Vegetable Oils Microwave Heating – CUPRAC, TEAC and FRAP Values in Relation with Oxidative Parameters

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Microwave heating is a common cooking procedure.Heating can accelerate oxidative processes in oil sand oxidation products are known to have potential toxic effects on human health.Therefore it would be useful to have a method to anticipate the vegetable oils behaviour under thermal conditions. Several methods to evaluate total oxidant activity were developed but unfortunatelly their ability to predict the oxidative changes during heating are poorly estimated. In this study we evaluated the relation of TEAC FRAP and CUPRAC values together with total polyphenols and flavonoids content of several commercial available vegetable oils with conjugated dienes and TBAR's products during 15 min microwave heating. FRAP values were correlated with oxidative parameters after 15 min heating

Keywords: vegetable oils, total antioxidant capacity, lipid peroxidation, microwave heating, CUPRAC, FRAP

Antioxidants occur naturally in oils where they offer protection against oxidative damage. Endogenous antioxidants are part of the unsaponifiable components of fats and oils, representing less than 5% of the total lipid composition. The activity of antioxidative compounds is evaluated by chemical assays such DPPH (2,2-DiPhenyl-1-PicrylHydrazyl), ORAC(*Oxygen Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power*),TEAC (Trolox Equivalent Antioxidant Capacity)etc., reviewed by [1–5], but these indirect methods correlate poorly with the ability of compounds to inhibit oxidation in real food systems due to their inability to account for important variables such as the location of the antioxidant and its interaction with other food components [6].

On the other hand, many studies have shown that phenolics can act both antioxidatively and prooxidatively depending upon the physicochemical nature and the composition of the lipid system as well as antioxidant structure and concentration [7, 8].

Flavonoids are diphenylpropanes that commonly occur in plants (more than 5000 have been found) and components of the human diet. The effects of a flavonoid compound may depend upon its behavior as either an antioxidant or a prooxidant. It is extremely important to understand the antioxidant and prooxidant behavior of flavonoids and the related activity-structure relationships in order to understand the mechanisms involved in atntioxidant and prooxidant effects [9].

In the presence of copper, flavonoids that showed protection against peroxyl radicals and hydroxyl radicals, acted as prooxidants rather than antioxidants. The copper-initiated prooxidant activity of a flavonoid depended upon the number of OH substitutions in the flavonoid structure. Flavonoids which have no or only one OH substitution had undetectable prooxidant activity, whereas those which have four, five, and six OH substitutions, respectively, had increased prooxidant activity [10].

Some studies have shown that some flavonoids acting as prooxidants in specific conditionsalso have high FRAP values[11]. Because reduced metals are active propagators of radical chains via hydroperoxide reduction to free radicals, it would be interesting to evaluate whether high FRAP values correlate with the tendency of flavonoids to become prooxidants under some conditions.

The purpose of this study was to evaluate the variation in total antioxidant capacity, total polyphenols and flavonoids relative to lipid peroxides and the formation of conjugated dienes during microwave heating and to assess if TEAC, FRAP or CUPRAC values (having a similar principle) are correlated with possible prooxidant activity in microwave heated oils and can be used as predictors for oxidation processes in edible oils during cooking.

Experimental part

From a local supermarket (Bucharest, Romania) sunflower, corn, soybean, palm (not hidrogenated), canola, sesame, olive (extra virgin) and mixed oil (containing sunflower, grape, flax seed and rice oil) were purchased. Cupric Reducing Antioxidant Capacity (*CUPRAC*), *Ferric Reducing Antioxidant Power.* (*FRAP*), Trolox Equivalent Antioxidant Capacity (TEAC), vitamin E as , total flavonoids, total phenolics were determined in oil samples as they were. Lipid peroxides as thiobarbituric reactive substances (TBARS) and conjugated dienes along with total polyphenols and flavonoids were determined in oils samples after 15 min microwave heating at 2450 KHz.

Samples of 50 mL were heated in Erlenmeyer dishes at maximum potency (1200 W) in a domestic microwave oven (Maxwell). After cooling, the samples were transferred to Falcon tubes and refrigerated until analysis.

