Microwave and Electrical Oven Heating are Having Different Effects on Antioxidant/oxidative Stress Parameters of Vegetable Oils

Potential impact on human health

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Oxidative modified lipid products are associated with various pathological conditions like cardiovascular disease and different types of cancers. Cooking can accelerate the oxidative degradation of vegetable oils. The aim of our study was to assess lipid oxidation parameters together with antioxidant parameters in oils heated in microwave and electric oven in order to better estimate the degree of oils deterioration in these conditions. Conjugated dienes and thiobarbituric reactive substances were used to asses lipids oxidation processes and TROLOX Equivalent Antioxidant Activity (TEAC) and vitamin E concentrations were determined as a way to asses antioxidant consumption under heating treatments of oils. Sunflower, corn, soybean, palm anda commercial mixed oil containing sunflower, grape, flaxseed and rice oil were tested in our experiments at different exposure times. The oxidative modifications are higher for oils containing higher concentrations of unsaturated fatty acids and lower antioxidant activity. There was no remarkable difference in terms of oxidative damages between microwave and electric oven heating.

Keywords: vegetable oils, total antioxidant capacity, vitamin E, lipid peroxidation, microwave heating, convection heating

Lipids are essential components for human health. They have structural,energetical, metabolic and signalling roles[1].

Vegetable oils are important sources for lipids in human diet. Triglycerides constitute more then 98% of lipid components of vegetable oils while the rest is represented by fat soluble vitamins like vitamine E and beta carotene, minerals, phytosterols, polyphenols. The relative quantities of these compounds depends on the crops cultivation conditions(soil and climate),as well as by the oil extraction and refining processes, storage time and storage conditions [2, 3]. The high content of unsaturated fatty acids makes vegetable oils extremely sensitive to oxidative alterations mainly by generation of lipid peroxides. Lipid peroxides and conjugated dienes formation-primary oxidation products- is usually followed by their decomposition to yield potential toxic aldehydes, ketones and volatile compounds(secondary oxidation products). Oxidation of lipids is decreasing the nutritional value of the oil. [4] Oxidative modified lipid products- advanced lipoxidation end products(ALE) and lipid peroxides are associated with various pathological conditions like cardiovascular disease and different types of cancers[5].

The degree of oxidation depends on the quality and quantity of: compounds with potential antioxidant actions like polyphenols and lipid soluble vitamins; compounds with potential prooxidant actions like transition metals and solvent rests from extraction and refining processes; specific fatty acids composition of oils 4)cooking method.

Cooking can accelerate the oxidative degradation of vegetable oils. It is already known that different cooking methods can differently impact the nutritional values of food[6][7].

Even oxidative processes takeing place under microwave or electric oven cooking have been studied extensively due to health concerns the results are still far from elucidating the complex situation of different types of oils used in different cultures[8][9]. This is why we have decided to study the oxidative modifications of 5 types of oils found on Romanian market and used by local population – sunflower oil, corn oil, soybean, palm and a mixed oil containing sunflower, grape, flaxseed and rice oil. The aim of our study was to asses lipid oxidation parameters together with antioxidant parameters in oils heated in microwave and electric oven in order to better estimate the degree of oils deterioration in these conditions because microwave and electric oven cooking are widespread used today in modern house holdings due to their efficiency.

Conjugated diens and thiobarbituric reactive(TBARS) substances were used to asses lipids oxidation processs and TROLOX Equivalent Antioxidant Activity (TEAC) and vitamine E concentrations were determined as a way to asses antioxidant consumption under heating treatments of oils.

Experimental part

Sample processing

Sunflower, corn, soybean, palm and mixed oil (containing sunflower, grape, flaxseed and rice oil) were purchased from a local supermarket in Bucharest, Romania. To simulate conventional times used in home cooking, different exposure times were tested, namely, 5, 10 and 15 min. For all samples, and for each exposure time, samples of 50 mL were individually heated in Erlenmeyer dishes in a domestic microwave oven (Maxwell) at maximum potency (1200 W) and in a convection oven (ARLI) (1300 W) and after each heating interval the temperature was taken using a metallic thermometer (BiTh 63 K). After cooling, the samples were either analyzed or transferred to Falcon tubes and stored at -80°C until analysis. TROLOX Equivalent Antioxidant Capacity (TEAC), lipid peroxides as thiobarbituric reactive substances (TBARS), oxidation index as conjugated dienes and vitamin

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E as ⟨-tocopherol were determined initially and in oils samples after 5, 10 and 15 min microwave (MW) heating at 2450 KHz or after convection heating at 250°C respectively.

