EFFECT OF PHENOLIC COMPOUNDS EXTRACTED FROM *Camelina sativa* OIL ON OXIDATIVE STABILITY OF LIPID SYSTEM

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Abstract
Phenolic compounds obtained from *Camelina sativa* oil were analysed according to their antioxidative properties in bulk lipid system. The extract of phenolic compounds was prepared by use of solvent extraction. The results indicate that camelina oil extract added to model lipid system retarded the process of lipid peroxidation. The extract was able to decrease the formation of primary oxidation products determined as conjugated dienes and peroxide value in lipid system incubated at temperature 65 °C. Additionally, the induction time (*IT*) of bulk lipid system enriched with extract as determined by Rancimat test was extended by 10 %.
Keywords: *Camelina sativa,* false flax, phenolic compounds, oil, antioxidant activity, lipid peroxidation, peroxide value, conjugated dienes, Rancimat

UČINEK FENOLNIH SPOJIN EKSTRAHIRANIH IZ OLJA *Camelina sativa* NA OKSIDATIVNO STABILNOST LIPIDNEGA SISTEMA

Izvleček
Antioksidativne lastnosti fenolnih spojin, ki smo jih pridobili iz olja *Camelina sativa*, smo analizirali v lipidnem sistemu. Izvleček fenolnih spojin smo pripravili s solventno ekstrakcijo. Rezultati kažejo, da izvleček, ki ga dodamo modelnemu lipidnemu sistemu, zavira proces peroksidacije. Izvleček je v lipidnem sistemu, ki smo ga inkubirali pri temperaturi 65 °C, zmanjšal tvorbo primarnih oksidacijskih produktov. Primarne oksidacijske produkte smo določili kot vsebnost konjugiranih dienov in kot peroksidno število. Poleg tega smo z metodo Rancimat določili indukcijski čas (*IT*) in ugotovili 10 % podaljšanje *IT* za lipidni sistem z dodanim izvlečkom.
Ključne besede: *Camelina sativa,* navadni riček, fenolne spojine, olje, antioksidativna učinkovitost, lipidna peroksidacija, peroksidno število, konjugirani dieni, Rancimat

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1 INTRODUCTION

Camelina sativa, with the common names false flax or gold of pleasure, is an oilseed plant and belongs to the *Brassicaceae* family. Slovenia is one of the few countries where the tradition of producing it has been preserved until today (Abramovič and Abram, 2005; Hrastar et al., 2012). *C. sativa* seed consists of about 43 % oil in dry matter. The oil produced from the seeds is partly used as edible oil but most of it is used as a traditional home remedy. From the nutritional point of view, camelina oil is a rich source of essential fatty acids (linoleic and α-linolenic) as well as ω-3 fatty acid (α-linolenic). The content of unsaturated fatty acids in the oil is about 90 % (Jankowski et al., 2019). About 50 % of the total fatty acids are polyunsaturated-linoleic acid (18:2*n*−6) and α-linolenic acid (18:3*n*−3). The content of erucic acid (22:1*n*−9) in the oil is about 3.0 % (Hrastar et al., 2009). According to Hrastar et al. (2012) and Abramovič et al. (2007) the content of tocopherols in camelina oil is about 700 mg/kg oil with predominating γ-tocopherol. In one of our papers (Terpinc et al., 2012) we published results concerning the occurrence and characterisation of phenolic compounds in camelina oil obtained from the seeds grown in the Koroška region (Slovenia). It has been found that during the pressing of the seeds most of the phenolics remain in the seed residues (cake), only a small portion is transferred into the oil reaching the value expressed in chlorogenic acid equvalents (CAE) around 100 mg CAE/kg. In the same study the phenolic compounds catehin, *p*-hidroxybenzoic acid, ellagic acid, sinapic acid, salicylic acid and quercetin were identified.

Initial studies assumed that lipid oxidation in bulk oil takes place in a homogeneous medium. Today is known that systems like vegetable oils are complex multiphase systems which contain small amounts of monoacylglycerols, diacylglycerols, free fatty acids, phospholipids, sterols, cholesterols, phenolic compounds and oxidation products. These amphiphilic molecules can self-assemble due to hydrophobic interaction to form a variety of different types of association colloids, including lamellar structures and reverse micelles. However, safflower oil used in this investigation was refined. Thus above mentioned amphiphilic compounds are removed, but this bulk oil system can still contain small amounts of water what alter the structure and characteristics of oil, and behaviour of antioxidants added (Chaiyasit et al., 2007).