Materials

All reagents were purchased from Santa Cruz Biotechnology, Inc (Dallas, Texas U.S.A). All solvents (methanol, ethanol, and hexane) were purchased from Sigma Aldrich Inc. (Germany). A UV/Vis spectrophotometer (Perkin Elmer, U.K), a Tecan Sunrise (Tecan Group Ltd, Switzerland) and a SONOREX SUPER RK255H sonicator (General Sonic, U.S.A.) were used. Ultrapure water 18.2 MΩ was used for dilutions.

CUPRAC method

CUPRAC was performed according to Celik S E et al., (2010) [12] trough a reaction which is based on the reduction of copper from copper chloride in the presence

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of neocuproin at *p*H 7. A buffer containing 1 M ammonium acetate, *p*H = 7, a solution of 10 mM $CuCl_2$ and a solution of 35 mM neocuproine (No) were made. Working Reagent contained: Buffer: No: $CuCl_2$ = 1: 1: 1 (v: v: v). 1 mL oil and 1 mL hexane were vortex mixed for 5 min. On oil/hexane mixture 5 mL of methanol were added and samples were sonicated on ice. The tubes were centrifuged at 10,000 rpm for 15 min. The supernatant was further diluted in MetOH and 10 µL of diluted sample were added in a 96 well plate on 290µL working reagent. The plate was incubated for 30 min at 37°C. Absorbance was read at 450 nm. A calibration was made with TROLOX 0.15-2.5 mM. Results were expressed in mM equivalent Trolox per liter.

FRAP method

FRAP was performed according to Alam (2013) [5] trough a reaction that is based on the reduction of iron from ferric chloride in the presence of 2,4,6-Tripyridyl-s-triazine (TPTZ) at *p*H 3.6.. A buffer containing CH3COOH / CH_3COONa, 0.3 M, *p*H = 3.6, a solution of TPTZ 10 mM and a solution of 20 mM $FeCl_3$ were made. Working Reagent contained: Buffer: TPTZ: Fe III = 10: 1: 1 (v: v: v). 1 mL oil and 1 mL hexane were vortex mixed for 5 min. On oil/hexane mixture 5 mL of methanol were added and samples were sonicated on ice. The tubes were centrifuged at 10,000 rpm for 15 min. The supernatant was further diluted in MetOH and 10 µL of diluted sample were added in a 96 well plate on 290 µL working reagent. The plate was incubated for 30 min at 37°C. Absorbance was read at 450 nm. A calibration was made with TROLOX 0.15-2.5 mM. Results were expressed in mM equivalent Trolox per liter.

TEAC method

Total antioxidant activity was determined based on the 6-hydroxy-2,5,7,8-tetramethyl -chroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay developed by Miller and Rice-Evans [13], with modifications[14]. The TEAC assay measures the relative abilities of antioxidants to scavenge the 2,22 -azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in comparison with the antioxidant power of standard amounts of Trolox, the water soluble analogue. The ABTS radical was generated from the interaction between ABTS and potassium per sulfate. At the beginning of the analysis day, an ABTS - working solution was obtained by the dilution in ethanol of the stock solution for the lipid soluble fraction or water for hydro soluble fraction. The marking 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 min against ethanol and water. The percentage inhibition of absorbance was calculated. A calibration curve using TROLOX 0.5-2.5 mM/L was constructed. The results were expressed as µmol eq.Trolox/l product.

TBARS method

The sample along with SDS 10%, BHT 2% and TBA 0.8% was incubated 60 min at 100° C. [15] After exactly 60 min the reaction was stopped by cooling the tubes in an iced water bath. The absorbance was read at 532 nm against a reagent blank. A calibration curve with tetraethoxypropane (TEP) 0.5-4 mM was constructed. The results were expressed asµM MDA eq/l product.

