Lipid Oxidation of Fat Blends Modified by Monoacylglycerol

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Abstract

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Model dispersions of fat blends (FBs) with monoacylglycerols (MAG) of saturated fatty acids with different lengths of the acyl chain (MAG10–MAG18) and 1-octadecenoylglycerol and without MAG (as blank) were prepared. We find out the influence of the addition of monoacylglycerol on oxidation of the fat dispersion. Trihexadecanoylglycerol (tripalmitoylglycerol – TAG48) was used as the dispersive phase and soybean oil was used as the dispersive medium. Primary (conjugated diens) and volatile secondary (by SPME in connection with GC-MS) lipid oxidation products and oil stability index (OSI) were measured during autoxidation of the fat blends in storage conditions. MAGs with a shorter (or the same) acyl chain length (MAG10–MAG16) than the acyl chain length of the structured fat (TAG48) arrange tightly on the interface oil/crystals of structured fat, thus prevent lipid oxidation.

Keywords: conjugated diens; secondary oxidation products; oil stability index – OSI; structured fat; trihexadecanoylglycerol; tripalmitoylglycerol

Oxidation of lipids is one of the major reactions resulting in the decrease of food quality and acceptability, because it reduces the nutritional value and generates rancidity, causing undesirable flavours. Primary oxidation products are hydroperoxides which have no effect on flavour quality of foods. Hydroperoxides are unstable being decomposed to secondary oxidation products, a complex mixture of volatile (aldehydes, methyl ketones, hydrocarbons) and non-volatile compounds under storage temperatures. The extent of oxidation, formation of oxidation products, and oxidative stability are primarily dependent on the degree of unsaturation of the fatty acids present in triacylglycerols and on the structural differences beween the various triacylglycerols. Double bonds and methylene groups near to double bonds in unsaturated fatty acids are the active sites for free radicals formation and oxidation reaction is subsequently started (Shahidi 1998; Choe & Min 2006; Laguerre *et al.* 2007).

Lipid oxidation in oils and in emulsions of oil-in-water (O/W) type has been well described in many publications (Velasco & Dobarganes 2002; Anwar *et al.* 2003; Osborn & Akoh 2004; Beltran *et al.* 2005; Haiyan *et al.* 2007). Nevertheless the information on the oxidation of lipids in emulsions of water-in-oil (W/O) type or dispersive systems like fat blends is not sufficient (Frankel 2001; Pokorná *et al.* 2004).

Oxidation and oxidative stability of lipids in model fat blends–fat dispersions were studied in this work. The simple fat dispersion consists of fat crystals and/or their clusters which create three-dimensional network as the dispersive phase and

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liquid oil as the dispersive medium (Narine & Marangoni 1999). High melting fat crystals such as fully hydrogenated fats can exhibit gel-like behaviour in fat blends. Three-dimensional fat crystal network then exhibits the properties of organogel (Higaki *et al.* 2004). Liquid triacylglycerols closed in three-dimensional network are oxidised. The network itself can make a specific barrier against oxygen diffusion and adsorbed emulsifiers can intensify this barrier (Spěváčková *et al.* 2009). It has been found out that monoacylglycerols retarded lipid oxidation in margarine emulsion, a system of three phases (two limitation miscibility liquids and solid phase) (Pokorná *et al.* 2004). In margarine emulsions the emulsifier is adsorbed not only on the interfaces emulsion/air and water/oil but also on the interface liquid lipid/solid lipid of the fat blend (Garti *et al.* 1998). In the shortenings the emulsifier can be accumulated on the interfaces fat blend/air and liquid lipid/solid lipid. Emulsifiers such as monoacylglycerol can improve their physicochemical properties in two main ways. The first function of an emulsifier is to enable the formation of two distinct phases (formation of a stable pseudo-homogenous state) and the second one is to modify the behaviour of continuous oil phase (Martini & Herrera 2008).