Determination of TEAC

Total antioxidant activity (TEAC) was determined based on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay developed by Miller and Rice-Evans[10], with modifications[11]. A dilution of 1:10 (v:v) oil in n-hexane was used. The TEAC assay measures the relative abilities of antioxidants to scavenge the 2,22 -azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation ($ABTSx^+$) in comparison with the antioxidant potency of standard amounts of Trolox, the water soluble vitamin E analogue. The ABTS radical was generated from the interaction between ABTS and potassium persulfate. At the beginning of the analysis day, an ABTS - working solution was obtained by the dilution in ethanol of the stock solution. The working ABTS solution had an absorbance of 0.70 +/- 0.02 AU at 734 nm. The absorbance of the samples was read at 734 nm at exactly 1 minute against ethanol and n-hexane. The percentage inhibition of absorbance was calculated. A calibration curve using TROLOX 0.5-2.5 mM was constructed. The results were expressed as µmol eq. Trolox/l oil.

Determination of TBARS

The sample containing 100 µL oil, SDS 10%, BHT 2% and TBA 0.8% was incubated 60 min at 100° C. After exactly 60 min the reaction was stopped by cooling the tubes in an iced water bath. The pink MDA-TBA (malondialdehyde-thiobarbituric acid) adduct was placed in a 96-wellplate and absorbance of the organic layer was read at 532 nm. A calibration curve with 1,1,3,3-tetraethoxypropane (TEP) 0.5-4 mM was constructed.The results were expressed as µM MDA eq/L oil. The absorbance measured in this way can come from all preexisting MDA and lipid peroxides as well as any other substances that give rise to MDA or TBARS in the hot acid. BHT was added to the sample prior to assay to ensure that no lipid oxidation occurs during the assay procedure [12].

Conjugated dienes

The absorption of conjugated dienes, was followed spectrophotometrically (UV/Visible Perkin Elmer, U.K.) at 234 nm. The oil sample was diluted (1:50) with hexane (HPLC grade). An extinction coefficient of 29,000 mol/L was utilized to quantify the concentration of conjugated dienes formed during oxidation [13]. The oxidation index was calculated and expressed as mM/L oil using the formula:

Cox = Absorbance of sample /Conc. Oil*Extinction coef. *100% (1)

alfa-Tocopherol content

A sample volume of 0.1 mL of oil in n-hexane (1:10, v:v) was mixed in a test tube with 1 mL of reagent solution (H_2SO_4 0.6M, NaH_2PO_4 28mM, $(NH_4)_2MoO_4$ 4mM) and incubated at 37°C for 90 min with vigorous shaking. Absorbance at 695 nm was measured against the appropriate blank. A typical blank contained 1 mL of reagent solution and 0.1 mL of pure hexane, and it was incubated under the same conditions as the samples. A calibration curve was constructed using α-tocopherol acetate (0.5-5 mM) as standard. Results were expressed as mM/L oil [14].

Results and discussions

Vegetable oils composition

The content of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids(SFA), total fat(TF) and caloric value for 100 mL oil according with the manufacturer labels are listed in table1.

Commercial palm oil also contained salt and fiber (manufacturer claim).

Time dependent microwave versus convection heating influence on oils temperatures (fig. 1 and fig. 2)

As expected oils temperatures increased with increasing time exposure. There are no significant

per 100 mL	(KCAL)	PUFA (g)	MUFA (g)	SFA (g)	FAT (g)	SALT (g)	FIBER (g)
CORN oil	900	56	30	14	100	0	0
SOYBEAN oil	900	63	23	14	100	0	0
SUNFLOWER oil	900	55	34	11	100	0	0
MIXED oil	828	27	56	9	92	0	0
PALM oil	810			42	90	0.2	2.5

