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Investigation of Peroxidation Kinetics in Oil-in-water Emulsions İnduced by Cu(II)

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Abstract: In this study, peroxidation of olive oil, corn oil, sunflower oil, walnut oil, argan oil, rosehip oil emulsions was carried out in the presence of copper (II) ion at 37 ° C and pH 7 in a ventilated incubation environment. Primer products (hydroperoxides) were monitored by Fe (III) SCN method and secondary products (malondialdehyde) were analyzed by TBARS analytical methods. In addition, GC-MS analysis were performed on the oils studied to identify compounds that behave as free radical scavengers or hydrogen donors. Before preparation of the oil emulsions in water, iodine index determinations of the degrees of unsaturation in the oils were made and found to be 86.28, 128.12, 140.22, 164.97, 97.29 and 183.58 gI2 / 100g for olive oil, corn oil, sunflower oil, walnut oil, argan oil and rosehip oil respectively. The rate constants were calculated kwalnut oil > kargan oil > krosehip oil > ksunflower oil > kcorn oil > kolive oil for FeSCN method and krosehip oil > kwalnut oil > kargan oil > kolive oil > ksunflower oil > kcorn oil for TBARS method, respectively. As a result, pseudo first order kinetics of hydroperoxides and aldehydes were observed in copper-catalyzed oil emulsions at 37 ° C and pH 7, and the absorbance values obtained as a function of the incubation period gave sigmoidal curves. This study showed that the oxidation rates of fats are closely related to the conjugated fatty acids. It was thought that the kinetic data obtained could be used to accurately calculate the shelf life of oils used as food components.

Keywords: TBARS Method, Ferric Thiocyanate Method, Olive oil, Corn oil, Sunflower oil, Walnut oil, Argan oil, Rosehip oil, Lipid emulsion

Introduction

Nutritional oils, which have an important place among daily consumables, are among the most frequently discussed topics in our country (Çabukel et al. 2009). Oils are one of the main nutrients necessary for people to carry on their vital activities. An adult person has about 93 gr daily. oil is required. Apart from the contents of other nutrients, the total amount of fat that should be taken directly is 63 grams. According to European norms, if you consume about 24 kg of fat per person per year, it can be mentioned without a healthy diet (Kolsarıcı et al. 2005).

Deterioration of lipid compounds is accelerated by increased oxidation in many circumstances such as increase of metals in the medium, pH and temperature change (Das and Pereira, 1990). Lipids become aggravated as the result of oxidation, and this oxidative aggravation is the main cause of food impairment. The acceptability of a food product depends on the degree to which this degradation occurs. For this reason, it is very important to evaluate the degree of oxidation (Grey, 1978). In addition, flavonoids found naturally

