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## MONITORING LIPID OXIDATION EVENTS AT FRYING TEMPERATURES THROUGH RADICAL SCAVENGING ASSAYS

*This communication proposes an alternative approach for monitoring oils during thermal stress at frying temperatures through radical scavenging assays. Oxidation events for extra virgin olive, pomace, sesame, sunflower, soybean, corn and of a commercial blend of oils are followed through the DPPH assay during heating at 100, 150 and 190 °C. Radical scavenging activity decrease expressed as trolox equivalent antioxidant capacity ( $\Delta$ TEAC, mmol trolox kg<sup>-1</sup> oil) is found to be linearly related to increases in total oxidation ( $\Delta$ TOTOX) values. This relationship is valid down to a certain  $-\Delta$ TEAC value cutoff that is different for different oils. Considerable consumption of antioxidants demonstrated by high  $-\Delta$ TEAC values renders the linear relationship invalid indicating that antioxidants cannot control late events of oxidative damage. Radical scavenging activity is found to increase upon sesame oil heating in contrast to all other oils. It is postulated that sesamol, a phenolic antioxidant, decomposes during heating to the more potent antioxidant sesamol accounting for the increase of radical scavenging activity upon heating. This paper demonstrates prospects of radical scavenging activity assays as a tool for monitoring oxidation events during frying and warrants further research and evaluation.*

*Key words: Oils, Frying, Heating, DPPH, TOTOX, Edible oil oxidation, Food quality control, Antioxidants.*

Frying is one of the most popular methods of preparing food throughout the world. Various oils and fats, usually vegetable ones, are being used. The selection of frying oils and fats depends mainly on factors such as price, availability, flavor and stability during storage.

Frying is a process that causes fast deterioration of oils as several physical and chemical changes take place. These changes are mostly oxidation, hydrolysis, polymerization, cis/trans isomerization, conjugation, pyrolysis, and cyclization [1,2]. These chemical reactions are known to change organoleptic and nutritional properties of oils and are modulated by the presence of air and moisture.

Thermal stressing of edible oils accelerates lipid oxidation reactions leading to primary oxidation products such as free radicals and peroxides. These further decompose to secondary products such as ketones, aldehydes, alcohols and hydrocarbons, that are responsible for the off-flavors [3].

Furthermore, some oxidation products are known to be harmful to human health. For example 4-hydroxy-2-nonenal, detected during frying of soybean oil [4], is a registered carcinogenic and mutagenic  $\alpha,\beta$ -unsaturated aldehyde [5]. Monitoring lipid oxidation is essential for evaluating the quality of frying oils in the food industry, as proposed in the 3<sup>rd</sup>

and 4<sup>th</sup> International Symposiums on deep fat frying held at Hagen, Germany [6]. Rapid tests that are easy to use and correlate with standard methods for the evaluation of oil quality during frying are recommended. Tests should be rugged, simple and safe for use in food processing/preparation areas providing an objective index in accordance to oil degradation.

Parameters commonly used for monitoring oils oxidative deterioration are peroxide value (PV) and p-anisidine (p-AV) along with acid value (AV), carbonyl value (CV), 2-thiobarbituric acid value (TBA) and total polar materials (TPM) [2]. Antioxidants in diet affect human health by influencing a number of physiological processes: apoptosis, metabolism, cell differentiation and growth, DNA repair, hormone regulation, etc. and also they seem to lower oxidative stress [7]. Antioxidants protect food systems delaying the formation of oxidation products through free radical scavenging. Recently, an inverse relationship between antioxidant content and peroxides formed during soybean oil heating at 110 °C, has been implied [8]. Another study indicates a linear relationship between radical scavenging activity (RSA) and some physicochemical parameters monitored during frying of French fries in palm olein mixtures with sunflower and cottonseed oils [9]. This linear relationship was derived using an extremely narrow range of parameters values undermining correlation validity. These studies [8,9] indicate that RSA could be related to oil oxidative deterioration during frying. This work is the initial evaluation of the use of RSA measurements for monitoring oxidation of extra virgin olive (EVO), pomace (P), sesame (SE), sunflower (SF), soybean (S), corn (C) and a commercial blend of oils

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(CB) during heating. The concept is that total antioxidant capacity, measured through the DPPH radical scavenging assay, is inversely related to the extent of lipid oxidation as assessed through the TOTOX value that is considered to be an appropriate physicochemical index for monitoring edible oil oxidation [10].

