Measurement of Peroxide Contents in Radiooxidized Dietary Fats by Chemiluminescence Technique

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Summary: Chemiluminescence (CL) method was employed for estimation of peroxide contents in red palm oil (RPO), sunflower oil (SFO) and vegetable ghee (VG), generated by radiolytic stresses. To evaluate the suitability of this method, a set of samples were irradiated 5-120 krad with gamma rays using Co-60 source. The untreated samples were considered as control. Radiolytic oxidative stresses significantly ($p<0.05$) increased the formation of peroxides. A linear response of CL intensity was observed with the increase in radiation doses. A positive correlation was observed between chemiluminescence intensity and peroxide value. The RPO was found to be more stable than other oils owing to the presence of tocopherol contents and β - carotene.Chemiluminescence technique is sensitive, convenient, fast, and may be used as a supplement to the standard chemical methods for quality assessment.

Introduction

Vegetable oils are very important because of their high content in mono- and polyunsaturated fatty acids when compared to animal fats [1]. Oxidation of unsaturated fatty acids is the main reaction responsible of the degradation of lipids. The oxidation level of oil and fat is an important quality criterion for food industry. Oxidation of the oil not only produces rancid flavors but can also decrease the nutritional quality and safety by the formation of oxidation products, which may play a role in the development of various diseases [2-4]. Among the existing technologies for food preservation, irradiation is recognized as a safe and effective method for a range of specific applications. The use of this treatment on fresh food could extend shelf life of the food and protect the host against pathogenic bacteria [5]. The gamma radiation has been successfully used against fungal spoilage of oil seeds. On the other hand, irradiation treatment brings about some biochemical changes that can affect the nutritional adequacy of food. It particularly specially affects the quality of oil by increasing its oxidation rate. It may produce active species (free radicals) which initiate certain chemical reactions and thus result in the rancidity of oils or fat [6]. Several investigators suspected the lipid fraction as a major contributor to the irradiated odor of complex fat containing foods [7].

Radiolytic stress speeds up the oxidative reactions, and thus lipid oxidation is a major concern in irradiation of lipid containing foods. Various carbonyl compounds have been isolated and characterized from irradiated meat fats [8]. Radiation brings about changes in the composition of oils and fats but the most volatile components such as terpene hydrocarbons were found resistant to irradiation [7]. Ionizing radiations effects the fatty acid composition of natural fats and the lipid peroxide formation as a result indicate that the peroxide value of fats and oils would increase with radiation [9]. Generally, it is conceded that the principal route of this deterioration and possible economic loss of vegetable oils is through rancidity resulting from oxidation which takes place at the double bond in triglycerides [10]. The irradiated fat odor produced in beef and pork fats was detectable at 2 Mrad was more intense at higher doses. Alkanes, alkenes and alkadienes were major radiolytic product in these samples [11].

Several methods have so far been reported for the estimation of deterioration of edible oils, such as peroxide value, carbonyl value, thiobarbituric acid value and detection of volatiles [12-18]. Among these chemical indices, peroxide value is the most commonly used because of its direct indication of the hydroperoxide content of oils [17,19]. Some earlier workers [20] observed extra weak photon emission, or low-level chemiluminescence, one of the physicochemical phenomena observed during oil deterioration, generally originates from electrically excited states, such as singlet molecular oxygen and

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triplet carbonyls, provoked by radical chain reactions in lipid per oxidation. Chemiluminescence method can be applied for the detection of low levels of lipid hydroperoxides [21].

In this study we examined the applicability of chemiluminescence method for the rapid estimation of peroxide contents of red palm oil (rich source of β-carotene and tocopherol natural antioxidants) sunflower oil and vegetable ghee under radiolytic stress. The changes in chemiluminescence intensity with the increase of radiation dose were compared with peroxide value, conventional parameter used to determine the extent of oxidation in oils.

Results and Discussion

Chemiluminescence (CL) method is based on the reactions of free radicals produced as a result of oxidation. The light is emitted in the form of short pulses, correlates with the amount of radicals produced [22], it can therefore, be used to estimate the extent of oxidation. Luminescence was recorded after the addition of luminol sensitizers to the sample. The data regarding POV is presented in Table-1, revealed an increasing trend with increase of absorbed dose of radiation . The increase in peroxide value is a strong indicator of continuous deterioration of the oil [23]. Peroxides and their oxidative products are also associated with the off-odors [24]. Maximum increase was observed in case of SFO (4.3 to 23.3 meq/kg), followed by VG (3.49 - 12.50 meq/ kg) and a slight increase was observed in RPO (12.49 - 14.93meq/kg). The results showed that SFO oxidized more than other test oils, due to presence of unsaturated fatty acids. These observations are in line with the work of A. S. Huang [25], who reported that high polyunsaturated fatty acids, especially linolenic acid (18:3n-3), is prone to oxidation, hydrolysis, and thermal degradation during heating

The effects of irradiation on fatty acid, ester, triglycerol, natural fats and fat containing foods and cooking oils have also been reported earlier [26]. Irradiation enhances lipid oxidation, as triglycerides in the oils are not stable under ionizing radiation and convert into free fatty acids. Unsaturated fatty acids are also prone to auto-oxidation in the presence oxygen [27].