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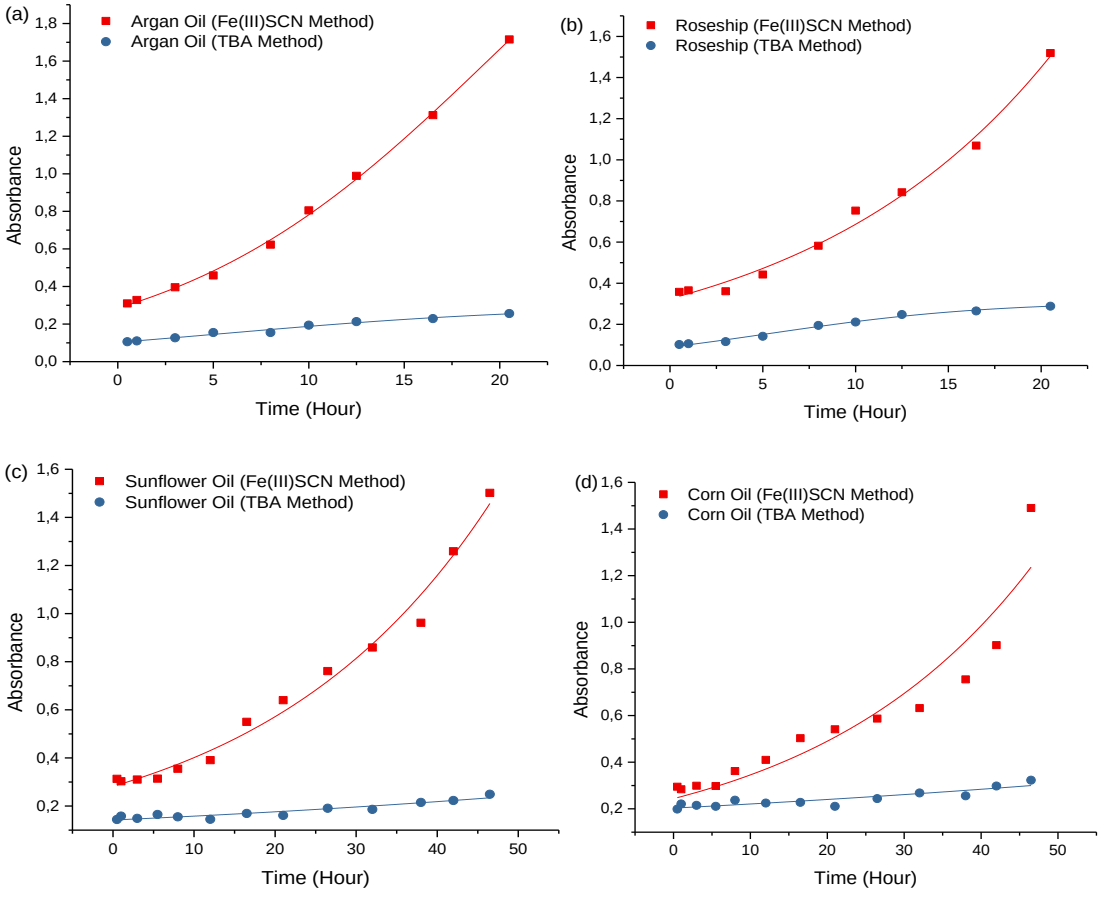
in plants such as argan, rosehip, walnut and carnation oils are regarded as important compounds in order to maintain stability against otoxidation on the lipids of plant sources (Das and Pereira, 1990). It is very important to examine the antioxidant action mechanisms of these compounds which inhibit or delay lipid oxidation in the lipid oxidation process.

In this study, lipid peroxidation rates of natural walnut oil, sunflower oil, olive oil, corn oil, Argan oil and Rosehip oil were examined at 37 ° C and pH 7. In the experimental phase of this study, Fe (III) SCN was used to examine the primary oxidation products and TBARS analytical methods were used to examine the secondary oxidation products. Trans fat rates of these oils were also examined by chromotographic methods and iodine numbers were determined.

Materials and Methods

Absorption measurements were recorded with SHIMADZU UVM-1240 UV-Visible spectrophotometer (Shimadzu Corp., Kyoto, Japan). All experiments were performed at 37 ° C in a NUVE BM 30 Circulation Water Bath. Chromotographic measurements were performed using a Shimadzu GCMS QP 2010 ULTRA instrument at RTX-5MS Capillary column (30m; 0.25mm; 0.25µm) and 200oC ion source temperature. All chemical compounds of the analytical reagent grade were purchased from Sigma-Aldrich Co. LLC. Distilled water was used for all operations. The oils used were fresh from Doğavita İlaç Gıda Sanayi ve Ticaret A.Ş. (Çiftcizade, Türkiye) and the production methods are summarized in Table 1.



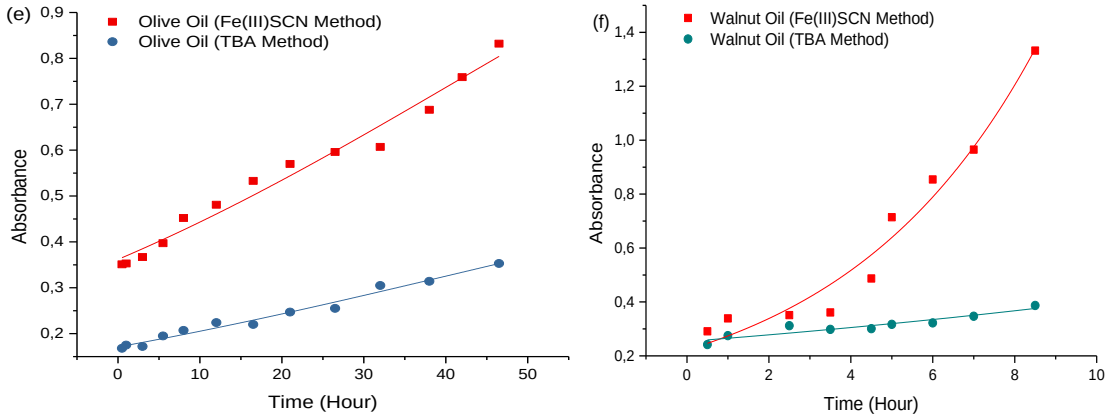


Figure 1: Absorbance-time graph of oxidation of (a)Argan, (b)Rosehip, (c)Sunflower,(d) Corn,(e) Olive, and (f)Walnut oils (Fe (III) SCN, and TBARS method measurements)