Antioxidant activity of camelina oil phenolics extract determined as free radical scavenging activity, metal ion reducing capacity, chelating ability and inhibitory action against β-carotene discolouration in an emulsified system has been investigated extensively (Terpinc et al., 2012). In comparison to the extract of phenolics obtained from camelina seeds and camelina cake the extract from camelina oil exhibited the highest iron-chelating capacity and was the most

effective in inhibiting β-carotene discolouration in an emulsified system. However, its antioxidant activity in bulk oil system was far less investigated.

Our model lipid system consisted of safflower oil, commercial standard without added antioxidants. Due to the high content of polyunsaturated fatty acids, it was expected that safflower oil would be exposed to the formation of undesirable off-flavors and potentially toxic oxidation products. Since one of the most effective means to delay lipid oxidation in oils is the incorporation of antioxidants, the aim of this study was to evaluate the protective effect of phenolic compounds extracted from camelina oil on the oxidative stability of the model lipid system (safflower oil). For that purpose, the Rancimat test was performed, and the formation of primary oxidation products in safflower oil incubated at elevated temperature was the followed method.

2 MATERIALS AND METHODS

2.1 Materials

The camelina oil used in this study was produced from seeds of *Camelina sativa* plants grown in the Koroška region, Slovenia. After pressing the heat-treated seeds, the obtained camelina oil was filtered and stored in the refrigerator until analysis. The commercially available refined, bleached, deodorised safflower (*Carthamus tinctorius*) oil from Sigma (S5007; Sigma-Aldrich GmbH, Steinheim, Germany) was employed as model lipid system. According to the product specification provided by the producer of this safflower oil, the fatty acid content (in weight percent; %) was: palmitic acid (16:0), 7 %; stearic acid (18:0), 2 %; oleic acid (18:1n-9), 16 %; linoleic acid (18:2n-6), 71 %. The peroxide value (*PV*) of safflower oil prior to the experiments was 3.8 mmol O_2/kg. All other chemicals and solvents were of analytical grade.

2.2 Preparation of phenolic extract from camelina oil

Extract of phenolic compounds from camelina oil was prepared according to the method described by Terpinc et al. (2012) by use of methanol-water mixture (80:10, v/v) as extraction solvent. The oil-to-solvent ratio was 1:4 (w/v). The residue after condensation in a rotary evaporator was redissolved in ethanol. The extraction was performed in triplicate. The repeatability of the extraction process amounted to 94 %.

2.3 Determination of total phenolic content in extract from camelina oil

The content of total phenolic compounds in extract from camelina oil was determined according to the Folin-Ciocalteu method described by Gutfinger

(1981). The amount of total phenolic compounds was expressed in CAE. The determination was conducted in triplicate and results were averaged. The standard deviation of determination was less than 5 %.

2.4 The protective effect of phenolic extract obtained from camelina oil on the oxidative stability of model lipid system

Appropriately diluted extract was added to bulk safflower oil, producing the desired concentration of 200 mg CAE/kg. Ethanol was then evaporated using N_2. The samples (70 mL) were transferred to transparent glass beakers (4 cm in diameter) covered with a watch glass and exposed to storage for 13 days at (65 ± 0.5) °C in the dark. Stored samples were subjected to determination of *PV* and conjugated dienes over a period of 13 days (after 2, 5, 11 and 13 days of experiment).

2.5 Peroxide value determination of model lipid system

The AOAC Official Method 965.33 (AOAC, 1999) was used for peroxide value (*PV)* determination. The value was expressed as mmol O_2/kg of safflower oil. The determination was carried out in triplicate. The standard deviation of determination was less than 2 %.

2.6 Conjugated dienes determination of model lipid system

Conjugated dienes in the safflower oil were determined according to method described by (IUPAC, 1987). Weighed safflower oil samples were dissolved in 5 mL cyclohexane, diluted, and the absorbance was measured at 234 nm (A_{234}) against cyclohexane as the blank. Results were expressed as specific extinction *E* (1 %, 1 cm) calculated by Equation 1:

$$E(1\ \%, 1\ \text{cm}) = A_{234} / \gamma_{\text{oil}} \quad \ldots(1)$$

where γ_{oil} is the concentration of safflower oil in cyclohexane (g/100 mL). The determination was carried out in duplicate. The standard deviation of determination was less than 5%.