This issue has been studied in a simple system of fat dispersions with fully hydrogenated zero-erucic rapeseed oil (containing more then 90 % (w/w) of stearic acid) as a structured fat. Positive barrier effect of 1-hexadecanoylglycerol was determined in these fat blends (Spěváčková *et al.* 2009). The aim of this paper was to study the influence of monoacylglycerol emulsifiers (with naturally occurring fatty acids) on lipid oxidation of model fat blends (FBs) with trihexadecanoylglycerol (TAG48).

MATERIAL AND METHODS

Chemicals. Glycerol (purity ≥ 99.5%; Sigma-Aldrich, Darmstadt, Germany), hexadecanoyl chloride (palmitoyl chloride, purity ≥ 98%; Sigma-Aldrich, Darmstadt, Germany), pyridine (purity ≥ 99.8%; Fluka, Germany), chloroform (purity ≥ 99.5%; Lach-Ner, Brno, Czech Republic), 3-dimethylamino-1-propylamine (purity ≥ 98%; Fluka, Germany), hydrochloric acid (purity ≥ 35%; Lach-Ner, Brno, Czech Republic), calcium chloride (purity ≥ 96 %; Lach-Ner, Brno, Czech Republic), *n*-hexane (purity ≥ 99 %, Penta, Prague, Czech Republic).

Synthesis of trihexadecanoylglycerol (tripalmitoylglycerol – TAG48). TAG48 was synthesised by the reaction of glycerol with hexadecanoyl chloride by the modified method (Sidhu & Daubert 1947; Täufel *et al.* 1960). The reaction was stopped after 12 h by the addition of 3-dimethylamino-propylamin. The reaction mixture was rinsed with 5% (w/w) aqueous solution of hydrochloric acid and subsequently with distilled water to neutral pH, and then the organic fraction was dried over calcium chloride. The chloroform was evaporated and the product was purified by crystallisation in *n*-hexane (two-times). The purity of TAG48 was higher than 98 % by HTGC/FID analysis while the contents of FFA, MAG and DAG were negligible.

FBs composition. 19.7 % (w/w) structured fat – TAG48; 79.7% w/w liquid oil – half-refined soybean oil (Setuza a.s., Ústí n. L., Czech Republic) (characterisation and fatty acids composition in Table 1); 0.6% w/w monoacylglycerol emulsifier. The emulsifiers 1-decanoylglycerol (MAG10), 1-dodecanoylglycerol (MAG12), 1-tetradecanoylglycerol (MAG14), 1-hexadecanoylglycerol (MAG16), 1-octadecanoylglycerol (MAG18), 1-octadecenoylglycerol (MAG18:1) were synthesised (Martin 1953). Purity of all MAGs was higher than 95%.

FB without emulsifier (WE) was prepared as a blank sample (20% w/w structured fat and 80% w/w liquid oil).

FBs preparation and storage conditions. FBs were prepared in duplicate vessels in argon atmosphere

Table 1. Characterisation and fatty acids composition [% w/w] of soybean oil

AV (mg KOH/g)	0.12
PV (meq. act. O/kg)	1.03
IV (g I_2/100 g)	130.4
CD (% w/w)	0.350
IP Rancimat (h)	4.06
IP Oxidograph (h)	3.60
C16:0	10.9
C18:0	4.5
C18:1	23.4
C18:2	53.0
C18:3	7.1
Others	1.1

AV – anisidine value; PV – peroxide value; IV – iodine value; CD – conjugated diens content; IP – induction period

(Piska *et al.* 2006). The mixture of structured fat, liquid oil and emulsifier was heated to 80°C. The liquid mixture was emulsified during 5 min with an emulsifying stirrer (1000 min^{-1}). FBs were stored over a long term in the darkness under an oxygen atmosphere (oxygen atmosphere was > 84% (v/v) – sensor CellOx 325, WTW; Weilheim, Germany) at temperature 20 ± 1°C.