## MATERIALS AND METHODS

### Materials

EVO, P, SE, SF, S, C, and CB were purchased from a local supermarket. DPPH, 90%; trolox, 97% and p-anisidine, 99.5% were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), KI, 99.5% and 0.1000 N titrisol  $\text{Na}_2\text{S}_2\text{O}_3$  standard solution were from Merck (Darmstadt, Germany). All solvents were of analytical grade from Merck.

### Oil samples and heating protocol

Oil aliquots of 7.5 gr were placed in open glass vials of 4 cm height and 2.5 cm internal diameter. Vials were placed in oil baths heated at 100, 150 and 190 °C and temperature was controlled within  $\pm 1$  °C. A vial was removed from each oil bath at 30 min, 2, and 8 h. Unheated oil aliquots were also obtained. All samples were stored in sealed glass vials at  $-18$  °C until analysis.

### Analytical methods

PV and p-AV were determined through the official EU [11] and the standard 2504 IUPAC [12] method, respectively. RSA of the oils were determined using the DPPH assay [13] as follows: DPPH solution was prepared by dissolving 5 mg of DPPH in 100 ml of ethyl acetate. Absorption at 515 nm was adjusted in the range of 1.4–1.5 and the solution was kept in dark. Oil aliquots of 80 – 130 mg, according to their RSA, were weighted in screw-capped glass vials. 4.0 ml of DPPH solution was added, the vials were closed, shaken for 10 s and the reaction was allowed to proceed for 60 min in the dark. The absorbances of the mixtures and the DPPH blank solution were measured at 515 nm in 1.0 cm quartz-cuvettes, using a Jasco (Japan) V-550 UV-Vis spectrophotometer. To express absorbance changes as TEAC values a trolox calibration curve was measured.

## RESULTS AND DISCUSSION

PV follows the evolution of peroxides and hydroperoxides while p-AV follows secondary oxidation products, principally 2-alkenals. At 100 °C p-AVs were low ranging from 2.2 to 5.7 indicating that the main oxidative event is the formation of peroxides. Table 1 depicts the linear relationship between the increase of PV versus the decrease of TEAC values for C, CB, EVO, SF and P at 100 °C. At elevated temperatures secondary oxidation processes lead to rapid decomposition of peroxides, restricting the linear relationship to SF and P

Table 1. Linear relationship between the increase of PV vs the decrease of TEAC values

		Slope $\pm$ SD (n=4) $\Delta\text{PV} \times \Delta\text{TEAC}^{-1} \times 10^{-1}$	r	PV max value	%RSD (n=3) of PV
100 °C	C	$7 \pm 1$	0.96	29	1.0 – 4.5
	CB	$6.0 \pm 0.5$	0.994	43	0.9 – 4.3
	EVO	$5.4 \pm 0.4$	0.995	39	0.5 – 1.8
	SF	$5.1 \pm 0.4$	0.995	77	0.1 – 2.5
	P	$1.5 \pm 0.2$	0.99	17	1.5 – 5.4
150 °C	SF	$3.1 \pm 0.3$	0.992	71	0.5 – 1.4
	P	$1.4 \pm 0.2$	0.97	37	0.5 – 2.4
190 °C	SF	$1.2 \pm 0.2$	0.96	25	0.8 – 2.6

at 150 and SF at 190 °C. It is interesting to note that the slope decreases along temperature.

In contrast to the increase of PV, the linear relationship between the increase of p-AV versus the decrease of TEAC values, shown in Table 2, is valid only at 190 and 150 °C. Linear correlations ( $r > 0.9$ ) between  $\Delta\text{p-AV}$  versus  $\Delta\text{TEAC}$  values for CB and EVO are also observed at 100 °C. However p-AV maximum values achieved at 100 °C are 5.7 for CB and 5.2 for EVO. That is, at low temperatures the decomposition rate of peroxides to secondary products measured through the p-AV is lower than the formation rate, therefore p-AVs are quite low. This indicates that prediction of p-AV through measurements of TEAC values at 100 °C is not feasible. It should be noted that slopes decrease from 190 to 150 °C.