The formation of peroxide contents of oxidized and nonoxidized oils and fats has also been determined by chemiluminescence technique using Luminol photosensitizer. The CL results for RPO, SFO and VG under the influence of gamma irradiation (0, 10,40,60,80,100 and 120 Krad) are shown in Tables-2. It reflected that maximum CL response was observed in SFO, followed by VG and minimum response was observed in RPO. The radiolytic stress accelerated the production of free radicals, and the amount of light emitted in the form of CL intensity, which can be correlated with amount of radical produced.

Table-1: Effect of irradiation on peroxide value (meq/ kg).

	Dietary Fats		
Radiation Dose(k rad)	SFO	VG	RPO
0	4.3466^{e}	3.49d^{d}	0.61^{f}
5	10.226^{c}	4.90^{d}	1.18^{e}
20	8.23^{d}	7.50^{c}	1.45^{d}
40	9.81^{c}	8.85^{bc}	2.04^{c}
60	10.56^{c}	9.46^{b}	2.28^{b}
80	13.45^{b}	11.40^{a}	2.87^{a}
100	23.33^{a}	12.50^{a}	3.75^{a}
LSD, 5 %- sample	0.0052	0.0052	0.0345

Values sharing common letters are not significantly different

Table-2: Comparative effect of irradiation on chemiluminescence intensity of different oils.

	Dietary Oils			Mean
Radiation Doses (krad)	RPO	SFO	VG	
0	1005^{q}	2323^{g}	1428^{mno}	1586^{g}
10	1173^{p}	2700^{f}	1627^{kl}	1833^{f}
40	1323^{op}	3347^{e}	1880^{ij}	2183^{e}
60	1370no	3600^{d}	1937^{i}	2302^{d}
80	1503^{lmn}	4093^{c}	2030^{hi}	2542^{c}
100	1563^{lm}	4833^{b}	2147^{h}	2848^{b}
120	1730^{jk}	6507^{a}	2337^{g}	3524^{a}
Mean	1381^{c}	3915^{a}	1912^{b}	
LSD value radiation dose = 87.54				
LSD value oils x irradiation =151.6				

Values sharing common letters are not significantly different

To see the existence of a significant regression of the increased CL values with the increase of absorbed doses, the correlation coefficient, and regression coefficient and regression equation of data were worked out. The good to strong correlation were observed for all samples as indicated in Fig. 1.

Radiolytic, stresse significantly ($p<0.05$) increased the production of peroxides and hydroperoxides so the CL intensity linearly increased with the increase in radiation dose It was thought worthwhile to further investigate whether a significant regression in the increase of peroxide value and CL intensity exist under influence of irradiation, and the regression lines of peroxide value of the test samples have been drawn (Fig. 2).

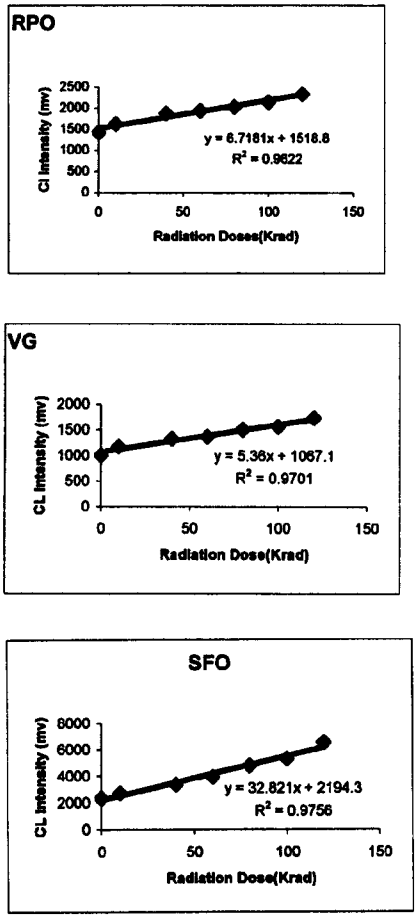

Fig. 1: Correlation between Radiation dose and CL intensity.