Determination of conjugated diens content. Conjugated diens (CD) content was determined by the standard IUPAC 2.206 method (IUPAC 1987) every two weeks.

Measurement of oxidative stability. The oxidative stability of FBs was determined by the instruments Rancimat 743 (Metrohm Ltd, Herisau, Switzerland) and Oxidograph$^{\text{TM}}$ (Mikrolab Aarhus A/S, Højbjerg, Denmark) every two weeks.

Rancimat procedure: 2.5 g of FB in the reaction vessel were bubbled through with air flow of 20 l/h at a temperature of 120 ± 1.6°C (Oil Stability Index method – OSI; AOCS Official Method Cd12b-92 1997).

Oxidograph procedure: 5 g of FB in the reaction vessel under oxygen atmosphere (filling of vessel by oxygen: flow of O_2 was 2–4 l/min for 20 s) were heated to 110°C (Velasco *et al.* 2004; Nogala-Kalucka et al. 2005).

Analyses of volatile compounds. The volatile compounds were extracted by the headspace-solid phase extraction (HS-SPME) and determined by gas chromatograph Agilent Technologies 7890 (Agilent Technologies, Santa Clara, USA) with mass detector Agilent Technologies 5975C (Agilent Technologies, Sant Clara, USA) (GC/MS). GC/MS with HP-5MS column (max. temperature 325°C, 0.25 mm × 30 m, thickness of stationary phase 0.25 µm; J&W Scientific, St. Louis, USA) was used.

HS-SPME procedure: SPME manual holder (Supelco, Bellefonte, USA) with 50/30 µm DVB/CAR/PDMS 1 cm fiber (Supelco, Bellefonte, USA). The fiber was conditioned for 60 min at 270°C as recommended by the manufacturer. 5.6 g of FB were capped with PTFE/Silicone septa in 15 ml vial (Supelco, Bellefonte, USA). The fiber was exposed to the sample headspace for 20 min at 20°C.

HS-SPME/GC/MS: The fiber was desorbed for 7 min in the splitless injection port at 240°C. The column temperature program was 40°C for 7 min and was followed by anincreased to 200°C at 5 K per minute. The flow rate of helium carrier gas was 0.9 ml/minute. The temperatures of the ion source and quadrupole were 230 and 150°C, respectively. The mass spectrometer operated in the electron impact (EI) ionisation mode at 70 eV and mass spectral data were acquired in the range 25–400 amu. The scan and selected ion monitoring (SIM) were used as the data acquisition mode, the chosen ions were 44 and 56 for hexanal and 44 and 58 for pentanal. The identification of the compounds was carried out by comparing their spectra with those of the NIST Mass Spectra library.

Calibration curves were prepared for two major volatile products – pentanal (valeraldehyde, purity ≥ 97%) and hexanal (purity ≥ 98%; both Fluka, Taufkirchen, Germany). These aldehydes were analysed in oil without volatile compounds. The calibration data were obtained under the same analysis conditions as were those for the samples and they are listed in Table 2.

Calculation of the area under the curve (AUC). The area under the curve for the individual MAGs was calculated from: the dependence of the CD content (expressed in % w/w) on storage time (expressed in weeks) – AUC_{CD}, the dependence of the oxidative stability (expressed in h) on storage time ($\text{AUC}_{\text{Rancimat}}$, $\text{AUC}_{\text{Oxidograph}}$), the dependence of the pentanal/hexanal concentration (expressed in µmol/kg) on storage time ($\text{AUC}_{\text{pentanal}}$, $\text{AUC}_{\text{hexanal}}$) by trapezoid method. The AUC correlated with the effects of MAGs on the formation of CD, pentanal, hexanal, and oxidation stability during storage.

Statistical analysis. The results were expressed as mean ± standard deviation (the corresponding error bars were displayed in the graphical plots). Statistical data analysis was performed by using Student's *t*-test at a confidence level of 95% ($P = 0.05$).

