Oxidative Changes of Vegetable Oils during Microwave Heating

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Abstract

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The oxidative stabilities of pork lard, sunflower, zero-erucic rapeseed, peanut and high-oleic peanut oils were tested under microwave heating conditions. Vegetable oils and lard were heated in a microwave oven for up to 40 min between 25°C and 200°C. The peroxide value, the contents of conjugated dienoic and trienoic acids, and polymers were used as markers of lipid degradation. Sunflower oil was found the least stable oil because of a high polyenoic acid content and a low content of γ-tocopherol. Rapeseed oil was more stable because of a lower polyenoic acid content and a high γ-tocopherol level. Conventional peanut oil was relatively stable, but substantially less stable than high-oleic peanut oil. Pork lard and high-oleic peanut oil formed only low levels of polymers due to a low polyenoic acid content.

Keywords: microwave heating; oxidation; polymerisation; pork lard; vegetable oils

Microwave heating is a modern and widely used method for food preparation. Changes of fats and oils have been intensively studied as the temperature of fat and oils can substantially increase during the operation. The temperature of the fat phase increases twice as fast during the microwave heating than the temperature of water or water-containing foods under comparable conditions (Barringer 1995). High levels of oxidation were observed in model systems containing sunflower, soybean, and virgin olive oils during microwave heating, while lipid hydrolysis was only moderate (Caponio *et al.* 2001). Oxidative changes depend on the unsaturation degree of oils, therefore, rapeseed oil was found more stable than soybean or safflower oils because of its lower polyenoic acid content (Yoshida 1993). Free fatty acids present in oil enhance the oxidation (Yoshida 1993), which may be important if cold-pressed unrefined oils are used in microwave treated systems. Similar results were obtained on comparison of sunflower, soybean, and peanut oils, and a mixture of soybean and peanut oils (Hassanein *et al.* 2003). Five vegetable oils were treated in a microwave oven, and their stabilities were tested under Rancimat conditions at 100°C; the stability decreased by 29–65% of the original value, and the tocopherol content by 5–61% during 15 min heating (Marinova *et al.* 2001). Changes of olive oil due to microwave heating were only small because of the low contents of polyenoic acids and relatively high contents of natural antioxidants, especially in virgin oils (Cossignani *et al.* 1998). Soybean oil, sesame oil, butter, and margarine were microwave heated for 5–20 min; tocopherol losses were only about 20% during the first 5 min,

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but naturally, they increased more rapidly during further heating (Kim & Joo 1995). The effects of synthetic antioxidants and citric acid were tested in experiments using microwave heating of canola oil (Vieira & Regitano-D'Arce 2001). At relatively low temperatures, i.e. during the first 10 min, no changes were observed, but pronounced changes followed afterwards (Vieira & Regitano-D'Arce 2001). The power of the microwave oven had a great effect on the changes of corn and soybean oils (Tan *et al.* 2001).

Oxidative changes were greater during microwave heating of sunflower, high-oleic sunflower, and olive oils and lard than in a conventional oven under comparable conditions (Albi *et al.* 1997a, b). Deep frying of cottonseed and hydrogenated palm oils was compared with microwave heating, and a higher oxidation degree and lower sensory scores were observed in the case of microwave heating (Farag *et al.* 1992). During microwave frying of potato chips, oxidative changes were higher than in a conventional oven (Regulska-Ilow *et al.* 1997). Similar results were obtained in experiments with soybean oil and palmolein (Zhang *et al.* 2000).