Results presented in Tables 1 & 2 show that TEAC values could be used to follow oxidation processes at elevated temperatures. However, oxidation of oils during frying is not adequately monitored by parameters that follow only primary (PV) or secondary (p-AV) products. On the contrary TOTOX value, calculated as  $2 \text{ PV} + \text{p-AV}$ , is a better index of oxidation status [10].  $\Delta\text{TOTOX}$  versus  $\Delta\text{TEAC}$  values relationship is linear at all temperatures (Fig. 1). This relationship is valid till a  $\Delta\text{TEAC}$  value cut-off point that for EVO and P is about 1.6 and 2.1 mmol trolox  $\text{kg}^{-1}$  oil, respectively (Fig. 1 b,c).

Table 2. Linear relationship between the increase of p-AV vs the decrease of TEAC values

		Slope $\pm$ SD (n=4) $\Delta\text{p-AV} \times \Delta\text{TEAC}^{-1} \times 10^{-1}$	r	p-AV max value	%RSD (n=3) of p-AV
190 °C	S	$12.2 \pm 0.4$	0.998	294	0.3 – 4.8
	CB	$8.7 \pm 0.6$	0.97	236	0.2 – 1.6
	C	$8 \pm 1$	0.98	218	0.4 – 2.8
	SF	$8 \pm 1$	0.97	137	0.3 – 1.9
150 °C	S	$6.0 \pm 0.1$	0.9997	156	0.2 – 4.8
	CB	$5.7 \pm 0.7$	0.98	162	0.1 – 1.6
	SF	$5.0 \pm 0.5$	0.99	107	0.9 – 4.6