2.7 Rancimat test of model lipid system

The test was performed on a 679 Rancimat apparatus (Metrohm model 743, Herisau, Switzerland). Safflower oil samples (3 mL) were subjected to a temperature of 110 °C at an air flow rate of 20 L/h. The oxidative stability was expressed as induction time (*IT*), which represents the time needed for decomposition of hydroperoxides produced by oil oxidation (Läubli et al., 1988).

The antioxidant activity of added antioxidants was expressed as protection factor (*PF*) that was calculated by Equation 2:

$$PF = 100\% \cdot (IT_{extr} - IT_{control}) / IT_{control} \quad \ldots(2)$$

where IT_{extr} and $IT_{control}$ mean the value for induction time for safflower oil with and without antioxidants, respectively. The determination was carried out in triplicate. The standard deviation was less than 3 %.

3 RESULTS AND DISCUSSION

From nutritional point of view, oils containing high proportion of polyunsaturated fatty acids are highly desirable. On the other hand, these oils are susceptible to oxidation. Oxidative stability of lipid system is one of the most important oil quality parameters that determine its usefulness as well as shelf life.

Lipid oxidation is a free radical chain reaction that includes common phases of initiation, propagation and termination. The generation of primary free radical causes the formation of another radical, resulting in the formation of the primary oxidation product, i.e. hydroperoxide. In addition, many free radical formed by decomposition of hydroperoxides are highly reactive. That leads to formation of the secondary and tertiary products which influence sensorial and nutritional properties of food. Besides, they react with the surrounding food components, thereby extending the undesirable effects of lipid oxidation (Zamora and Hidalgo, 2016).

Beside on fatty acid composition the oxidative stability of lipid system depends on its physical state and the presence of antioxidants (Martinović et al., 2019). The phenolic compounds act as antioxidants due to their capability to scavenge free radicals, chelate transitional metals and quench singlet and triplet oxygen molecules. However, chemical structure of phenolic compounds and reaction environment are of high importance.

In our investigation the ethanolic solution of extracted phenolic compounds from camelina oil was added to bulk lipid system (commercially available safflower oil) and the influence of these phenolic compounds on hydroperoxide formation during safflower oil storage at 65 °C was elucidated. As can be observed on Figure 1 the *PV* of the control (oil without added antioxidants) increased from 3.8 to 14.9 mmol O_2/kg in the first six days, meanwhile *PV* of the safflower with added extract reached 11.3 mmol O_2/kg in the same period. However, after 13 days i.e. at high *PV*s, the difference between the control and oil with added extract was reduced, what shows that extract obtained from camelina oil inhibited peroxidation more

effectively at the early stages of lipid peroxidation process. The same observation has been published previously (Deiana et al., 2002).

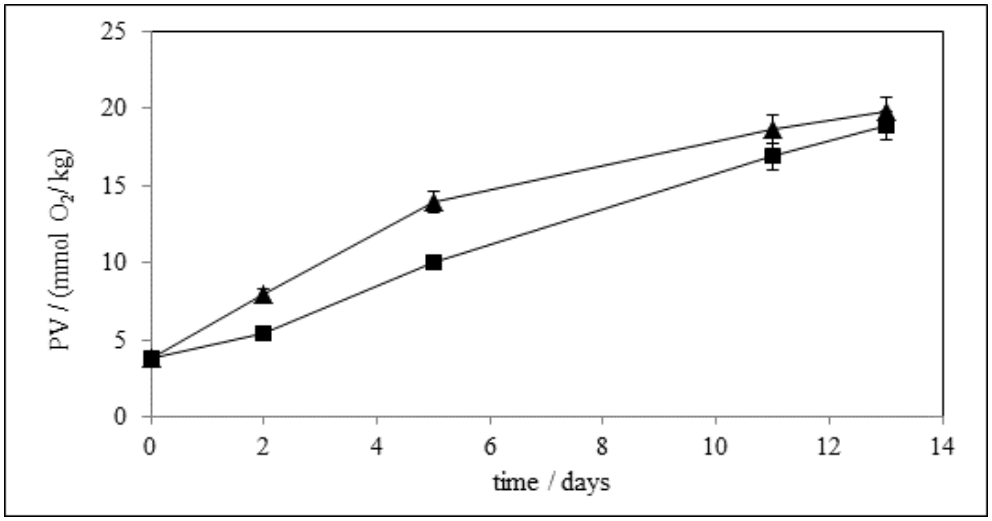

Figure 1: *Dependence of PV of safflower oil on time of storage at 65 °C in the dark; (▲-control lipid system; ■- lipid system with camelina oil extract). Values are means of three determinations ± standard deviation.*