Another important factor was the content of natural antioxidants, which were only tocopherols in sunflower and rapeseed oils. Tocopherols, especially α-tocopherol, are decomposed during microwave heating to a pronounced degree (Yoshida *et al.* 1991). On the contrary, δ-tocopherol was found more efficient than other tocopherols (Yoshida *et al.* 1993). The resistance of vegetable oils against oxidation under microwave heating conditions was proportional to the tocopherol content (Marinova *et al.* 2001). In pork lard, only traces of α-tocopherol were present. Vegetable oils contained mixtures of tocopherols, which increased their resistance against oxidation. Highly unsaturated sunflower oil was oxidised at a high rate because of a high linoleic acid content and because the relatively less active α-tocopherol was mostly present. While tocopherols and linoleic acid were rather stable during microwave heating of intact sunflower seeds (Yoshida *et al.* 2002), oxidation proceeded fast in refined oil. On the contrary, the major tocopherol in rapeseed oil was the relatively active γ-tocopherol, and the total content of tocopherols was also high. Rapeseed oil and Virginia peanut oil were relatively stable because of the medium polyenoic acid content. Peanuts were found stable on microwave roasting (Yoshida *et al.* 2003), but significant oxidative deterioration was observed after 20 min microwave heating of peanut oil. No substantial difference in the fatty acid composition was found during the microwave heating for 5–20 min (Kim & Yoo 1995). It means that not more than about 1% polyenoic acid was destroyed, as a change in the fatty acid composition would be otherwise observed. On the contrary, small changes of essential fatty acids and the formation of epoxides were observed in other experiments with several vegetable oils (Hassanein *et al.* 2003). In the case that natural antioxidants of oils used for microwave heating have no satisfactory effect, it is possible to add other permitted antioxidants (Gertz 2004). Sekretár *et al.* (1997, 1998) reported interesting positive results after the application of natural antioxidants.

In this study, we compared oxidative and polymerisation changes of several vegetable oils of different unsaturation, and of lard during the microwave heating.

MATERIAL AND METHODS

Material. Dry rendered pork lard was not refined. Double-zero winter rapeseed oil and traditional sunflower oil were produced from Czech crops using conventional plant technology, and physical refining. Virginia peanuts were of Chinese production, high-oleic SunOleic peanuts (O'Keefe *et al.* 1993; Knauft *et al.* 1993) were produced in Japan. Peanuts were crushed and extracted with hexane (Parkányiová *et al.* 2000; Sakurai *et al.* 1999). The fatty acid and tocopherol compositions of lipids used in our experiments are shown in Table 1.

Analytical methods. The following standard analytical methods of IUPAC (Paquot & Hautfenne 1987) were used: the acid value (Method 2.206) was determined by titration, and the results are expressed in mg KOH/g fat; the peroxide value (Method 2.501) was determined iodometrically, and the results are expressed in meq/kg (= 1/2 mmol per kg, which is not an allowed unit, but this unit is recommended after IUPAC); conjugated dienoic and trienoic acids were determined by ultraviolet spectrophotometry, and the results were converted into % using the coefficients suggested by IUPAC (Method 2.206); the polar fraction was determined using method 2.507; the fatty acid composition was determined by gas chromatography (Method

Table 1. Composition of lard and vegetable oils used for the microwave heating

Component	Pork lard	Oil			
		sunflower	rapeseed	Virginia peanut	SunOleic peanut
Main fatty acids (% of peak area)					
Palmitic	28	4	2	12	6
Stearic	11	3	2	3	2
C_{20}–C_{22} saturated	1	1	2	5	5
Monoenoic	52	34	49	48	82
Dienoic	6	57	34	30	3
Trienoic	trace	trace	8	trace	trace
Eicosenoic	–	–	1.5	1	2
Trans-unsaturated	trace	0.8	0.5	0.8	0.3
Peroxide value (meq/kg)	1.2	0.2	0.58	1.5	0.05
Acid value (mg/g)	0.6	0.06	0.07	1.5	1.4
Conjugated dienes (% m/m)	trace	0.6	0.4	2.1	1.4
Polar compounds (% m/m)	1.2	0.4	0.8	2.1	1.7
Tocopherols (mg/kg)					
Tocopherol α	5	373	294	162	201
Tocopherol β+γ	—	34	392	134	214
Tocopherol δ	—	14	12	6	11
Total tocopherols	5	418	698	303	426

2.302) after conversion into the respective methyl esters (Method 2.301). The results are expressed in % of areas of methyl ester peaks.

Determination of polymers. For the determination of polymeric products, 100 µl of the sample were dried with anhydrous sodium sulfate and dissolved in 3 ml of the mobile phase. The polymer content was determined using the high-performance size-exclusion chromatography (HPSEC). The modified procedure proposed by Réblová (1999) was used. The micropump LCP 4000.1 (Ecom, Prague, CR), a 300 mm × 75 mm column packed with PL-gel Mixed E (3 µm), and a refractometric detector HP 1047 A (Laboratorní přístroje, a. s., Prague, CR) were used. Tetrahydrofuran (HPLC grade, E. Merck, Darmstadt, D) was used as the mobile phase; the flow rate was 0.6 ml/min. The signal was processed by the chromatographic station CSW 1.7 (Data Apex, Prague, CR) using the method of inner standardisation. The repeatability was 0.11%, the detection limit 0.14%, and the lower detection limit 0.35%.