Table 1
VALUES OF PUFA, MUFA, SFA, FAT, SALT AND FIBER AS STATED ON THE MANUFACTURER LABELS

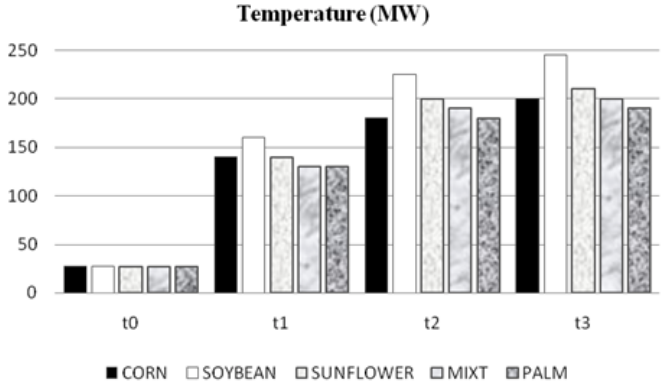

Fig. 1. Temperature values evolution in correlation with microwave exposure time and oil type (t0-room temperature, t1-5 min heating, t2-10 min heating, t3-15 min heating)

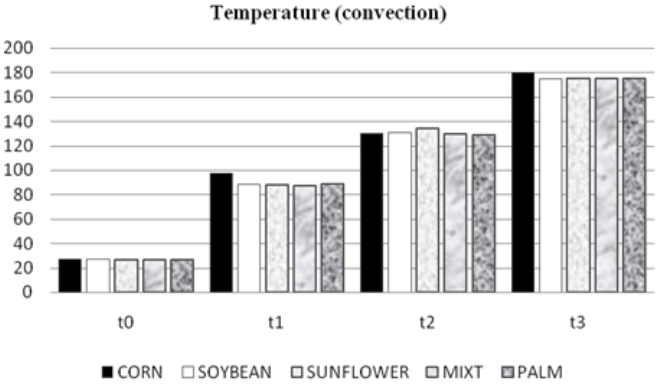

Fig. 2. Temperature values evolution in correlation with convection heating time and oil type (t0-room temperature, t1-5 min heating, t2-10 min heating, t3-15 min heating)

Table 2

EXPERIMENTAL VALUES OBTAINED FOR α– TOCOFEROL, ABTS, TBARS AND CONJUGATED DIENES FOR ALL FIVE OILS USED IN THE STUDY

Oxidative Marker		α – tocoferol (mM/L)		ABTS (mM eq trolox/L)		TBARS (uM/L)		Conjugated dienes (mM/L)	
Heating source		MW	Conv	MW	Conv	MW	Conv	MW	Conv
Corn oil	T0	22.04	22.04	9.92	9.92	19.28	19.28	9.51	9.51
	T1	12.00	20.38	6.57	8.09	11.55	30.02	9.52	10.05
	T2	10.69	17.20	5.05	8.03	30.35	25.65	14.34	12.79
	T3	9.74	16.50	4.62	6.98	20.50	19.17	16.54	17.60
Soybean oil	T0	16.11	16.11	9.56	9.56	19.17	19.17	7.99	7.99
	T1	12.48	15.70	7.32	8.41	30.02	22.08	7.94	9.63
	T2	10.63	15.35	5.73	8.25	66.17	28.31	12.15	11.90
	T3	7.06	11.40	4.22	7.44	83.41	20.62	17.13	19.50
Sunflower oil	T0	16.43	16.43	6.95	6.95	14.13	14.13	11.17	11.17
	T1	7.41	13.57	7.07	8.08	13.90	20.17	12.76	9.48
	T2	7.19	12.74	5.83	7.96	12.33	24.20	14.21	11.59
	T3	6.55	8.09	4.10	6.64	21.06	21.41	16.89	22.04
Mixed oil	T0	15.89	15.89	6.57	6.57	35.51	35.51	11.92	11.92
	T1	14.87	15.48	6.17	8.27	33.15	47.37	10.93	10.19
	T2	11.90	12.16	4.90	7.90	42.00	22.41	14.13	11.80
	T3	7.76	6.11	4.62	6.91	40.32	54.42	14.25	18.02
Palm oil (unhidrogenated)	T0	16.88	16.88	6.04	6.04	24.87	24.87	7.03	7.03
	T1	13.94	16.31	6.18	9.00	15.13	17.60	8.32	9.40
	T2	13.37	14.90	4.86	8.60	12.67	17.82	11.33	11.28
	T3	11.71	9.62	4.62	6.50	18.15	22.86	16.45	16.69

differences between oils temperatures when samples where heated in the electric oven. We can remark a small increase of corn oil temperature compared with the temperatures reached by the others oils tested (fig. 2).

The situation is completely different for microwave treatment. The temperatures increased differently for the different types of oils, corn oil having again the highest temperature at every time point (fig. 1).

