (*Micić et al.*, *2014*). Parry and Yu (*2004)*, Parry et al., (*2005*) found significant amounts of α-linolenic acid, tocopherols, polyphenols and carotenoids in marionberry, boysenberry, red raspberry and blueberry seed oils.

Oxidation of polyunsaturated fatty acids is the main reaction that affects the quality of oil and oil products during storage and use. Since oil oxidation is an exothermic reaction, it releases heat that can be measured using Differential Scanning Calorimetry (DSC). Because it is a simple, time saving method that does not require the use of toxic chemicals, DSC could be used for routine analysis in the oils and fats industry. Thus, in recent times, DSC has been one of the most commonly used techniques for examination of the oxidative stability of different types of oils (*Arain et al.*, *2009*; *Litwinienko et al.*, *1999*; *Tan et al.*, *2002*).

The objective of the present work was to evaluate and compare the oxidative stability of blackberry (*R. fruticosus* L., Čačak Thornless cultivar) and raspberry (*Rubus idaeus* L., Willamette cultivar) seed oils and to determine the kinetic parameters related to the oxidation process by DSC in non-isothermal conditions.

MATERIAL AND METHOD

Materials

Blackberry (*R. fruticosus* L., Čačak Thornless cultivar) and raspberry (*Rubus idaeus* L., Willamette cultivar) were obtained from Fruit Research Institute, Čačak, Serbia. Oil was extracted from milled berry seeds, using hexane as described in literature (*Oomah et al.*, *2000*). The sample (5 g) was stirred for 2 h at 4 ^{o}C with hexane (50 ml). The solvent was removed by vacuum filtration and the sample was extracted two additional times. After the last filtration, the extracts were pooled and the hexane was removed by rotary vacuum evaporation at 30 ^{o}C. The oil was stored at -20 ^{o}C until analysis.

Physicochemical characterization

The ISO Standard Methods were employed for determinations of free fatty acid content, acid, peroxide and saponification values(ISO 660, 2009; ISO 3960, 2001; ISO 3657, 2002), and Wijs Method was used for determinations of iodine value in the oil samples. Both oil samples were analyzed in triplicate.

DSC analysis

Oxidative stability of the oil samples was determined by TA Instruments DSC Q1000, Differential Scanning Calorimeter (Delaware, USA), with TA Universal analysis 2000 software. The apparatus was calibrated with a high-purity indium standard. Oil samples of (3.0 ± 0.3) mg were weighed into open aluminum pans and placed in the equipment's sample chamber. An empty open aluminum pan was used as reference. Experiments were performed under an oxygen (99.95 % purity) flow of 50 ml/min.The oil samples were heated at different heating rates, β (2, 5, 7.5, 10, 15, 20 ^{o}C/min), starting from 40 ^{o}C to the beginning of the oxidation process. During oil oxidation, the oxygen consumption can be neglected due to the large excess of oxygen generated by a constant flow rate. Such conditions allow the formation of peroxides independent of the oxygen concentration, which also means that the autoxidation is a first order reaction. This is an essential assumption for the calculation of kinetic triplet parameters: activation energy, E_a (J/mol), pre-exponential factor or frequency factor, A (min^{-1}), and reaction rate constant, k (min^{-1}). A commonly used non-isothermal method is the Ozawa-Flynn-Wall (OFW) method. Applying this method, kinetic parameters were calculated from the data set obtained from DSC curves (onset temperature (T_{on}) and peak temperature (T_p) at different heating rates) using the following equations:

$$\log\beta = a\cdot\left(1/T\right)+b \tag{1}$$

wherein β is heating rate (K/min) and T is temperature, T_{on} or T_p (K). By plotting $log\beta$ vs. $1/T$, the activation energy and the pre-exponential factor were determined directly from the slope and the intercept according to:

$$a = -0.4567\cdot\left(E_a/R\right) \tag{2}$$

$$b = -2.315+\log\left(A\cdot E_a/R\right) \tag{3}$$

wherein a and b are the slope and the intercept from Eq. (1), respectively, and R is the universal gas constant (8.314 J/molK). Therefore, the activation energy is calculated from:

$$E_a = -2.19\cdot R\cdot\frac{d\log\beta}{d\left(1/T\right)} \tag{4}$$

Values of E_a and A can be used to calculate the rate constant of reaction, given by the Arrhenius equation:

$$k = A\cdot\exp\left(-E_a/RT\right) \tag{5}$$

Statistical analysis

All DSC experiments and measurements were performed in triplicate and the values are presented as mean values ± SD. T_{on} andT_p were used to calculate Arrhenius parameters (E_a, A, k). All kinetic data were subjected to analysis of variance (ANOVA) for the comparison of means, and significant differences are calculated according to post-hoc Tukey's HSD ("honestly significant differences") test at p<0.05 significant level, 95 % confidence limit. The linear regression analysis for fitting of kinetics data, was performed using STATISTICA 10.0® (StatSoft Inc., Tulsa, OK, USA)software. The accuracy of T_{on} andT_p was tested based on coefficients of variation.

RESULTS AND DISCUSSION

The initial characteristics of edible oils used in the present study are given in Table 1. The acid value (AV) is a common parameter in the specification of fats and oils.When the acidity of the oil is lower, its quality is better. These parameters show how the seed was processed before and during seed oil extraction. An increment in the amount of FFA in a sample of oil indicates hydrolysis of triglycerides. Peroxide value, as indicator of the primary oxidation process, was about two time higher in the raspberry than in the blackberry seed oils, whiche indicates that the raspberry seed oil is more susceptible to oxidation.Iodine value is a measure of the degree of unsaturation in the fatty acids of triacyglycerol and it can be used to determine the type and source of oils. The obtained iodine value are relatively high which suggest that examined oils are highly unsaturated oilsand therefore prone to oxidation. The saponification value shows the amount of KOH needed to saponify all free and bound fatty acids. These values of raspberry and blackberry seed oils were similar to previously reported values for raspberry seed oil (191 – 211 mg KOH/g) (*Oomah et al., 2000*; *Šućurović et al., 2009*).

Table 1. Physicochemical characteristics of blackberry and raspberry seed oils

Parameter	**Blackberry seed oil**	**Raspberry seed oil**
Acid value (mg KOH/g)	2.34±0.08	6.44±0.08
Free fatty acids (FFA) (%)	0.83	1.4
Peroxide value (meqO$_2$/kg)	5.2±.02	9.5±0.2
Saponification value (mg KOH/g)	192.3±0.2	188.5±0.2
Iodine value (gJ$_2$/100g)	188.3±0.2	162.3±0.2

Fig. 1. shows a typical non-isothermal DSC curve during heating of raspberry seed oil sample at the rate of 10 ^{o}C/min in oxygen flow. Three characteristic points can be observed: onset temperature (T_{on}), first peak (T_{p1}) and second peak (T_{p2}). DSC curves at other heating rates show the same shape for both oils. Since two exothermic peaks occur, it is clear that two main processes take place during oil oxidation. Litwinienko and Kasprzycka-Guttman (*1998*) have shown that the model of sequential reactions with autocatalytic onset is the best explanation for the shape of the DSC signal during oil oxidation. This model demonstrates that the first peak is caused by hydroperoxide formation, while the second peak is the result of decomposition of hydroperoxides to further products.

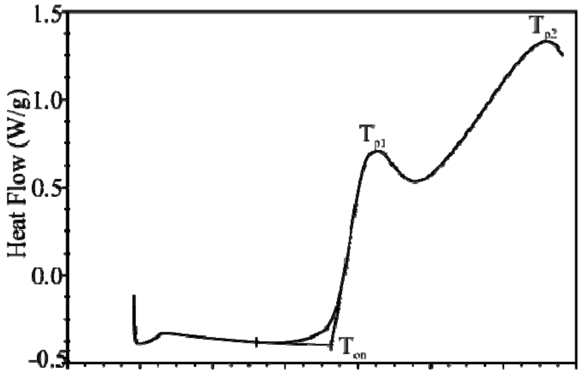

Fig. 1. DSC curve during heating of raspberry seed oil sample at rate 10 ^{o}C/min in oxygen flow 50 ml/min (T_{on} – onset temperature, T_{p1} – the first peak and T_{p2} – the second peak)

Based on this, it is clear that only the onset of oxidation and the first peak should be considered in assessing the oil oxidative stability from non-isothermal DSC method.