Microwave heating. The sample (25.0 g ± 1.0 g) was weighed into a 100 ml beaker (inner diameter d = 50 mm), and the beaker was placed in the center of the rotating plate of the microwave oven MT-243/UKM 347, 2450 MHz, maximum power 1000 W (manufactured by Whirlpool, UK). The samples were heated at the input of 500 W for 3, 6, 9, 12, 15, 20, 25, 30, and 40 min. The course of temperature changes is evident from the example of rapeseed oil, given in Figure 1. Changes of the temperature during the microwave heating of other oils and of lard were almost the same (the temperatures between duplicates of the same oils differed by less than 1.5%, in the case of different oils, differences did not exceed 2%). Therefore, changes of temperatures in individual cases are not given here.

Two independent series of experiments were carried out under the same conditions. At the defined intervals, the samples were taken out of the oven, cooled rapidly, and stored at –18°C till the analysis. Separate samples were used for different heating times; the sample was used for analyses, and was not returned back to the oven. Temperature changes were recorded using a double-channel optical temperature system (Nortech Fibronic, Inc., Canada), monitoring the temperatures between –40°C and 250°C in 30 s intervals

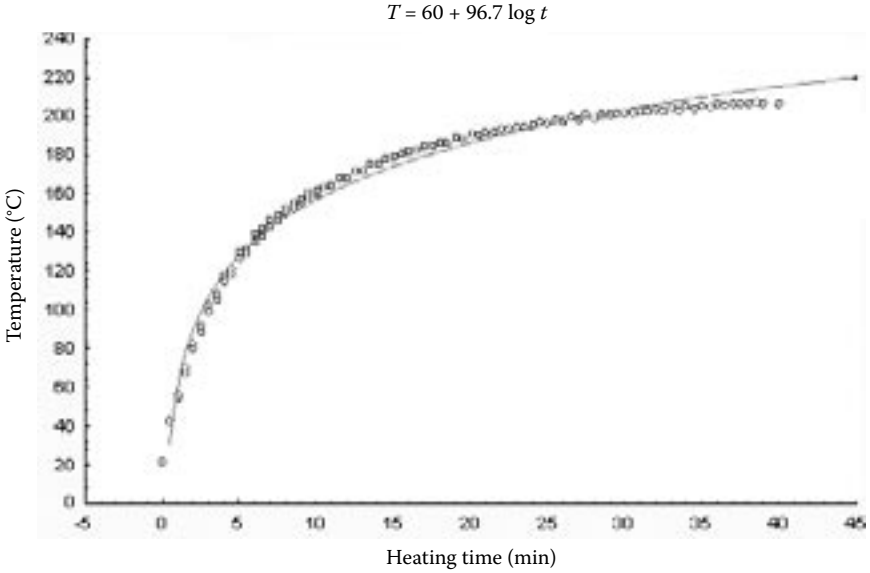


Figure 1. Course of temperature increase during microwave heating of rapeseed oil

Circles = measured values; full line = curves approximated using the logarithmic expression given above

at two predetermined places. Arithmetic means of the duplicates were calculated.

Statistical analysis. Standard deviations and statistical significance of differences between the mean values were calculated using one-way ANOVA. Statistical significance between two experimental series was calculated using the sign test. Linear, semilogarithmic and doublelogarithmic regressions and correlation coefficients were calculated using the programme HP STATISTICA, Version 7. The probability level of $P = 0.95$ was used. The validation of the analytical methods (including z-values) was performed after the recommendation of The German Society of Fat Science, 6th Interlaboratory Comparison Test (DGF, Frankfurt a/M, 2000).

RESULTS AND DISCUSSION

General considerations

The procedure of microwave heating simulated the process of deep frying. During 20 min, most often used for food frying in a microwave oven, the temperature of lard or oils increased to nearly 200°C, and further increase was only negligible (Figure 1). The temperature of oil rises at a substantially higher rate than in the case of water, due to its lower permitivity and specific heat (Prosetya & Datta 1991). The course of temperature changes was nearly the same in all cases.