Microwaves heated the oils in 15 min to temperatures over 200°C, while the convection oven only above 150°C. Also the temperatures reached by the oils were more uniform for the convection oven than for the microwave oven, most likely because microwaves are influencing all the molecules of the oils and the electric oven only transfers the heat by the convection of the heated air inside.

In the case of microwave heating is clear that the type of oil is influencing the temperature obtained.

Our results are different from the results obtained by Abd El-Moneim Mahmoud et al. [15] who observed lower temperatures for oils under microwave treatments. This can be explained by the different setted power and temperatures of the heating equipments. We used the highest power and temperatures available for our cooking ovens.

Vitamine E content, TEAC, conjugated diens and TBARS values obtained as a function of time, heating method and type of oil can be found in table 2.

Vitamin E content

The values obtained for vitamin E suggest a strong tendency to its degradation during both types of heat treatment. We could observe that α-tocopherol degradation is more pronounced in the case of exposure

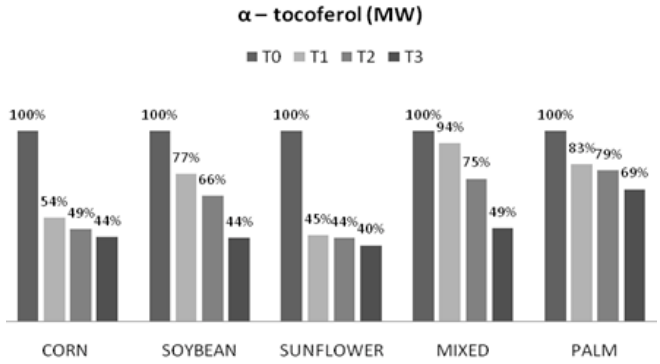

Fig. 3. Vitamin E % evolution in correlation with microwave exposure time and oil type

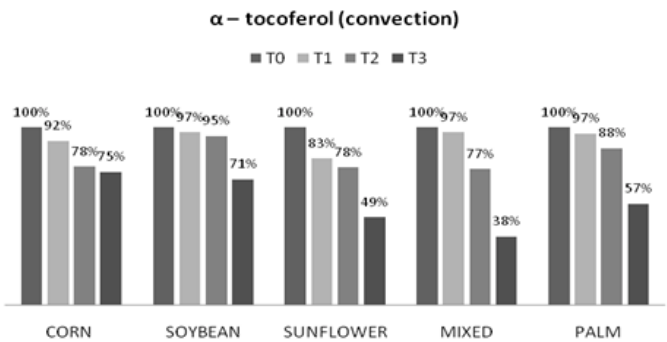

Fig. 4. Vitamin E% evolution in correlation with convection heating time and oil type

to microwave (fig. 3 and fig. 4). The highest content before microwave treatment was observed in corn oil (22.04 mM/L). The best retention of vitamin E after 5 min of MW heating was shown by the mixed oil (94%) followed by

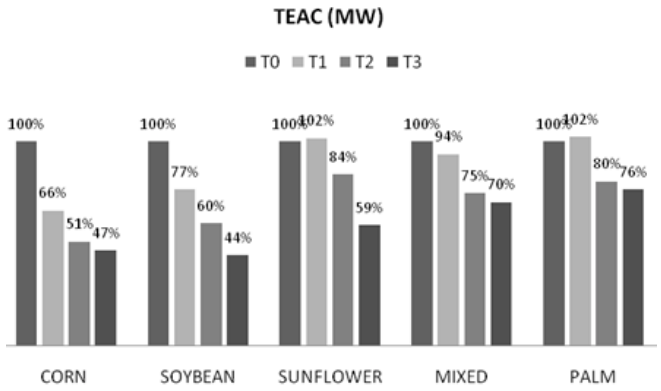

Fig. 5. TEAC content evolution in correlation with microwave exposure time and oil type