Phenolic compounds suppress lipid peroxidation mostly by scavenging radicals. The inactivation of free radicals is achieved through donation of hydrogen to a free radical and hence its transformation to unreactive species (Zamora and Hidalgo, 2016). In one of our previous studies (Abramovič et al., 2007) we have clearly shown how the content of natively present (not added) phenolic compounds in vegetable oil linearly decreased with increased *PV* during incubation at elevated temperatures (50 °C and 65 °C). In the same investigation it was observed that the rate of degradation with increased *PV* was much more profound at 65 °C than at 50 °C. When phenolic compound donates phenolic hydrogen to radical it oxidizes to quinone, which is not effective as radical scavengers. Further, there is also possibility for the formation of dimers of phenolic compounds among which some are antioxidatively active, but others are not. As a consequence, the antioxidant capacity of phenolic compounds in oil decreases as lipid peroxidation increases.

The formation of primary oxidation products formed during storage of safflower oil at 65°C was assessed also through determination of conjugated dienes. Lipids containing methylene-interrupted dienes or polyenes show a shift in their double bond position during oxidation. The resulting conjugated dienes, which relate to the production of hydroperoxides, exhibit intense absorption at 234 nm. The formation of conjugated dienes in control and in safflower oil containing

antioxidants as a function of storage time is presented on Figure 2. In the first 5 days, no significant difference in the formation of conjugated dienes between the control sample and the samples treated with extract was observed. At day 0 slightly higher *E* (1 %, 1 cm) value for control than for safflower with added extract was determined. According to Koski et al. (2002) who investigated antioxidant activity of another oil extract from *Brassica* family (i.e. rapeseed) in methyl linoleate at 100 ppm level, higher value was not due to initial higher content of hydroperoxides. It was attributed to the presence of other compounds, such as sinapic acid and its derivatives (Figure 3) with conjugated double bonds absorbing at 234 nm. Differences between the control and oil with added extract became more evident as storage time progressed. After 11 days incubation significantly higher contents of conjugated dienes were observed for control than for safflower oil with added extract which succeeded to inhibit lipid peroxidation for almost 20 %.

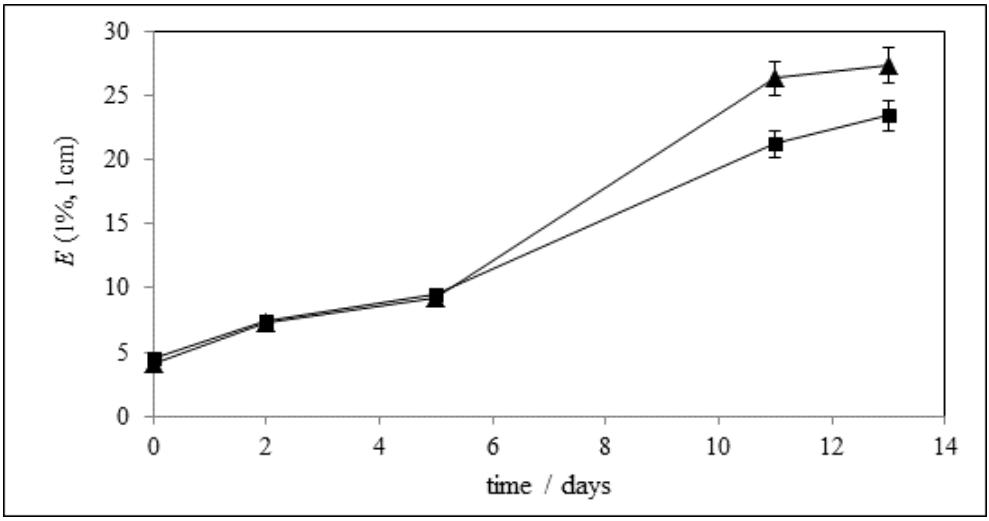

Figure 2: *Dependence of E (1 %, 1 cm) at 234 nm of safflower oil on time of storage at 65 °C in the dark; (▲- control lipid system; ■- lipid system with camelina oil extract). Values are means of three determinations ± standard deviation.*

As we can see on Figure 2, at the end of the storage the concentration of conjugated dienes in control sample tend to decline. At later phases of lipid oxidation conjugated diene hydroperoxides are expected to decompose to secondary products. The results are in agreement with those reported by Peña-Ramos and Xiong (2003).