Differences between two duplicate experiments were determined in preliminary experiments, when only the initial value and the value of a sample after 40 min heating were analysed. The average difference between duplicates of peroxide value determination was equal to 0.7 meq/kg in the case of two reference samples used for the validation (the z values of 0.55 and –0.58, respectively). The acceptable difference was 0.4 meq/kg ($\alpha = 0.05$). Analogous study of the repeatability of the determination of conjugated dienes was 0.04% (m/m); ($\alpha = 0.05$). In the case of polymeric lipids, the average difference between duplicates was 0.03% at the polymer level of 0.66%, and 0.05% at the polymer level of 8.13%; the z-scores found were –0.33 and 0.66, respectively. The respective standard deviations were 0.02% and 0.04% in low-polymer and medium-polymer reference samples, respectively. As the differences between the duplicates were only very low, not exceeding the levels obtained in the case of the reference samples, only the means of duplicates are shown in the experimental part.

Oxidative degradation of fats and oils during microwave heating depends on the polyenoic acid content and on the content of natural antioxidants. From the standpoint of unsaturated fatty acid composition, lard was highly saturated, containing oleic acid as the major unsaturated fatty acid, and only 6% linoleic acid, the only polyenoic acid. Vegetable oils contained higher levels of linoleic acid (and a small amount of linolenic acid in the case of rapeseed oil) than lard, which increased their sensitivity against oxidative degradation.

We were interested mainly in the contents of oxidation and polymerisation products which are important from the standpoint of food safety. The acid value changes were negligible (less than

0.2 mg/g between the original sample and the last one) under the experimental conditions of this study in preliminary experiments. This observation is in agreement with the literature data (Tan *et al.* 2001). Therefore, the acid value was not included as a marker of deteriorative changes in our experiments. In our preliminary experiments, changes of the fatty acid composition were found very low between the original sample and the last one (40 min microwave heating), even in the case of linoleic and linolenic acids. The results with the original and the heated samples did not differ significantly, and were not due to systematic changes. The difference did not exceed twice the standard deviation of the analysis. They were not statistically significant ($\alpha = 0.05$). On the contrary, degradative changes were very pronounced in the case of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) under similar microwave heating conditions (Bednářová 2004). Therefore, it was not found necessary to determine the time course of the changes in the fatty acid composition under our experimental conditions. The changes of tocopherols during microwave heating of edible oils were studied by Yoshida *et al.* (1991, 1993, 2002). Therefore, it was considered as unnecessary to repeat their experiments under our heating conditions.

Changes of hydroperoxides content during microwave heating

The changes of the peroxide value during microwave heating are shown in Table 2. Differences between the duplicate series of experiments were not statistically significant. The peroxide value is a measure of the content of hydroperoxides, which are primary oxidation products. The peroxide value rose at high rate only after the end of the induction period, i.e. after 6 min heating in the case of lard, when the temperature reached about 150°C. Lard has a low polyenoic acid content so that hydroperoxides in lard, being mostly monoenoic products, are relatively stable. Therefore, the peroxide value continued to increase till the end of heating. Contrary to lard, the linoleic acid content is high in

Table 2. Changes of the peroxide value (meq/kg) during microwave heating

	Heating time (min)	Pork lard	Oil			
			sunflower	rapeseed	Virginia peanut	SunOleic peanut
Series 1	0	0.41	0.30	0.25	2.7	3.4
	3	0.41	0.35	0.29	2.8	3.4
	6	0.48	1.14	0.36	3.4	3.4
	9	1.52	7.39	2.53	3.0	2.5
	12	2.45	9.54	5.48	2.7	2.6
	15	4.09	10.59	6.61	1.3	1.4
	20	4.85	10.64	6.77	1.3	1.0
	25	6.13	9.69	6.38	1.3	1.0
	30	6.73	10.00	6.04	1.5	0.9
	40	7.13	6.00	4.61	2.0	1.0
Series 2	0	0.41	0.30	0.25	2.7	3.4
	3	0.41	0.49	0.29	2.9	3.8
	6	0.49	5.65	0.34	3.6	3.6
	9	1.75	6.69	2.65	2.6	2.2
	12	3.81	9.49	5.84	2.5	2.6
	15	4.49	8.97	6.32	1.3	1.3
	20	5.04	7.36	7.04	1.1	0.8
	25	5.68	6.30	6.82	1.5	1.0
	30	6.88	6.45	6.18	1.7	1.1
	40	7.66	7.58	5.06	2.4	0.8