Table 2. The calibration data of standard volatile compounds by HS-SPME/GC/MS

Parameter	Hexanal	Pentanal
Average of retention time t_R (min)	9.123	5.169
Calibration curve	$y = 205.1x$	$y = 2838.8x$
Correlation coefficient (R^2)	0.9760	0.9626
Range of concentration of standard solution (µmol/kg)	1.14–1022.74	1.16–51.32

RESULTS AND DISCUSSION

The influence was studied of monoacylglycerol emulsifier type on the extent of oxidation and oxidative stability in model fat blends. Oxidative changes of model fat blends were investigated and the selected oxidation products were determined over 16-week period.

Primary oxidation products

Primary oxidation products are hydroperoxides. The polyunsaturated fatty acids have pentadiene system of double bonds. During oxidation, the conjugated system of double bonds is formed due to the rearrangement of double bonds (energy of he system is lowered). Thus, conjugated diens content correlates with the peroxide value (Shahidi 1998; Spěváčková *et al.* 2009).

The conjugated diens content increases with time (Figure 1). The areas under the curves of the dependence of CD content on time are presented in Table 3. Fat blends with MAG18, MAG18:1 and fat blend without emulsifier had the highest contents of conjugated diens and were statistically significantly different from the other fat blends.

Table 3. The area under the curve of dependence of CD content on time (AUC_{CD})

	AUC_{CD}		AUC_{CD}
MAG10	6.812 ± 0.037	MAG16	6.892 ± 0.079
MAG12	6.268 ± 0.074	MAG18	8.449 ± 0.057
MAG14	6.524 ± 0.005	MAG18:1	9.634 ± 0.236
WE	9.458 ± 0.238		

Oxidative stability

Oxidative stability is an important factor for the prediction of fat/oil quality. It is represented as the resistance to oxidation under defined conditions and is expressed as the induction period (IP). The induction period was determined by two different methods: in the first one, Oil Stability Index method was measured by Rancimat, and in the second one, the decrease of oxygen pressure in the headspace was monitored by Oxidograph (Velasco *et al.* 2004; Nogala-Kalucka *et al.* 2005).

Oxidative stability of FBs decreased during storage (Figure 2). The results obtained from Rancimat and Oxidograph are related, the IP determined by Rancimat instrument is higher than IP determined by Oxidograph instrument. The minimum oxidative stability was determined in fat blends with MAG18, MAG18:1 and in the fat blend without emulsifier by both methods (Table 4). These three FBs were statistically significantly different from the other FBs. Oxidative stability of FBs with MAG10, MAG12, MAG14, and MAG16 was generally higher. The differences in oxidative stability were not significant in this group.

Volatile secondary oxidation products

Volatile oxidation products were detected and the major products were quantified by HS-SPME/