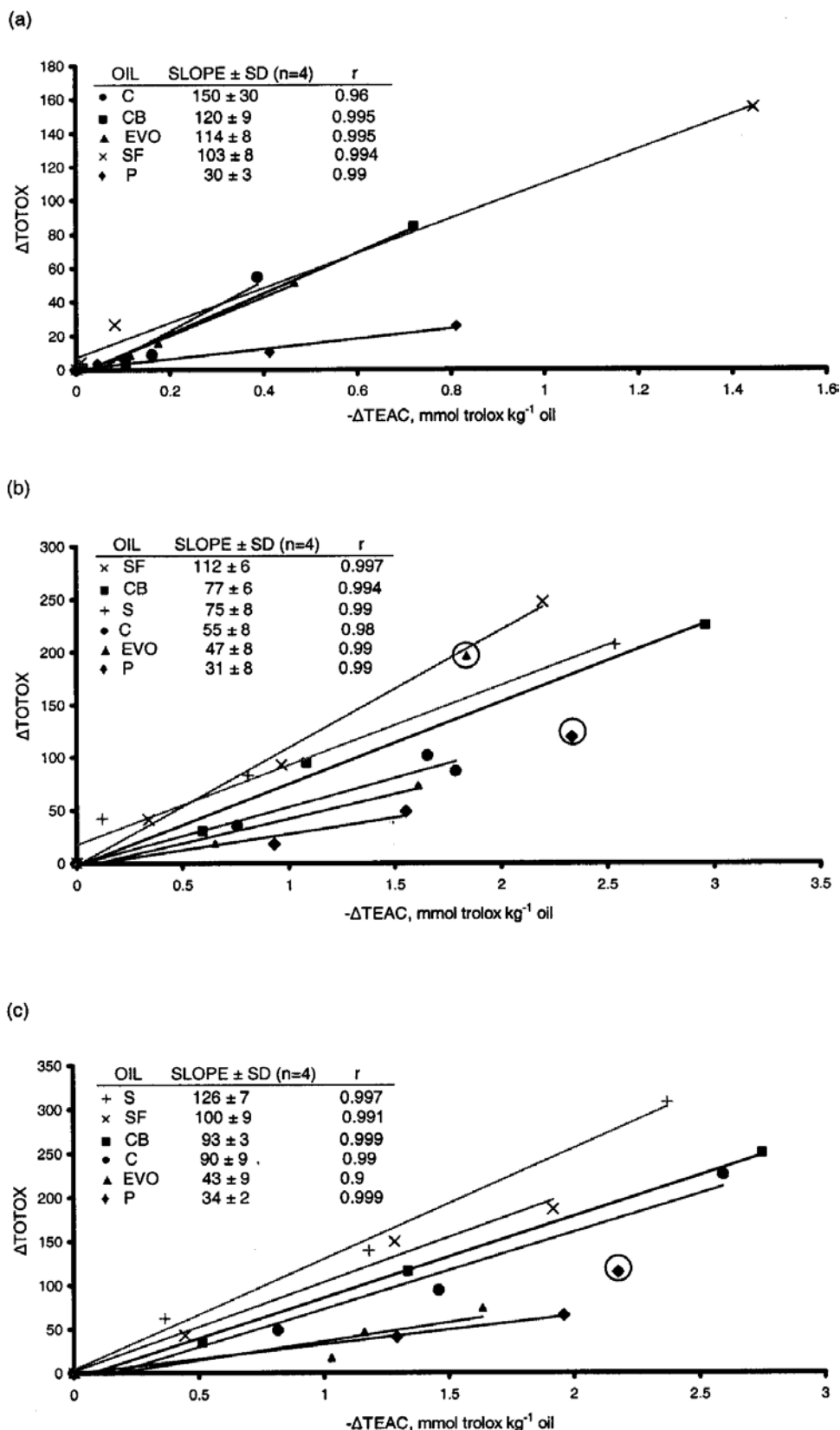


Figure 1. Changes of TOTOX values as related to changes in TEAC values during oil heating: (a) 100, (b) 150 and (c) 190 °C. Slopes derived through linear regression data fits, along their correlation coefficients are shown in the inserts. Data points shown in circles, corresponding to higher  $\delta$ TEAC than the cut-off values, were excluded from the calculation. PV and p-AV values for TOTOX calculation are means of triplicate measurements. %RSD values, n=3, calculated for TOTOX through error propagation formulas (14) were in the range of 0.8–3.7 for P, 0.3–2.5 for C, 0.5–4.8 for S, 0.2–3.5 for SF and 0.6–3.6 for EVO.

These cut-off points correspond to low TEAC values *i.e.*, 0.3 and 0.6 mmol trolox kg<sup>-1</sup> oil, respectively. For low TEAC values the small antioxidant content cannot control efficiently the oxidation processes. The cut-off point seems to be different for different oils. It is interesting to note that slopes for CB, C, EVO and S are statistically different at the three temperatures. This implies that temperature differentiates the antioxidants control on the oxidative processes. Slopes for these relationships are also highly affected by the ratio: surface (exposed to air) to mass of oils [15]. Therefore these relationships cannot be directly applied to different systems and a field adjustment by calculating the slopes of the linear relationships is required according to the frying conditions of the operation. This could result to a useful tool for the industry. For SE oil  $\Delta$ TOTOX do not correlate with  $\Delta$ TEAC values. Moreover, TEAC values at 150 °C for 30 min and 2 h and at 190 °C for 30 min increase from the baseline level while at 100 °C TEAC values were almost constant, minimally increasing after 8 h. SE oil main antioxidant compounds are tocopherol, sesamol and sesamol [16]. Sesamol acts as a precursor for sesamol and sesaminol [17]. Sesamol, that has a rather small RSA, decomposes during heating to sesamol that has 30/fold higher activity towards the DPPH radical [16,18]. This probably accounts for the increase of RSA upon heating and could explain the model inadequacy for TOTOX prediction. It is interesting to note that a) S oil TOTOX values upon heating at 100 °C are constant during the initial two hours and b) TEAC value increases by 5% after 30 min declining by just 8% after eight hours heating. This could be attributed to the protective action of antioxidant compounds formed upon 100 °C heating: genistein formed through hydrolysis of genistin [19]. This hydrolytic reaction increases the number of phenolic hydroxyls by one per molecule increasing thus antioxidant potential.

## CONCLUSION

Single photometric measurements of RSA can replace two parameters (*i.e.*, PV and p-AV) used for monitoring lipid oxidation processes during frying. The proposed procedure through the DPPH assay is simpler from multistep titrimetric methodologies used for PV evaluation. Beyond simplicity, the fewer manual operations of the DPPH assay result in increased

precision. Moreover, the proposed methodology is cost effective and environmental friendly replacing big volumes of chlorinated and toxic solvents used in PV and p-AV assays with just 4 mL ethyl acetate per assay.

Results presented in this communication point out good prospects for thermal stress monitoring and warrant further research and investigation.

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