Preparation of Oil Emulsions

Span 80 and Tween 80 emulsifier mixture for all O / W emulsions was prepared as HLB value 10. A stock solution of CuCl2, as a chelator for catalytic oxidation of oils, was prepared at a Cu(II) concentration of 0.01 M. Amonium acetate buffer (1M, pH = 7) solution were used for incubation medium. (The concentration in the emulsion solution is 0.9 M as 90 mL NH4Ac in 100 mL emulsion solution).

$CH_3COONH_4 \rightarrow CH_3COO^- + NH_4^+$

According to $I = \sum \frac{1}{2} c_i . z_i^2$ equation, I = 0.9.

0.3 g of oil was weighed into a 100 mL volumetric flask. 3 mL of stock solution was added as an emulsifier (Tween 80 + Span 80, HLB: 10, 0.3 g in 3 mL ethanol) (Hassan, 2015). 2 mL of ethanol and 90 mL of NH4Ac (1M, pH = 7) buffer solution was slowly added to the mixture and emulsified by stirring through a magnetic stirrer.

Measurements by GC-MS for fatty acid methyl ester (FAME) analysis

The oils were analyzed by GC-MS fatty acid methyl ester analysis (FAME) according to the IUPAC standard method (IUPAC, 1992). Accordingly, the esterification pretreatment was carried out by treating 0.1 g of sample with 10 ml of n-hexane and adding 0.5 ml of 2N methanolic KOH solution. The oven temperature program is 5 minutes at 90˚C, 4˚C / min increase from 90˚C to 250˚C, 5 minutes at 250˚C.

Kinetic Measurements:

For spectrophotometric measurements, 0.1 mL samples were taken at different time intervals throughout the incubation. This 0.1 mL sample; 4.7 mL of liner containing 0.02 M iron (II) chloride solution prepared in 0.15 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 3.5% HCl was added. The mixture was then allowed to stand for 3 minutes and the absorbance at 500 nm was read across the blanks containing all the components except the oil emulsion and sigmoidal curves were obtained giving changes in absorbance versus time (Yıldoğan-Beker et al., 2011).

Secondary oxidation products, symbolized as malondialdehyde in the oil emulsion, were determined by the TBARS method. At certain time intervals from the standard and sample solutions incubated for this, 0.1 mL of the sample was mixed with 0.15 mL of Trichloroacetic Acid (TCA, 2.8%), 0.1 mL of Thiobarbituric Acid (TBA, 1%). And then 2.65 mL purified water was added to test tubes to give a total volume of 3 mL. It was

stored for 15 min in a water bath at 95-100 ° C. It was cooled and 1 mL of ethanol was added. Shaken and the absorbance was measured against a control band of 532 nm (Ohkawa et al., 1979).

Results and Discussion

Iodine Indexes in Oils

Iodine indices, a measure of the degree of unsaturation of the oils, were assigned to six different fat samples. According to this, as shown in Table 1, the iodine indices are bigger and smaller for Kuşburnu, Walnut, Sunflower, Corn, Argan and Olive oils 183,58> 164,97> 140,22> 128,12> 97,29> 86,28 was found. As can be seen, Rosehip and Walnut oils have more unsaturated fatty acids, while Argan and Olive oil have less unsaturated fatty acids. Although nutritional experts appreciate the high levels of unsaturation of fatty acids found in vegetable oils, they cause serious technological problems due to their greater susceptibility to oxidation (Kowalski, 2007).