sunflower oil. This is the reason why the peroxide value started to increase rapidly, already after 3 min heating. However, polyenoic hydroperoxides are rather unstable at temperatures over 60°C, the hydroperoxide maximum is soon reached (after 15 min), and thus the peroxide value started to decrease again after prolonged heating because the rate of hydroperoxide decomposition exceeded that of hydroperoxide formation. The same behaviour was observed with rapeseed oil, only the peroxide maximum was lower than in sunflower oil because of a lower concentration of polyenoic fatty acids. Another high-polyenoic acid oil used for food consumption – corn oil, showed a similar behaviour on heating (Vieira & Regilano-D'Arce 1999). Sekretár *et al.* (2000) determined the changes of the peroxide value during the microwave heating of lard, rapeseed, and sunflower oils (i.e. the same oil as used in the present experiments). They obtained higher peroxide values, which can be explained by a lower temperature (155°C) used in their experiments, in comparison with 180–200°C used in our experiments. The decomposition of polyenoic hydroperoxides proceeds very rapidly with increasing reaction temperature. Oxygen dissolved in the lipid fraction was exhausted less rapidly at 155°C than in our experiments at 200°C, and the oxygen consumed in oxidation reactions was more easily supplied by diffusion from the atmosphere. Both peanut oils used in these experiments were not refined, therefore, the original peroxide value was relatively high, but it changed only a little because of the lower content of linoleic acid (especially in the case of SunOleic oil), the absence of linolenic acid, and the presence of phospholipids which accelerate the hydroperoxide decomposition (Pokorný & Schmidt 2001). On the contrary, the acid value was higher than that in refined oils, and free fatty acids increase the rate of oxidation (Yoshida *et al.* 1992).

Changes of conjugated double bond systems

During the formation of linoleic acid hydroperoxides, the pentadienoic double bond system becomes conjugated. The content of conjugated dienes is

Table 3. Changes of the content of conjugated dienes (% w/w) during microwave heating

	Heating time (min)	Pork lard	Oil			
			sunflower	rapeseed	Virginia peanut	SunOleic peanut
Series 1	0	0.18	0.31	0.25	0.51	0.03
	3	0.18	0.31	0.25	0.52	0.03
	6	0.20	0.32	0.26	0.55	0.04
	9	0.20	0.38	0.27	0.53	0.04
	12	0.21	0.41	0.32	0.57	0.05
	15	0.21	0.35	0.34	0.58	0.06
	20	0.21	0.38	0.37	0.56	0.06
	25	0.23	0.42	0.39	0.58	0.09
	30	0.27	0.49	0.43	0.59	0.08
	40	0.29	0.68	0.52	0.66	0.07
Series 2	0	0.18	0.31	0.25	0.51	0.03
	3	0.18	0.31	0.31	0.51	0.03
	6	0.18	0.39	0.31	0.53	0.04
	9	0.19	0.38	0.32	0.53	0.06
	12	0.20	0.34	0.36	0.51	0.03
	15	0.20	0.38	0.37	0.56	0.07
	20	0.20	0.42	0.40	0.56	0.09
	25	0.24	0.55	0.41	0.56	0.10
	30	0.30	0.55	0.47	0.57	0.12
	40	0.31	0.57	0.56	0.70	0.09