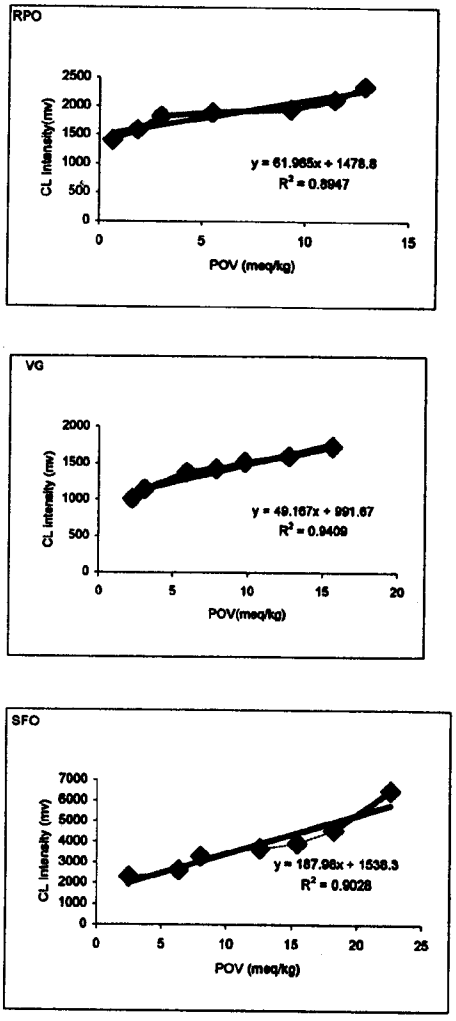

Fig. 2: Relationship of CL intensity (mv) and peroxide value (meq/ kg) under radiolytic stress.

The chemiluminescence intensities of RPO, SFO and VG showed a proportional increase with peroxide values and the relationship was expressed by equation indicated in the Figures, where x is peroxide value (meq/kg) and y is the amount of CL value in mv. The increase in CL was found positively correlated to the peroxide value as evident from R-values. Lipid hydroperoxide is the primary product of lipid oxidation. The detection of low levels of lipids hydroperoxides is very important to estimate the progress of oxidation. Chemiluminescence is sensitive and convient method, can be applied to the detection of low levels of lipid peroxides [28-31].

Different plant nuts were radiated with gamma rays of 0.5, 1.0 and 1.5 KGy. The lipids were extracted and it was observed that peroxide, anisidine and FFA values were higher in Irradiated samples [32].The organic peroxides in irradiated and non irradiated pork were found to increase. The increase was of 7.4 times greater than background in 1 KGy irradiated minced meat of pork [33].

The CL intensity of RPO showed low level of peroxide formation as compared to other test oils. This is due to presence of carotenoids and tocopherols. Carotenoids have antioxidant properties as radical scavengers and singlet oxygen quenchers in lipid oxidation [34]. The presence of natural antioxidants in RPO could inhibit the further

decomposition of hydroperoxide by reacting with alkoxyl radicals (RO˙), which are mainly responsible for the generation of volatile compounds, contributing to rancidity. our results are in agreement with findings of other reseacers [35], used Fourier transform Raman spectroscopy for direct minitoring of lipid oxidation in edible oils and observed that corn oil showed more stability due to presence of carotenoids and tocopherols, while peanut and rapeseed oil showed lesser degree of stability probably due to the higher content of linoleinic acid, which is much more prone to oxidation than linoliec and oleic acid [36].

Experimental

Sample collection and irradiation, The red palm oil was obtained from the Malaysian Palm Oil Board (MPOB) through their regional office at Karachi-Pakistan.The sunflower oil and vegetable ghee was procured from a super store of Peshawar. The samples were irradiated @ 0,10,40,60,80,100 and 120 Krad with gamma rays usining cobalt-60 source (ISSLE DO VATEL, CIS) installed at Nuclear Institute for food and Agriculture (NIFA), Peshawar Pakistan. The non-irradiated samples were considered as control. The quality changes induced by different doses of gamma radiation were determine by analysis of the oils for the quality parameter such as POV determined by the AOCS method [37].

Chemiluminescence Measurement

Chemiluminescence's response of Irradiated and control samples was measured using luminol sensitizer. The samples were put in sample port of Bio-orbit 1250 luminometery system. The CL response was recorded as peak height on the chart recorder. The chemiluminescence measurements can serve as a simple and rapid procedure for estimation of peroxide content of red palm oil (RPO) sunflower oil (SFO) and vegetable ghee (VG) samples stressed by high dose of gamma radiation

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