Considerable body of research has been dedicated to oxidative stability of vegetable oils and the efficiency of various extracts in prevention of lipid peroxidation has been extensively analyzed. However, this is the first report on antioxidant activity of camelina oil phenolic extract studied through Rancimat test. The Rancimat test determines the induction time of samples by measuring the increase in the volatile acids released from the oxidizing oil exposed to elevated temperatures and air flow. The concentration of these degradation products, which are transferred into distilled water, is assessed by measuring the conductivity. Longer *IT* indicates higher oxidative stability of lipid system and suggests stronger antioxidant activity of the added antioxidants. The Rancimat test is a commonly used procedure in the food industry to examine the oxidative stability of edible oils and for prediction of their shelf life. Gordon and Mursi (1994) reported for rapeseed oil that *IT* of 1 h determined at 100 °C was equivalent to 2 days storage at 20 °C. Maszewska (2018) found that the time of incubation at 63 °C at which selected *PV* was achieved was highly correlated to *IT* determined in Rancimat at 120 °C.

In our study the protective factor of camelina oil extract on the susceptibility to oxidation in Rancimat of safflower oil expressed as percentage extension of the *IT* amounted to 10 %. For safflower oil without added extract the *IT* was 12.3 h. During incubation at temperature used in Rancimat (110 °C) the transformation of phenolic compounds occurs more rapidly than at 65 °C which was the temperature of storage conducted in the previous part of this study. At 110 °C some of phenolic acids most probably underwent to so-called thermal decarboxylation and are transformed into their more volatile counterparts (Castada et al., 2020). The main phenolic compound in camelina oil is sinapic acid (Terpinc et al. 2012). As already published (Terpinc et al., 2011), during heat treatment, its decarboxylation product, 4-vinylsyringol (Figure 3), an important antioxidant, is formed. However, the content of this volatile compound, named also canolol, was decreased for 80 % when the oil was exposed to high temperatures (Koski et al., 2003; Mikołajczak et al., 2019). In this respect, next to evaporation of 4-vinylsyringol, its transformation to dimer, phenylindane (Figure 3), was proposed (Harbaum-Piayda et al., 2010).

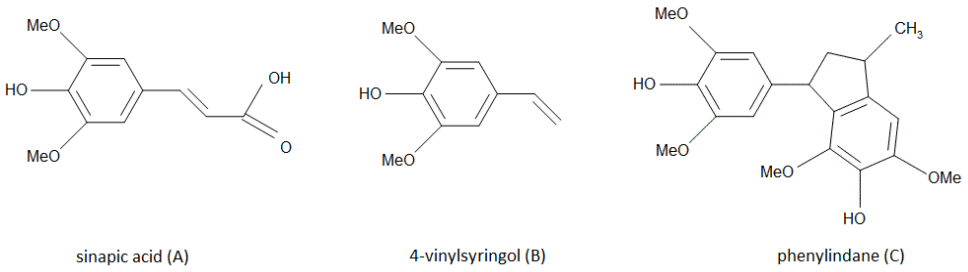

Figure 3: *Structures of sinapic acid (A), 4-vinylsyringol (B) and phenylindane (C).*

In bulk lipid system, beside free radicals scavenging capability of phenolic compounds the polar paradox should be considered. The polar paradox states that lipophilic antioxidants (e.g. 4-vinylsyringol) are more effective in oil-in-water emulsions, while polar antioxidants (e.g. sinapic acid) are more effective in bulk lipid systems as confirmed in our previous study (Martinović et al., 2019). In this study after 20 days of incubation at 25 °C sinapic acid and 4-vinylsyringol added to lipid system at concentration 2 mmol/kg succeeded to supress UV induced conjugated diene formation for 33 % and 9 %, respectively.

4 CONCLUSION

Data presented in this study showed that phenolics obtained from camelina oil were able to decrease the formation of primary oxidation products in bulk lipid system stored at accelerated conditions (65 °C). Furthermore, the oxidative resistance of model lipid system (measured by Rancimat at 110 °C) was improved in the presence of extract of camelina oil. These findings suggest that the phenolic compounds extract from camelina oil has protective effect on the oxidative stability of lipid system.

5 REFERENCES

Abramovič H., Abram V. Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil. Food Technology and Biotechnology. 2005; 43(1): 63-70.

Abramovič H., Butinar B., Nikolič V. Changes occuring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. Food Chemistry. 2007; 104: 903-909.

AOAC. Official methods of analysis (16th ed.), AOAC International, Gaithersburg.1999.