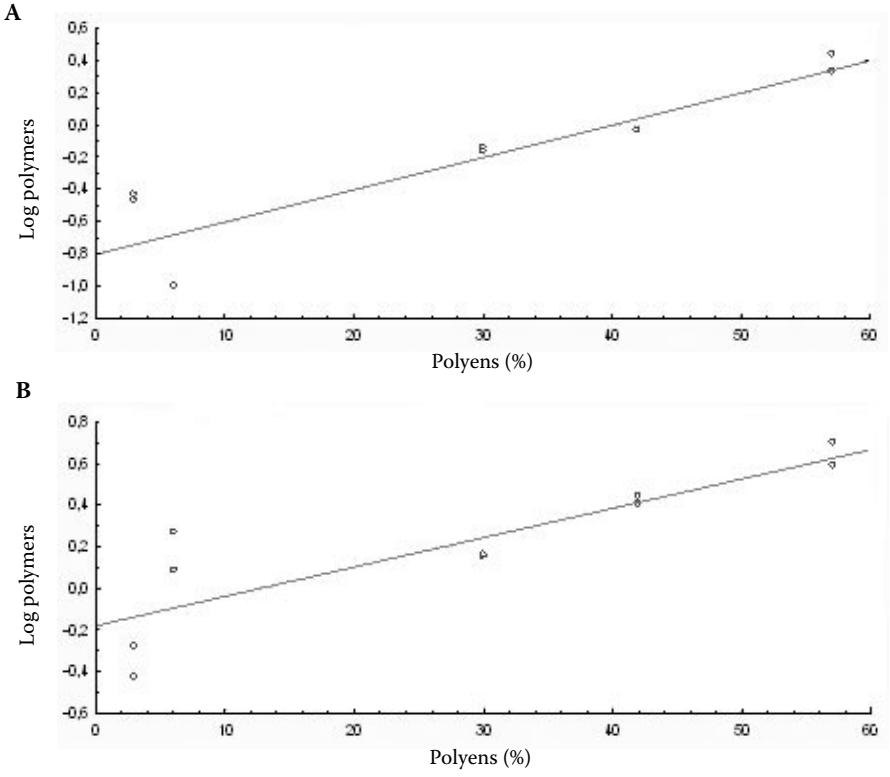
shown in Table 3. Differences between the duplicate series of experiments were not statistically significant. Still more pronounced increase of the dienoic conjugation than that found in our experiments was observed in the case of microwave heating of corn oil (Vieira & Regitano-D'Arce 1999). Conjugated trienes were present only in traces below 0.05% (e.g. the conjugated trienoic derivatives in Virginia peanut oil were equal to 0.0044 at the beginning of heating, they rose to 0.0195% after 20 min and to 0.0375% after 40 min) so that their content is not shown in Table 3, even though the content rose very slightly during the heating. These experimental results are in agreement with the expectation because the formation of conjugated -C=C-C=C-C=C- double bond system is improbable even in the case of linolenic acid oxidation. The –C=C-C=C-C=O systems are more probable, and could interfere with the measurement at 268 nm.

Conjugated dienes were formed parallelly with hydroperoxide formation. About 1% of conjugated dienes corresponds to the peroxide value of 295 meq/kg (Pokorný & Mareš 1958). As evident from Table 3, the increase of the content of conjugated dienes was higher than the respective increase of hydroperoxides. Some hydroperoxides obviously decompose without losing the conjugated double bond system, e.g. by decomposition into 2, 4-alkadienals (Kamal-Eldin 2003). Some alkadienals are typical components of fried flavour (Pokorný 1989).

Low peroxide values and dienoic conjugation could explain the negligible changes of essential fatty acids observed during the microwave heating as reported in the literature (Cossignani *et al.* 1998; Kim & Joo 1995; Hassanein *et al.* 2003), which is in agreement with the results of our preliminary experiments.

Changes of polymers

Very important degradation products of frying and similarly heated oils are polymers. Their changes are presented in Table 4. Differences in the polymer content between the duplicate series were not significant. Fresh lard contains no polymers, and their formation on heating is only slow because of low polyenoic acid content, but at heating temperature of about 200°C even monoenoic and saturated fatty acids polymerise. The same was valid for non-deodorised peanut oils. Virginia peanut oil containing 33% linoleic acid polymerised quicklier than SunOleic peanut oil containing only 3% linoleic acid. Slow polymerisation of high-oleic peanut oil is in agreement with



Contents of polyenoic acids
HOP = high oleic peanut oil (3% polyenoic acids)
COP = conventional peanut oil (30% polyenoic acids)
RPO = rapeseed oil (42% polyenoic acids)
SFO = sunflower oil (57% polyenoic acids)