T_{on} and T_{p1} values determined from DSC curves, which are obtained at different heating rates, are shown in the *Table 2*, for both oils. As can be seen, these values increase with an increase in the heating rate.

*Table 2. T_{on} and T_{p1} values obtained at different heating rates (β) for blackberry and raspberry seed oils**

Heating rate, β (K/min)	**Blackberry seed oil**		**Raspberry seed oil**	
	T_{on} (K)	**T_{p1} (K)**	**T_{on} (K)**	**T_{p1} (K)**
20	470.16 ± 3.15^{d}	509.72 ± 4.48^{c}	464.05 ± 4.15^{c}	502.22 ± 3.63^{d}
15	466.13 ± 5.30^{cd}	498.59 ± 5.28^{bc}	458.50 ± 5.45^{ac}	491.43 ± 5.35^{b}
10	459.52 ± 2.95^{bc}	490.09 ± 3.14^{ab}	453.31 ± 1.00^{a}	481.66 ± 1.69^{ab}
7.5	455.43 ± 1.29^{ab}	485.91 ± 1.79^{a}	449.78 ± 2.62^{ab}	478.55 ± 3.62^{a}
5	446.52 ± 2.65^{a}	484.81 ± 2.57^{a}	441.60 ± 1.67^{b}	475.21 ± 4.03^{a}
2	435.31 ± 3.12^{e}	461.69 ± 5.04^{d}	429.33 ± 3.33^{d}	453.29 ± 3.14^{c}
CV (%)	1.14	1.09	1.19	1.09

**Values printed in one column, with the same letters (a-e) in superscript are not statistically different at the p<0.05 level, 95 % confidence limit, according to Tukey's HSD test; CV – coefficient of variation.*

As T_{on} and T_{p1} are temperatures at which the oxidation process is on the same conversion degrees for different heating rate values, following kinetic parameters can be calculated, activation energy (E_a) and pre-exponential factor (A), for both degrees of conversion by iso-conversional method (OFW method), as explained above. *Table 3* shows the parameters obtained by linear regression. Good fitting to experimental results were obtained, for both samples, using linear regression with a high coefficient of determination of $R^2 > 0.95$ for both conversion degrees (at T_{on} and T_{p1}). Using the values of E_a and A obtained from the slope and the intercept of linear regression model, the oxidation rate constant was calculated at 25 °C and 120 °C by Arrhenius equation. These results are also presented in *Table 3*.

Table 3.Linear regression parameters obtained from plotting log β vs. $1/T_{on}$ and $1/T_{p1}$ for blackberry and raspberry seed oils, as well as oxidation rate constants at 25 °C (k_{298}) and 120 °C (k_{393})

Parameters	**Blackberry seed oil**		**Raspberry seed oil**	
	T_{on}	**T_{p1}**	**T_{on}**	**T_{p1}**
a	13.39 ± 0.47	11.43 ± 1.07	13.65 ± 0.40	11.05 ± 0.85
b	-5689 ± 216	-5141 ± 520	-5729 ± 178	-4875 ± 409
R^2	0.99	0.96	0.99	0.97
E_a (kJ/mol)	104 ± 4	94 ± 9	104 ± 3	89 ± 7
A ($\times10^{10}$min^{-1})	41	0.49	73	0.22
k_{298} ($\times10^{-7}$ min^{-1})	2.89	1.95	3.87	6.09
k_{393} ($\times10^{-3}$ min^{-1})	7.02	1.8	10.1	3.49

As can be seen, the constants calculated using T_{p1} are 3 to 4 times lower than those obtained using T_{on} at 120 °C, while their values are almost equal at 25 °C. This indicates that two different reactions are taking place at the onset and the peak of DSC curve: at lower temperatures, both reactions take place approximately at the same rate, while at higher temperatures the reaction at the onset of DSC curve becomes several times faster than one at the peak, so that the oxidative stability of oils is dominantly influenced by the reaction at the onset of DSC curve (reaction of radical formation).

Based on the differences of activation energy values of these two oils, it is impossible to accurately conclude which oil is more prone to oxidation, because they are almost equal within experimental error. However, by comparing the oxidation rate constants, it can clearly be seen that the k of raspberry seed oil is about 2 times higher than that of blackberry seed oil, which indicates that blackberry seed oil is more stable in terms of oxidation.