Table 1: Provision of oil used in working

Oil name	Oil Botanical Name	Obtaining Method	Iodine Indexes (gI_2 / 100g)
Walnut Oil	Juglans Regia	Cold Press	164.97
Rosehip Oil	Rosa canina	Cold Press	183.58
Argan oil	Argania Spinosa	Cold Press	97.29
Sunflower oil	Helianthus annuus	Extraction	140.22
Corn oil	Maize oil	Extraction	128.12
Olive Oil	Olea europaea	Precision and extraction	86.28

GC-MS data in fats

GC-MS analyzes carried out using IUPAC standard method are given in Table 2 for Argan, Sunflower, Corn, Olive, Walnut and Rosehip oils. The gas chromotograms of each oil found are shown by the nomenclature based on the carbon number. According to this, in each oil, the 18-carbon fatty acids are found as linoleic acid (9,12-Octadecadienoic acid (Z, Z)), oleic acid (9-Octadecenoic acid, (E)) and stearic acid methyl ester a variety of fatty acids are generally observed between 16 and 24 carbons. Unlike these oils, Argan oil also has fatty acids ranging from 8 to 14 carbon atoms.

Table 2: Fatty acid composition and trans fatty acid content (%) of Argan, Sunflower, Corn, Olive, Rosehip and Walnut oils

Fatty acids	Argan Oil	Sunflower Oil	Corn Oil	Olive Oil	Rosehip Oil	Walnut Oil
C 8:0	0.12					
C 10:0	0.06					
C 12:0	0.57					
C 14:0	0.25				0.05	
C 16:0	6.66	6.29	10.9	12.7	6.35	6.66
C 17:0	0.03	0.02	0.05	0.14		0.03
C 18:0	4.15	4.85	2.8	3.97	4.2	3.16
C18:0 c9,c10 (ep.-9)**	0.11					
C 20:0	0.53	0.27	0.46	0.56	0.35	0.14
C22:0			0.25			
C23:0 (iso-20)	1.09	0.82		0.15	0.92	0.06
C24:0		0.22				

Σ SFA*	**13.57**	**12.47**	**14.46**	**17.52**	**11.87**	**10.05**
C16:1w7	0.08	0.06	0.07	0.86	0.08	0.08
C17:1				0.21		
C18:1						31.07
C18:1w9	33.74	30.38	31.81	74.46	32.29	
C20:1w9	0.33	0.11	0.34	0.31	0.16	0.21
Σ MUFA*	**34.15**	**30.55**	**32.22**	**75.84**	**32.53**	**31.36**
C14:2 c1,c13 (T)*						
C16:3						0,3
C18:2w6	49.93	56.99	53.12	6.64	55.6	57.5
C18:3			0.15			
C18:3 c9,c12,c15-(Butyl)**						0.16
C20:2						0.06
Σ PUFA*	**49.93**	**56.99**	**53.27**	**6.64**	**55.6**	**58.02**
C18:1 t9 (1,3-Dielaidin)						0.27
C18:2 c9,t11			0.05			
C18:3 c9,t11,t13	2.22					
Σ Trans	**2.22**		**0.05**			**0.27**
Bicyclo[10.1.0]tridec-1-ene						0.18
Decyl cyclohexanecarboxylate						0.11
Σ Diğer						**0.29**

Evaluation of kinetic parameters for primary and secondary oxidation product formation:

In accordance with the pseudo first order equation during the oxidation of the oils, hydroperoxides and k_2 velocity constants as primary products and aldehyde and ketones as secondary products are formed with k_1 rate constant. In the following autocatalytic parallel and successive reactions;

$$L \xrightarrow{k_1} LOO^{\cdot} \rightarrow LOOH \rightarrow MDA \quad (1)$$

$$L \xrightarrow{k_2} MDA \quad (2)$$

L, LOOH and MDA respectively represent lipid, lipid hydroperoxide and malondialdehyde. Pseudo first order velocity constant (k) is calculated from the slope of the ln [(1 - A) / A] curve according to t ;

$$\ln (1 - A_t) / A_t = \ln (1 - A_0) / A_0 - kt \quad (3)$$

A_0 is the absorbance at the beginning and A_t is the absorbance (A_{500} nm or A_{532} nm), which is proportional to the total concentration of hydroperoxides or aldehydes at time t. Ferric thiocyanate (Fe (III) SCN) (pseudo first-order rate constants measured by Fe (III) SCN) and thiobarbituric acid-reactive substances (TBARS) methods were specifically named k1 and k2, respectively. In addition, the absorbance-time graphs of the oxidation of Argan, Rosehip, Sunflower, Corn, Olive and Walnut oil are shown in Figure 1 after Fe (III) SCN and TBARS measurements.