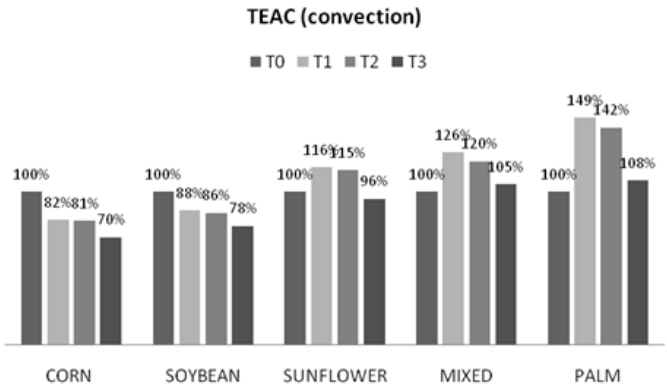

Fig. 6. TEAC content evolution in correlation with convection heating time and oil type

palm oil (83%), while the lowest was showed by sunflower oil (45%). After 10 and 15 min of microwave heating palm oil showed the highest retention in vitamin E (79% and 69%). The worst vitamin E percentage retention evolution could be observed for sunflower oil (down to 40%). The highest measured value for α-tocopherol after 15 min microwave treatment was for palm oil (11.71 mM/L). In the case of convection heating, the highest vitamin E retention after 5 min was observed for palm, soybean and mixed oil (97%). After 10 min of electric heating soybean oil showed a retention of 95% for vitamin E and after 15 min of heating corn oil still had 75% of the a-tocopherol initial quantity.

Our results are in agreement with several other studies found in literature [16].The higher reduction of vitamin E concentration in microwave heated samples can be explained by the fact that vitamin E has been reported to be unstable under microwave heating [17] . Alternatively, microwave heating may produce increased oxidative damage compared with convection heating and vitamin E could have been rapidly consumed in the attempt to stop lipid peroxidation. Interesting, palm oil having a high content of saturated fatty acids is having also the best retention percent of vitamin E after 15 min of microwave heating, while oils containing higher amounts of polyunsaturated fatty acids prone to oxidative damages are having lower retentions. This is sustaining the hypothesis of vitamin E consumption in order to lower lipids degradation.

TEAC evolution

Total antioxidant capacity showed a similar general evolution as α-tocopherol. Values for oil heated in the convection oven ranged from initial values around 10 mM eq. Trolox/L to about 6.5 mM eq. Trolox/L after 15 min of heating, while in the microwave oven the values decreased to 4 mM eq. Trolox/L.

In unheated oils, the highest total antioxidant capacity was determined for corn oil (9.92 mM eq. Trolox/L), followed closely by the soybean (9.56 mM eq. Trolox/L). After microwave heating for 5 and 10 min the highest value for TEAC was determined in the case of sunflower oil (102% and 84%) and after 15 min of MW treatment palm oil still had 76% of its antioxidant capacity.

20-30% higher values for TEAC were observed for electric oven heating compared to microwave heating. The highest values after 5, 10 and 15 min convection heating belong to palm oil (149, 142 and 108%).

In the case of MW heating, corn, soybean and mixed oil TEAC had the expected descending trend proportional with the duration of heating. Sunflower and palm oils had an increased antioxidant capacity after 5 min of heating, after which the values decreased.

Corn and soybean oil had a steady descending trend in antioxidant capacity content after heating in the electric oven. Sunflower, mixed and palm oil showed an increase in TEAC values after 5 min of convection heating and a descending trend afterwards, but the final values were very close to the initial ones.

The unexpected higher values obtained for total antioxidant capacity with increasing heating time were similar with those obtained in other study[18]. The increased values obtained can come from the contribution of polyphenols with high molecular weight released by hydrolysed complex lipids under heat treatment. The same study concluded that polyphenols present in oil can stabylise tocopherol under heat treatment. Interestingly, palm oil having the highest TEAC values in our experiment is also having the highest vitamin E retention during heating.

TBARS content

Thiobarbituric acid reactive substances (TBARS) highest initial value belonged to mixed oil (35.51 uM/L).This is not surprising takeing into account the high content of polyunsaturated acids present in this oil.

Remarkably, soybean oil showed an increase in TBARS of over 435% after 15 min of MW heating After convection heating for 5 min the highest increase was observed for corn oil (156%), at 10 min for sunflower oil (171%) and at 15 min for mixed oil (153%).

We could observe that in the case of TBARS each oil had a different evolution curve during heating, in most cases an ascendent one, but not always.