Conjugated dienes method

The absorption of conjugated dienes, was followed spectrophotometrically (at 234 nm. [16] The oil sample was diluted (1:50) with hexane (HPLC grade). An extinction coefficient of 29,000 mol/L was used to quantify the concentration of conjugated dienes formed during oxidation. The quantity was calculated and expressed as mM/L oil using the formula:

$$Cox = \text{Absorbance of sample} / \text{Conc. Oil} * \text{Extinction coef.} * 100\% \quad (1)$$

Total polyphenols method

One mL of oil sample was diluted with 1 mL of n-hexane and extracted three times with 1.6 mL EtOH:CH3Cl - 80:20 v/v and 400 ul distilled water [17]. A calibration curve of gallic acid 0.04±0.7 mg/mL in methanol was used. On 0.6 mL of a standard solution of gallic acid or sample, 0.125 mL of the Folin-Ciocalteau reagent, 0.25 mL of 7.5% Na_2CO_3 was added. The solutions were stored overnight and read at λ=765 nm.

Total flavonoids method

Total flavonoid content was determined by using the aluminum chloride colorimetric method as described by Hossain (2011) [18], with some modifications. Oil extracts, 10% aluminum chloride (10 µL), 1M potassium acetate (10 µL) and distilled water (200 µL) were mixed in a 96-well plate. After incubation at 37°Cfor 30 min, the absorbance was measured at 420 nm. Quercetin was used to make the calibration curve in concentrations between 12.5-100 µg/mL.

Pearson regressions and correlations between experimental data were determined using InStat GraphPad software (GraphPad Software Inc.La Jolla, United States). Pearson's correlations between the values obtained for each determination in unheated oils and the values obtained for unheated and heated oils were determined using the same software.

Olive oil showed the highest CUPRAC value determined in unheated oils (10.57 mM eq. T/L) while corn oil had the lowest (6.02 mM eq. T/L). It had only a negative correlation with TEAC (r = -0.73). No other relevant correlations with determinations in heated oils have been found.

FRAP in sesame oil had the highest value (9.02 mM eq. T/L) and the lowest was found in mixed oil (5.22 mM eq. T/L). FRAP measured in unheated oils had positive correlations with total flavonoids (r = 0.73) and CDV (r = 0.80) in 15 min heated oils. Negative correlations appeared with polyphenols (r = -0.86) measured in unheated oils and after 15 min of microwave treatment (r=-0.81).

Highest TEAC value was measured in corn oil (9.92 mM eq. T/L) while the lowest was found in sesame oil (4.14 mM eq. T/L). TEAC showed significant negative correlations with total flavonoids (r=-0.62) and CUPRAC (r=-0.73) before heating and a positive corellation with CDV after 15 min heating (r=0.68).

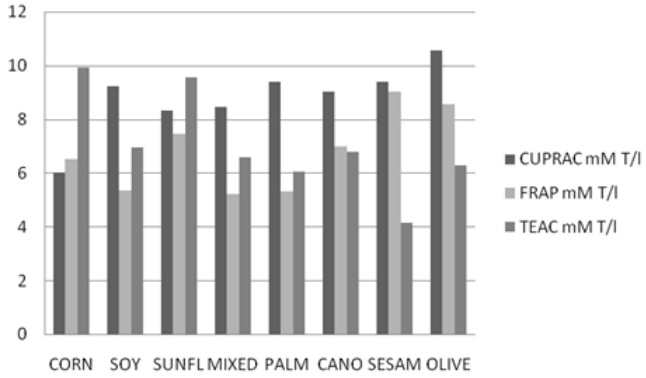

Fig. 1 CUPRAC, FRAP and TEAC values expressed as mM equivalent Trolox/L oil for all oils

Table 1

VALUES FOR: CUPRAC, FRAP, TEAC (EXPRESSED AS mM EQUIVALENT TROLOX/L OIL) BEFORE HEATING; TOTAL FLAVONOIDS, TOTAL POLYPHENOLS BEFORE AND AFTER HEATING; TBARS AND CONJUGATED DIENES (CDV) AFTER15 MINUTES MICROWAVE HEATING