Castada H.Z., Sun Z., Barringer S.A., Huang, S. Thermal degradation of *p*-hydroxybenzoic acid in macadamia nut oil, olive oil, and corn oil. Journal of the American Oil Chemists' Society. 2020; 97: 289-300.

Chaiyasit W., Elias R. J., McClements D. J., Decker E. A. Role of Physical Structures in Bulk Oils on Lipid Oxidation. Critical Reviews in Food Science and Nutrition. 2007; 47: 299-317.Deiana M., Rosa A., Cao C. F., Pirisi F. M., Bandino G., Dessì M. A. Novel approach to study oxidative stability of extra virgin olive oils: Importance of α-tocopherol concentration. Journal of Agricultural and Food Chemistry. 2002; 50: 4342-4346.

Gordon M.H. , Mursi E. A comparison of oil stability based on the Metrohm Rancimat with storage at 20 °C. Journal of the American Oil Chemists' Society. 1994; 71: 649-651.

Gutfinger T. Polyphenols in olive oils. Journal of the American Oil Chemists Society. 1981; 58: 966-968.

Hrastar R., Gams Petrišič M., Ogrinc N., Košir I. J. Fatty acid and stable carbon isotope characterization of *Camelina sativa* oil: implications for authentication. Journal of Agricultural and Food Chemistry. 2009; 57: 579-585.

Hrastar R., Abramovič H., Košir I. J. *In situ* quality characterization of *Camelina sativa* landrace from Koroška, Slovenia. European Journal of Lipid Science and Technology. 2012; 114: 343-351.

Harbaum-Piayda B., Oehlke K., Sönnichsen F. D., Zacchi P., Eggers R., Schwarz K. New polyphenolic compounds in commercial deodistillate and rapeseed oils. Food Chemistry. 2010; 123: 607-615.

IUPAC. Standard methods and recommended practices of the American Oil Chemists' Society, 4th ed., Champaign, IL: AOCS Press. 1987.

Jankowski K.J., Sokólski M., Kordan B. Camelina: Yield and quality response to nitrogen and sulfur fertilization in Poland. Industrial Crops and Products. 2019; 141: 111776.

Koski A., Psomiadou E., Tsimidou M., Hopia A., Kefalas P., Wähälä K., Heinonen M. Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. European Food Research and Technolology. 2002; 214: 294-298.

Koski A., Pekkarinen S., Hopia A., Wahala K., Heinonen M. Processing of rapeseed oil: Effects on sinapic acid derivative content and oxidative stability. European Food Research and Technology. 2003; 217: 110-114.

Läubli M. W., Bruttel P. A., Schalch, E. A modern method of determining the oxidative stability of fats and oils. International Food Marketing and Technology.1988; 1: 16-18.

Martinović N., Poklar Ulrich N., Abramovič H. Sinapic acid and its derivatives increase oxidative stability in different model lipid systems. European Journal of Lipid Science and Technology. 2019; 121(4): 1-10.

Maszewska M., Florowska A., Dłu˙zewska E., Wroniak M., Marciniak-Lukasiak K., Żbikowska A. Oxidative stability of selected edible oils. Molecules. 2018; 23(7), 1746.

Mikołajczak N., Tańska M., Konopka I.. Impact of the addition of 4-vinyl-derivatives of ferulic and sinapic acids on retention of fatty acids and terpenoids in cold-pressed rapeseed and flaxseed oils during the induction period of oxidation. Food Chemistry. 2019; 278: 119-126.

Peña-Ramos E.A., Xiong Y.L. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Science. 2003; 64: 259–263.

Schwarz K., Huang S.-W., German J. B., Tiersch B., Hartmann J., Frankel, E. N. Activities of Antioxidants Are Affected by Colloidal Properties of Oil-in-Water and Water-in-Oil Emulsions and Bulk Oils. Journal of Agricultural and Food Chemistry. 2000; 48, 4874-4882.

Terpinc, P., Polak, T., Poklar Ulrih, N., Abramovič, H. Effect of heat treatment (*Camelina sativa*) seeds on the antioxidant potential of their extracts. Journal of Agricultural and Food Chemistry. 2011; 59: 8639-8645.

Terpinc P., Polak T., Makuc D., Poklar Ulrih N., Abramovič H. The occurrence and characterisation of phenolic compounds in *Camelina sativa* seed, cake and oil. Food Chemistry. 2012; 131: 580-589.

Zamora R. and Hidalgo F. J. The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. Trends in Food Science & Technology. 2016; 54, 165-174.