Figure 2. Relationship between the intial polyenoic fatty acid content and the logarithm of polymer content during microwave heating for 20 min (A) and 40 min (B)

Table 4. Changes of the content of polymeric products (% total peak area) during microwave heating

	Heating time (min)	Oil				
		Pork lard	sunflower	rapeseed	Virginia peanut	SunOleic peanut
Series 1	0	trace	1.25	0.35	0.18	0.18
	3	trace	1.25	0.40	0.18	0.19
	6	trace	1.33	0.40	0.24	0.24
	9	trace	1.53	0.45	0.25	0.23
	12	trace	1.69	0.47	0.30	0.24
	15	trace	1.72	0.61	0.41	0.28
	20	trace	2.14	0.95	0.68	0.34
	25	0.35	2.60	1.28	0.83	0.37
	30	1.07	2.99	1.89	0.98	0.35
	40	1.29	5.00	2.75	1.41	0.38
Series 2	0	trace	1.25	0.35	0.18	0.16
	3	trace	1.25	0.35	0.22	0.20
	6	trace	1.44	0.39	0.27	0.27
	9	trace	1.60	0.38	0.26	0.26
	12	trace	1.71	0.56	0.33	0.26
	15	trace	1.87	0.60	0.42	0.32
	20	trace	2.71	0.94	0.72	0.37
	25	0.35	3.10	1.28	0.87	0.38
	30	1.09	3.71	1.59	1.03	0.40
	40	1.87	3.85	2.55	1.43	0.42

its better resistance against oxidation both in bulk oil (Parkányiová *et al.* 2000) and in emulsion (Zainuddin *et al.* 2004).

Rapeseed and sunflower oils were refined and deodorised at high temperatures, when traces of polymers are always formed. Therefore, small amounts of polymers were found even in fresh oils. Tocopherols prevented the oxidation and polymerisation at low temperatures so that polymers were formed at a higher rate only at temperatures above 150°C. Polymerisation was more rapid in sunflower oil because of the high linoleic acid content, especially during the microwave heating to 200°C. The degree of 10–12% polymers considered as limiting in frying oils (Márquez-Ruiz & Dobarganes 1996; Vitrac *et al.* 2003) was not obtained even after 40 min heating.

The degree of polymerisation was proportional to the logarithm of polyenoic acid content after both 20 min (Figure 2A) and 40 min heating (Figure 2B). It means that, at a higher polyenoic acid content, the oxidative degradation is faster, and therefore, the polymer formation is also faster. No kinetic conclusions can be made from the logarithmic dependence because the polymerisation reaction is a secondary reaction with no direct dependence on the polyenoic acid content. In the case of sunflower and rapeseed oils, slow polymerisation started from the beginning of heating when the temperature exceeded 100°C. In the case of lard, which is less unsaturated than rapeseed or sunflower oils, the content of free radicals was also lower in heated lard in comparison with edible oils. Therefore, the recombination of free radicals in lard, resulting in the formation of dimers or higher oligomers, was less probable than the formation of monomeric or cleavage products resulting from other secondary reactions (Pokorný 1987). A lag period was observed at the beginning of microwave heating. The polymerisation could proceed at a measurable rate only when sufficient amounts of hydroperoxides and their decomposition products had accumulated.

CONCLUSIONS

Pork lard and vegetable oils were oxidised only moderately during short microwave heating (up to 20 min), but more intensive oxidation was observed after 40 min. Hydroperoxides formed slowly at the beginning of heating, before the temperature increased to about 150°C, even in the case of polyenoic oils, as a short lag period was observed. At temperatures of about 200°C, however, the polyenoic hydroperoxides decomposed, and only lard showed a slow increase. Conjugated dienes were better markers of oxidative deterioration than hydroperoxides, especially at later stages of microwave heating. The content of conjugated trienoic acids was too low to be significant. Polymers were slowly formed in polyenoic vegetable oils, when the temperature increased to 200°C, but their content was higher only in sunflower and rapeseed oils; they were very scarce in lard and in high-oleic sunflower oil. The oxidative state and polymerisation of lipids after microwave heating under conditions commonly used in culinary technology leads to the conclusion that, in most cases, deteriorative changes are only small so that lipids after heating of up to 40 min may be considered as suitable for human consumption.

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