In the case of MW heating we could observe a decrease in TBARS values, followed in most cases by an increase after 10 and 15 min of heating. In the case of electric heating the trend was different, the majority of oils showing an increase in TBARS after 5 min of heating, followed generally by a decrease after 10 and 15 min , but usually to a higher value than the initial one. This is probably caused by the various oxidation product generation in each stage of heating. MDA obtained as a secondary oxidation product

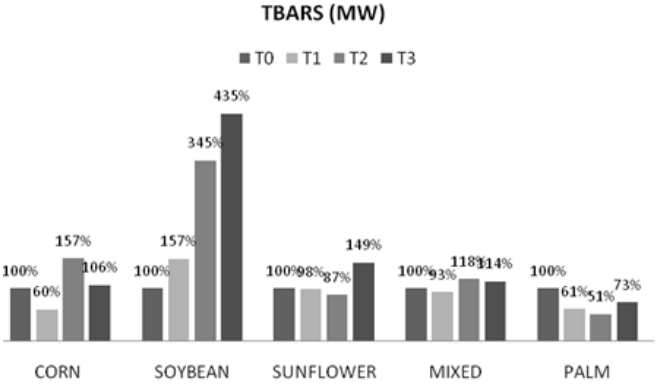

Fig. 7. MDA-TBA content evolution in correlation with microwave exposure time and oil type

can react further with other compounds present in the reaction mixture.. A special case was the palm oil that for both heating types had a steady decrease in TBARS values for all heating durations. This could be an effect of the presence of soluble fibre or amino containing phospholipids giving Maillard reaction

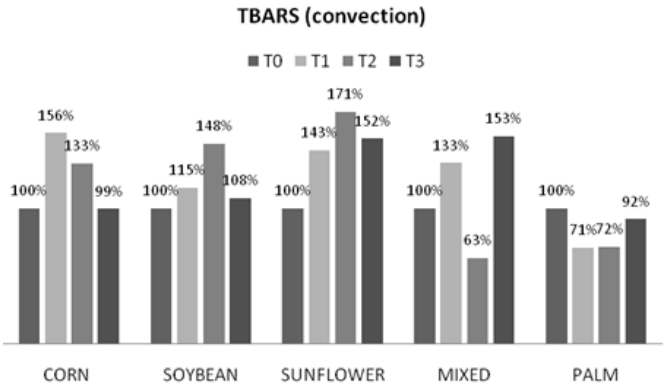

Fig. 8. MDA-TBA content in correlation with convection heating time and oil type

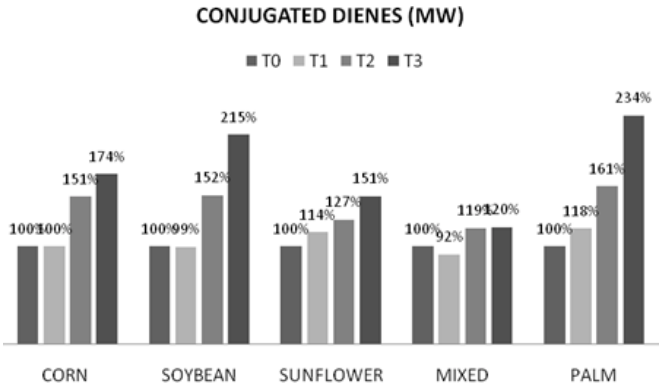

Fig. 9. Conjugated dienes content in correlation with microwave exposure time and oil type

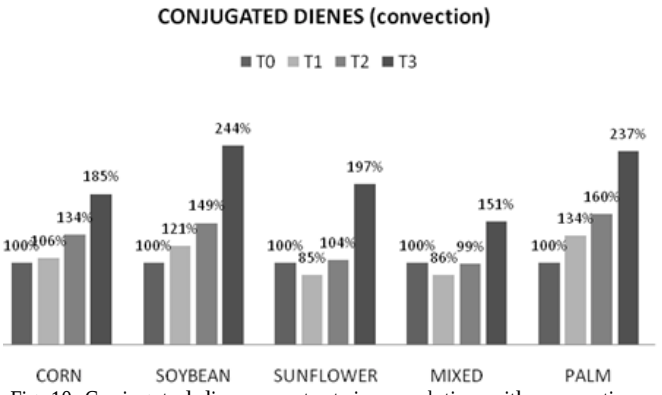

Fig. 10. Conjugated dienes content in correlation with convection heating time and oil type

Conjugated dienes content

Mixed oil was also having the highest initial conjugated diene content (11.92 mM/L). For MW heating for 5 min the highest value was observed in sunflower oil (12.77 mM/L); for 10 min to corn oil (14.34 mM/L). With the observation that sunflower oil and mixed oil had similar values; after 15 min heating maximum value was observed in soybean oil (17.14 mM/L). Using an electric oven, maximum values determined belonged to mixed oil (10.19 mM/l) and corn oil (10.05 mM/L) after 5 min heating. After 10 min of heating corn oil showed a value of 12.79 mM/L and a few other oils gave values close to 11 mM/L; after 15 min, sunflower oil had the highest value (22.4 mM /L).