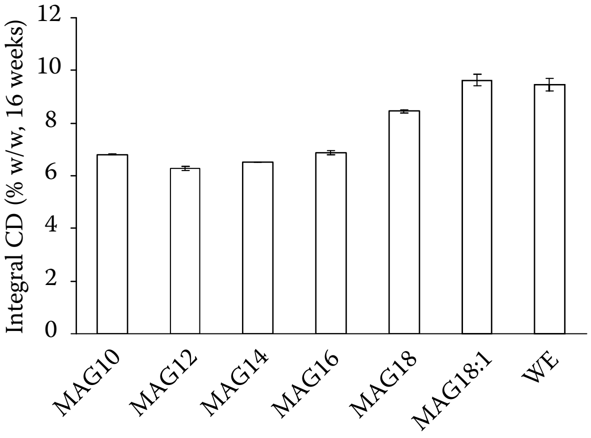

Figure 1. Conjugated diens content of FBs with MAGs during storage time

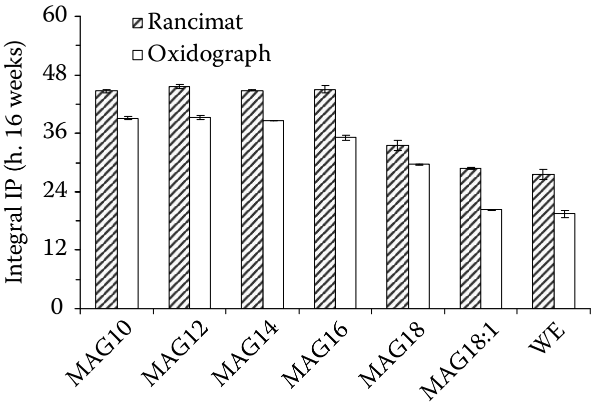

Figure 2. Oxidative stability of FBs with MAGs determined by Rancimat (A) and Oxidograph instrument (B) during storage time

Table 4. The area under the curve of dependence of $IP_{Rancimat}$ and $IP_{Oxidograph}$ on time ($AUC_{Rancimat}$, $AUC_{Oxidograph}$)

	$AUC_{Rancimat}$	$AUC_{Oxidograph}$
MAG10	44.71 ± 0.33	39.10 ± 0.26
MAG12	45.59 ± 0.46	39.20 ± 0.36
MAG14	44.83 ± 0.06	38.60 ± 0.05
MAG16	45.02 ± 0.77	35.10 ± 0.62
MAG18	33.49 ± 1.11	29.60 ± 0.09
MAG18:1	28.85 ± 0.16	20.30 ± 0.13
WE	27.57 ± 0.98	19.40 ± 0.79

Table 5. The area under the curve of dependence of pentanal and hexanal concentration on time ($AUC_{pentanal}$, $AUC_{hexanal}$)

SPME	$AUC_{pentanal}$	$AUC_{hexanal}$
MAG10	182.73 ± 10.96	2233.96 ± 63.81
MAG12	121.51 ± 7.29	888.05 ± 26.64
MAG14	166.86 ± 8.34	1389.08 ± 64.75
MAG16	182.88 ± 14.63	1226.56 ± 79.81
MAG18	213.98 ± 12.84	1781.49 ± 83.47
MAG18:1	129.61 ± 6.48	1784.03 ± 103.27
WE	186.66 ± 13.07	3919.39 ± 75.39

GC/MS. The major volatile compounds, as we had expected, were hexanal and pentanal because the liquid oil in the fat blends was soybean oil with a high content of linoleic acid (Table 1). Hexanal is formed from (9*Z*, 11*E*)-13-hydroperoxyoctadeca-9, 11-dienoic acid and pentanal is formed from (9*Z*, 12*Z*)-14-hydroperoxyoctadeca-9, 12-dienoic acid. The formation of pentanal from ω-6 polyunsaturated fatty acids may be also explained by the hexanal decomposition (Frankel 1993). Pentanal and hexanal were quantified using the calibration curve. These two compounds were detected at the beginning of storage. The content of hexanal stighly increased during storage (Figure 3). The areas under the curves of the dependence of pentanal and hexanal concentration on time in fat blends are shown in Table 5. The lowest concentration of both aldehydes was determined in fat blends with MAG12, MAG14, and MAG16. FB with MAG10 gave rise to a higher content of hexanal. On the other hand, the highest concentration of aldehydes (especially hexanal) was detected in the fat blend without emulsifier (blank – WE). This fat blend was statistically significantly different from the other fat blends (Tables 5 and 6).