The rate of hydroperoxide formation was reported to be higher than the rate of degradation in the first two hours of oxidation (Bondet et al., 2000). According to this, when the formation of primary oxidation products of oils were examined, it was seen that Walnut oil oxidation occurred in 0-8 hour period and the maximum speed constant was $(5.11\pm1.45)x10^{-1}$ hours^{-1}. The lowest oxidation rate constant was $(0.42\pm0.02)x10^{-1}$ hours^{-1}

in olive oil. Hydroperoxide formation rates for oils Walnut oil> Argan oil> Rosehip oil> Sunflower oil> Corn oil> Olive oil followed the order. The autoxidation rate is largely dependent on the rate of formation of the fatty acid or acylglycerol alkyl radical. The relative oxidation rate of oleic, linoleic and linolenic acids has been reported to be 1:40 to 50: 100 depending on the uptake of oxygen (Min et al., 1992).

Unsaturated fatty acids prevent atherosclerosis and coronary heart disease, and monounsaturated fatty acids have been shown to reduce plasma LDL-cholesterol levels (Wolfram, 2003). Although unsaturated fatty acids are highly effective in health, saturated fatty acids are subject to less peroxidation than unsaturated equivalents (Rael et al., 2004). Fatty acid composition is composed of unsaturated fats and oils are oxidized faster (Parker et al., 2003). Accordingly, we can associate the oxidation rates of walnut oil, argan oil, rosehip oil, sunflower oil, corn oil and olive oils with the saturated and unsaturated fat ratios in Table 3. When the total percentages of total polyunsaturated fatty acids (Σ PUFA) and total monounsaturated fatty acids (Σ MUFA) are calculated in Table 2, Walnut oil (89.38% + 0.27% (trans) + 0.29% other fatty acids), Argan (84.08% + 2.22% (trans)), Corn (85.49% + 0.05%), Rosehip (88.13%), Sunflower (87.54%), Olive (82.48%) was found. These results are consistent with the large to small sequence of lipid oxidation rates calculated for primary oxidation products.

Table 3. Kinetic data obtained from Cu (II) -induced oxidation of argan, rosehip, olive, sunflower and corn oil emulsions.

Oil Species	Fe(III)SCN Method *$k_1 \pm S_k$ (min $^{-1}$) x10^{-1}	r^2	TBARS Method *$k_2 \pm S_k$ (min $^{-1}$) x10^{-1}	r^2
Walnut Oil	(5.11±1.45)	0.620	(0.69±0.06)	0.919
Rosehip Oil	(1.91±0.23)	0.917	(0.69±0.07)	0.928
Argan oil	(3.67±0.81)	0.767	(0.54±0.05)	0.944
Sunflower oil	(0.99±0.08)	0.929	(0.13±0.01)	0.858
Corn oil	(0.63±0.05)	0.920	(0.11±0.01)	0.777
Olive Oil	(0.42±0.02)	0.960	(0.21±0.01)	0.975

*(k1and k2 are pseudo-first order rate constants with respect to hydroperoxides and malondialdehyde formation)

When the formation of secondary oxidation products of oils were examined, walnut oil and rosehip oil oxidation were found to have the same rate constant. In spite of this, Figure 1 shows that walnut oil has increased with higher absorbances in the range of 0-10 hours. Rosehip oil in the range of 0-20 hours compared to the walnut oil was observed to have lower absorbances. Corn oil was found to have the lowest rate constant in terms of secondary product formation.

Malondialdehyde and secondary product formation rates for oils Walnut oil = Rosehip> Argan oil> Olive oil> Ayçicek> Corn oil followed the order. The high proportion of unsaturated fats leads to the rapid development of secondary products, as in the case of primary products. Walnut and rosehip oils have been found to have nearly equal levels of unsaturated fat and equally equal rate constants. However, although olive oil has the lowest unsaturated fat content in total, it has a slightly higher rate constant than corn and sunflower oils in terms of secondary product formation. According to this, it is very difficult to explain secondary product formations with saturated and unsaturated fat ratios.

Conclusion

Today, many studies show that the degree of unsaturation in the oil is an effective factor in the rate of oxidation. However, we can see that in primary and secondary product formation in lipids, oxidation rates are associated with fatty acids in very few studies. These results, of course, alone are not sufficient to examine the rate constants. However, it can produce a comparative idea about the oxidation rates of oils. According to these results, the differences in the fatty acid structure, such as the level of unsaturation and the position of the double bonds and the stereoisomeric configuration, have shown that it can affect the fatty acid oxidation rate.

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