After 5, 10 and 15 min of MW heating the highest % increase in conjugated dienes levels was observed for palm oil (118, 161 and 234%). For convection heating the highest increases in conjugated dienes were observed for palm oil after 5 and 10 minutes (134% and 160%) and soybean oil after 15 min (244%).

In the case of MW heating we noticed a steady increase in conjugated dienes content with heating duration. In the electric oven soybean and sunflower oil showed a decrease in conjugated dienes after 5 min heating, longer times of heating giving an increasing trend. The % increase was higher for the conventional heating compared to MW. Sometimes the peak point in light absorption of the conjugated dienes may be covered by non-peroxidized fat absorption and contaminant extraction products. Moreover, moving the double bond can occur in the absence peroxidation reactions.

Conclusions

Considering the oxidative oils modifications(conjugated dienes and TBARS) there are not marked differences between microwave and electric oven heating

The most sensitive oil to oxidative damage is soybean oil, having also the highest PUFA content. The sensitivity can also be explained by low TEAC and tocopherol contents

Higher MUFA then PUFA present in the commercial mixed oil used in our experiment seem to have a good impact on oil oxidative stability

Also palm oil is seriously damaged in terms of oxidative modifications it is still retaining a high tocopherol and TEAC activity after 15 min of heating. Tocopherol retention is higher in microwave then in convection heating conditions

Adition of antioxidants in commercial oils having high contents of unsaturated fatty acids and also low TEAC activity can probably improve their oxidative stability

Aknowledgement: Liviu Iosif and Laura Gaman equally contributed to this work as first authors

References

1. J.D.W. PAUL G. DAVIS, in:, Lipid Metab. Heal., 2006, pp. 47–60.
2. MIHALACHE, M., BRATU, A., HANGANU, A., CHIRA, N. A., MAGANU, M., TODASCA, M.C., ROSCA, S., Rev. Chim.(Bucharest), **63**, no. 2012, no. 10, p. 984
3. COZEA, A., IONESCU (BORDEI), N., POPESCU, M., NEAGU,M., GRUIA, R., Rev. Chim. (Bucharest), **67**, no.3, 2016, p. 422
4. Y.H. CHU, Y.L. KUNG, Food Chem. **62** 1998 191–195.
5. J. KANNER, Mol. Nutr. Food Res. **51** 2007 1094–1101.
6. N. GERBER, M.R.L. SCHEEDER, C. WENK, Meat Sci. **81** 2009 148–154.
7.C. MIGLIO, E. CHIAVARO, A. VISCONTI, V. FOGLIANO, N. PELLEGRINI, J. Agric. Food Chem. **56** 2008 139–147.
8.S. CHANDRASEKARAN, S. RAMANATHAN, T. BASAK, Food Res. Int. **52** ,2013, 243–261.
9.G. KNOTHE, K.R. STEIDLEY, Bioresour. Technol. **100** ,2009, 5796–5801.
10.N.J. MILLER, C. A RICE-EVANS, Redox Rep. **2** ,1996, 161–171.
11.R. RE, N. PELLEGRINI, A. PROTEGGENTE, A. PANNALA, M. YANG, C. RICE EVANS, Free Radic. Biol. Med. **26** ,1999, 1231–1237.
12.H.H. DRAPER, M. HADLEY, Methods Enzymol. **186** ,1990, 421–431.
13. *** Food safety and Standards Authority of India, C. Products, Man. Anal. Oils Fats ,2012, 20–35.
14. P. PRIETO, M. PINEDA, M. AGUILAR, (n.d.).
15. E.A. EL-MONEIM MAHMOUD, J. DOSTALOVA, J. POKORNY, D. LUKESOVA, M. DOLEZAL, Czech J. Food Sci., 2009.
16. A. KAMAL-ELDIN, R. ANDERSSON, J. Am. Oil Chem. Soc. **74** ,1997, 375–380.
17. T. ALBI, A. LANZON, A. GUINDA, M. LEON, M.C. PEREZ CAMINO, J. Agric. Food Chem. **45**, 1997, 3795–3798.
18. N. PELLEGRINI, F. VISIOLI, S. BURATTI, F. BRIGHENTI, J. Agric. Food Chem. **49**, 2001 2532–2538

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