	CUPRAC	FRAP	TEAC	FLAVO 0	FLAVO 15	POLY 0	POLY 15	TBARS 15	CDV 15
	mM T/l	mM T/l	mM T/l	mg/ml	mg/ml	mg/ml	mg/ml	uM/l	mM/l
CORN	6.02	6.52	9.92	0.61	0.10	2.64	0.38	20.51	16.54
SOY	9.23	5.36	6.95	0.21	0.14	2.53	0.43	83.41	17.14
SUNFL	8.32	7.44	9.56	0.68	0.11	2.55	0.87	21.07	16.89
MIXED	8.45	5.22	6.57	0.51	0.15	3.28	0.99	40.32	14.25
PALM	9.40	5.31	6.04	0.65	0.10	3.10	0.45	18.16	16.46
CANO	9.01	6.98	6.78	0.36	0.04	2.88	0.82	71.33	8.40
SESAM	9.40	9.02	4.14	0.26	0.04	1.80	0.74	26.11	7.12
OLIVE	10.57	8.55	6.27	0.23	0.01	1.88	1.44	21.63	6.16

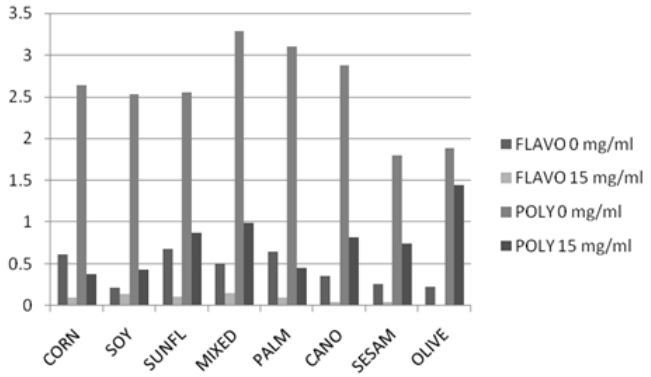

Fig. 2 Total polyphenols and flavonoids evolution with temperature for all the oils studied expressed as mg/mL

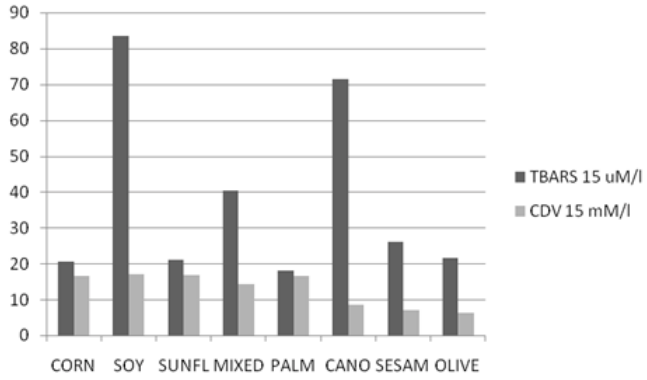

Fig. 3TBARS and CDV values for all the oils studied after 15 min of microwave treatment

Total polyphenol values decreased with temperature (fig. 2). The highest value was found in the mixed oil (3.28 mg / mL) before heating. The lowest value was found in corn oil (0.38 mg / ml) heated for 15 min. Total polyphenols in oils before microwave heating had a negative correlation with FRAP (r = -0.86) in unheated oils and positive corellations with CDV (r=0.70) and total flavonoids (r=0.60) after 15 min nof microwave treatment.

The same descending trend with temperature was observed for total flavonoids (fig. 2). Maximum values were observed in sunflower oil (0.68 mg / mL) before heating andmixed oil after 15 min of microwave heating (0.15 mg / mL). The lowest values were in unheated soybean oil (0.21 mg / mL) and olive oil after 15 min heating (0.01 mg / mL). Total flavonoids before heating showed negative correlations with TEAC (r = -0.62) in unheated oils and total polyphenols after 15 min of microwave treatment (r=-0.50).