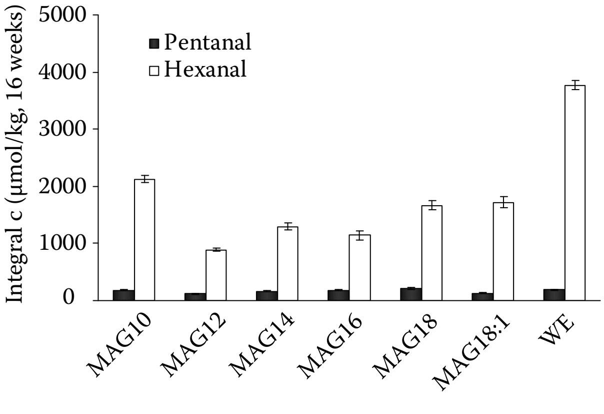

Figure 3. Hexanal concentration of FBs with MAGs determined by HS-SPME/GC/MS during storage time

CONCLUSION

The aim of this study was to find out the influence of MAG addition on the oxidation changes of model fat blends. A homological group was used of MAGs with saturated fatty acids (from MAG10 to

Table 6. Volatile secondary oxidation products of model fat blends by HS-SPME/GC/MS

FB with emulsifier	Volatile compounds				
MAG10	pentanal	hexanal	(*Z*)-2-heptenal	3,5-octadien-2-one	
MAG12	pentanal	hexanal	3,5-octadien-2-one		
MAG14	pentanal	hexanal	(*Z*)-2-heptenal	3,5-octadien-2-one	
MAG16	pentanal	hexanal	3,5-octadien-2-one		
MAG18	pentanal	hexanal	(*Z*)-2-heptenal	2,4-heptadienal	3,5-octadien-2-one
MAG18:1	pentanal	hexanal	(*Z*)-2-heptenal	2,4-heptadienal	3,5-octadien-2-one
WE	pentanal 2-octenal	hexanal 3,5-octadien-2-one	(*Z*)-2-heptenal 2,4-undecadien-1-ol	2,4-heptadienal 2-nonenal	2,4-octadiene (*E*,*E*)-2,4-decadienal

MAG18) and MAG18:1 (as a member of a homological group of MAG with unsaturated fatty acids).

From the point of view of primary oxidation products, volatile aldehydes (representing secondary oxidation products) and oxidative stability it is possible to split up the obtained results in three groups. The first one, FB without MAG (blank – WE) oxidises to the greatest extent in all monitored parameters. The second group, FBs with 1-octadecenoylglycerol (MAG18:1), 1-octadecanoylglycerol (MAG18), is a typical case of longer acyl chain length of MAG than the acyl chain length of the structured fat used. Primary oxidation products are formed to the greatest extent and their decomposition to secondary oxidation products is minimal in this group of FBs. Oxidative stability of this group of FBs is insufficient. The third group, FBs with MAG10, MAG12, MAG14, and MAG16 is a typical case of a shorter or the same acyl chain length of MAG as compared to the acyl chain length of the structured fat used. FBs of this group oxidise to the minimal extent in all monitored parameters.

There is a hypothesis that monoacylglycerols as surface active agents adsorb on the interfaces fat blend/air and oil/crystals of structured fat. MAGs with a shorter (MAG10–MAG14) or the same acyl chain length (MAG16) arrange on the interface oil/crystals of structured fat tightly and therefore the oxygen diffusion through the fat blend layer is retarded. MAGs with 18 carbon atoms in the acyl chain or with 18 carbon atoms and *cis* double bond in the acyl chain arrange on the interface oil/crystals of structured fat not so tightly and therefore the oxygen diffusion through the fat blend layer is accelerated.

In a previous paper (Spěváčková *et al.* 2009), where fat blends with different structured fats were studied, similar results were found out.

References

Anwar F., Bhanger M.I., Kazi T.G. (2003): Relationship between rancimat and active oxygen method values at varying temperatures for several oils and fats. Journal of the American Oil Chemists‘ Society, **80**: 151–155.

AOCS Official Method Cd12b–92 (1997): Oil Stability Index (OSI). Sampling and Analysis of Commercial Fats and Oils. American Oil Chemists’ Society, Champain.