Also, by comparing the oxidation rate constants obtained for blackberry and raspberry seed oils with those of commercial oils (olive and sunflower) in literature (*Adhvaryu et al., 2000*; *Ostrowska-Ligeza et al., 2010*), it can be seen that they are approximately of the same value or lower, which is contrary to expectations, being that berry oils are highly unsaturated oils. This is probably because these oils are very rich in tocopherols (*Van Hoed et al., 2009*), which are among the most effective natural antioxidants.

CONCLUSION

By comparing the oxidation rate constants, blackberry seed oil was shown to be more oxidatively stable than raspberry seed oil.Although both oils are highly unsaturated oils, they show similar oxidative stability as some commercial oils, probably due tohigh tocopherols content of berry seed oils.

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REFERENCES

Adhvaryu, A., Erhan, S. Z., Liu, Z. S., Perez, J. M. (2000). Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy. Thermochimica Acta, 364 , 87–97.

Arain, S., Sherazi, S. T. H., Bhanger, M. I., Talpur, F. N., Mahesar, S. A. (2009). Oxidative stability assessment of Bauhinia purpurea seed oil in comparison to two conventional vegetable oils by differential scanning calorimetry and Rancimat methods. Thermochimica Acta, 484 , 1–3.

Burdge, G. C. (2006). Metabolism of alpha-linolenic acid in humans. Prostaglandins, Leukotrienes, and Essential Fatty Acids, 75 (3), 161–168.

Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. The American Journal of Clinical Nutrition, 71 (1), 171S–175S.

ISO 660:2009 Animal and vegetable fats and oils -- Determination of acid value and acidity.

ISO 3960:2001 Animal and vegetable fats and oils -- Determination of peroxide value.

ISO 3657:2002 Animal and vegetable fats and oils -- Determination of saponification value.

Litwinienko, G., Daniluk, A., Kasprzycka-guttman, T. (1999). A differential scanning calorimetry study on the oxidation of

C12-C18 saturated fatty acids and their esters. Journal of the American Oil Chemists' Society, 76 (6), 655–657.

Litwinienko, G., Kasprzycka-Guttman, T. (1998). A DSC study on thermoxidation kinetics of mustard oil. Thermochimica Acta, 319 , 185–191.

Micić, D., Ostojić, S., Simonović, M., Simonović, B. R. (2014). Thermal behavior of raspberry and blackberry seeds oils followed by DSC. Journal on Processing and Energy in Agriculture, 18 (5), 204–206.

Oomah, B. D. D., Ladet, S., Godfrey, D. V., Liang, J., Girard, B. (2000). Characteristics of raspberry (Rubus idaeus L.) seed oil. Food Chemistry, 69 (2), 187–193.

Ostrowska-Ligeza, E., Bekas, W., Kowalska, D., Lobacz, M., Wroniak, M., Kowalski, B. (2010). Kinetics of commercial olive oil oxidation: Dynamic differential scanning calorimetry and Rancimat studies. European Journal of Lipid Science and Technology, 112 (2), 268–274.

Parry, J., Su, L., Luther, M., Zhou, K., Yurawecz, M. P., Whittaker, P., Yu, L. (2005). Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. Journal of Agricultural and Food Chemistry, 53 (3), 566–573.

Parry, J., Yu, L. (2004). Fatty acid content and antioxidant properties of cold-pressed black raspberry seed oil and meal. Journal of Food Science, 69 (3), 189–193.

Šućurović, A., Vukelić, N., Ignjatović, L., Brčeski, I., Jovanović, D. (2009). Physical-chemical characteristics and oxidative stability of oil obtained from lyophilized raspberry seed. European Journal of Lipid Science and Technology, 111, 1133–1141.

Tan, C. P., Che Man, Y. B., Selamat, J., Yusoff, M. S. A. (2002). Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. Food Chemistry, 76 (3), 385–389.

Tapiero, H., Nguyen Ba, G., Couvreur, P., Tew, K. D. (2002). Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomedicine & Pharmacotherapy, 56 (5), 215–222.

Van Hoed, V., De Clercq, N., Echim, C., Andjelkovic, M., Leber, E., Dewettinck, K., Verhé, R. (2009). Berry seeds: a source of specialty oils with high content of bioactives and nutritional value. Journal of Food Lipids, 16 (1), 33–49.

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