Maximum MDA values were found in soybean oil after 15 min of heating(83.41 μM / L). Minimal values were observed in palm oil warmed for 15 min (18.16 μM / L) (fig.3).

The maximum values for CDV observed were in soybean oil after 15 min (17.14 mM / L) of microwave treatment. Minimum values were all observed in olive oil (6.16 mM /Ll) (fig.3).

CUPRAC and FRAP did not correlate positively with eachother or TEAC. CUPRAC determined in unheated oils showed only negative correlations with TEAC.

FRAP from unheated oils had positive correlations with total flavonoids and CDV for 15 min heated oil. Negative correlations were found with polyphenols measured in unheated and heated oils.

An interesting result was that in corn oil we measured the highest TEAC value and the lowest CUPRAC. Another interesting fact was that in sesame oil we found the highest FRAP value and also the lowest TEAC.

Positive correlations between TEAC and polyphenols with conjugated dienes show that, in addition to the antioxidant effect, there are also pro-oxidative processes taking place at the same time, and the antioxidant stability of the oils is ultimately determined by the equilibrium that is reached between these two types of processes [9].

Flavonoids showed only negative correlations with TEAC in unheated oils. This suggests the existence of a prooxidative potential troughout the process. The prooxidizing activity of a flavonoid, as antioxidant activity that absorbs its peroxyl or hydroxyl moiety, depends on the number of free OH substitutions on its structure. The more OH substitutions occur, the more prooxidant activity [19, 8].Negative correlations of flavonoids with antioxidant capacity underline the important contribution of flavonoids to prooxidative processes taking place during microwave treatment.

Results suggest that flavonoids are first destroyed in the oxidative processes during the microwave treatment of oils and probably this has a protective effect for the total polyphenols**,** as shown by the negative correlation between the total flavonoids and total polyphenols after 15 minutes heating. The most eloquent example is the case of olive oil. After 15 min of heating in the microwave oven we found the lowest amount of flavonoids in olive oil and also the highest amount of total polyphenols. Olive oil showed also the highest initial CUPRAC and the lowest value for primary oxidation products as CDV. The TBARS value was also one of the lowest measured.

The highest values for both primary and secondary oxidation products were measured in soybean oil, that is a refined oil. From our selection sesame oil was cold pressed and olive oil was extra virgin(that is also obtained without chemical intervention), the rest being refined oils. As a general observation, the values for oxidation products were higher for the refined oils. An exception was palm oil that had the lowest TBARS value, and that can be explained from the lack of unsaturated fatty acids in its composition.

From the collected data only FRAP showed potential to be used as predictor for oxidation processes in edible oils having correlations with total polyphenols, total flavonoids and conjugated dienes after 15 min of microwave treatment.

Conclusions

Data collected confirms that, in most cases, the antioxidant capacity of oils is linked to increased amounts of polyphenols and flavonoids. Current data showed that before losing polyphenols during heating, oils will first lose the flavonoids content.

We could identify a class of oxidation-resistant oils due to the high retention of polyphenols - palm oil, sesame and extra virgin olive oil. These oils showed a lower increase in oxidation products (TBARS and conjugated dienes) after 15 minutes of microwave heating. A special case is olive oil which, having one of the lowest antioxidant capacity, showed excellent retention in polyphenols, which caused one of the smallest increases in oxidation products.

After 15 min it was found that a number of oils have huge increases in oxidation products, both from oils with lower antioxidant compounds retention - canola, soybean and sunflower oil, and the higher retention oils - palm, corn and mixed oil.

The occurrence of prooxidative effects during microwave heating was suggested in the case of flavonoids. Antioxidant stability of oils is ultimately established by the balance between antioxidant and prooxidative processes that are influenced by both heating and tocopherol and flavonoid concentrations.

It would be of interest to deepen the study of these processes by determining the individual contribution of various flavonoids involved in the balance of antioxidant / prooxidant processes occurring during microwave heating of food oils.

Knowing these interactions could lead to improved food oils so they have an increased resistance to oxidative processes and, implicitly, longer shelf life and possible unintended effects on the health of consumers as little as possible.

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