Beltran G., Aguilera M.P., Gordon M.H. (2005): Solid phase microextraction of volatile oxidation compounds in oil-in-water emulsions. Food Chemistry, **92**: 401–406.

Choe E., Min D.B. (2006): Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety, **5**: 169–186.

Frankel E.N. (1993): Formation of headspace volatiles by thermal decomposition of oxidized fish oils *vs.* oxidized vegetable oils. Journal of the American Oil Chemists‘ Society, **70**: 767–772.

Frankel E.N. (2001): Interfacial lipid oxidation and antioxidation. Journal of Oleo Science, **50**: 387–391.

Garti N., Binyamin H., Aserin A. (1998): Stabilization of water-in-oil emulsions by submicrocrystalline α-form fat particles. Journal of the American Oil Chemists’ Society, **75**: 1825–1831.

Haiyan Z., Bedgood Jr. D.R., Bishop A.G., Prenzler P.D., Roberds K. (2007): Endogenous biophenol, fatty acid and volatile profiles of selected oils. Food Chemistry, **100**: 1544–1551.

Higaki K., Koyano T., Hachiya I., Sato K. (2004): In situ optical observations of microstructure of β-fat gel made of binary mixtures of high-melting and low-melting fats. Food Research International, **37**: 2-10.

IUPAC (1987): Determination of di- and tri-unsaturated fatty acids by ultraviolet spectrophotometry. Standard Methods for the Analysis of Oil, Fats and Derivaties 2.206. 7th Ed. Blackwell Sci. Publication, Oxford.

Laguerre M., Lecomte J., Villeneuve P. (2007): Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in Lipid Research, **46**: 244–282.

Martin J.B. (1953): Preparation of saturated and unsaturated symmetrical monoglycerides. Journal of the American Oil Chemists‘ Society, **75**: 5482–5483.

Martini S., Herrera M.L. (2008): Physical properties of shortenings with low-trans fatty acids as affected by emulsifiers and storage conditions. European Journal of Lipid Science and Technology, **110**: 172–182.

Narine S.S., Marangoni A.G. (1999): Relating structure of fat crystal network to mechanical properties: a review. Food Research International, **32**: 227–248.

Nogala-Kalucka M., Korczak J., Dratwia M., Szczapa E., Siger A., Buchowski M. (2005): Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chemistry, **93**: 227–235.

Osborn H.T., Akoh C.C. (2004): Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. Food Chemistry, **84**: 451–456.

Piska I., Zárubová M., Loužecký T., Karami H., Filip V. (2006): Properties and crystallization of fat blends. Journal of Food Engineering, **77**, 433–438.

Pokorná I., Filip V., Šmidrkal J. (2004): Lipid oxidation in margarine emulsions. Czech Journal of Food Sciences, **22**: 140–143.

Shahidi F. (1998): Indicators for evaluation of lipid oxidation and off-flavor development in food. Food Flavors: Formation, Analysis and Packaging Influences, **40**: 55–68.

Sidhu S.S., Daubert B. F. (1947): X-Ray investigation of glycerides. IV. Diffraction analyses of synthetic triacid triglycerides. Journal of the American Chemical Society, **69**: 1451–1453.

Spěváčková V., Hrádková I., Ebrtová M., Filip V., Tesařová M. (2009): Lipid oxidation in dispersion systems with monoacylglycerols. Czech Journal of Food Sciences, **27** (Special Issue): S169–S172.

Täufel K., Franzke C., Dietze P. (1960): Darstellung von einigen zweisäurigen asymmetrischen triglyceriden. Fette, Seifen, Anstrichnittel, **62**: 926–928.

Velasco J., Andersen M.L., Skibsted L.H. (2004): Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. Food Chemistry, **85**: 623–632.

Velasco J., Dobarganes C. (2002): Oxidative stability of virgin olive oil. European Journal of Lipid Science and Technology, **104**: 661–676.

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