Effect of Saturation and Micro Nutritional Status on Stability of Dietary Oils under Photooxidative Stress Condition

TAUFIQ AHMAD*, SHAHEEN ATTA, AURANG ZEB AND SAEED GUL
Nuclear Institute for Food and Agriculture, *Tarnab Peshawar*, *Pakistan.*

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Summary: Various commercial fats were photooxidized. Objective test methods *i.e.*, peroxide value (POV), free fatty acid (FFA), anisidine value (AV), iodine value (IV), color index, fatty acid profile radical scavenging activity (RSA) and Chemiluminescence (CL) method were applied to observe the changes that take place when lipid is oxidised. Result revealed a significant ($P<0.05$) positive correlation between change in quality indices and storage conditions. Among the samples Red Palm Oi\l (RPO) showed greater stability as reflected by least 18:2 to 18:1 ratios, balanced fatty acids profile and presences of higher amount of natural antioxidants.The highest Radical Scavenging activity (RSA) was also noted in RPO followed by Vegetable Ghee (VG) and Sunflower Oil (SFO). A proportional positive relationship was observed between physicochemical characteristics of oxidized fats and their RSA while linear response of Chemiluminescence (CL) intensity was found with the increase in POV.

Introduction

Vegetable oils are becoming increasingly important because of their high content in mono- and polyunsaturated fatty acids as compared to animal fats [1]. Dietary oils are very prone to deterioration by the action of oxygen, light and temperature. 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 triglyceride molecule. Oxidation of oils not only produces rancid flavors but also decrease the nutritional quality and safety by the formation of oxidation products [2-6]. Auto and photo-oxidation results in rancidity of food items, whereby these processes convert fatty acid esters of oils into free fatty acids. Furthermore, the free fatty acids give rise to smell that is observed in many vegetable oils over time [7]. Lipid oxidation is one of the processes associated with the deterioration of quality in foods, especially in the fat products. The change in quality is manifested by the formation of a number of volatile secondary products, due to adverse changes in physico chemical properties and possible production of toxic compounds [8]. Auto-oxidation is the major oxidative reaction in oils and involves the formation of free radicals in the presence of initiators [9].

The other common oxidation reaction in oils is photo oxidation, which is a much faster reaction that involves the attack at double bond through a singlet $^1O_2$ species. It is has been postulated that the double bond within a fatty acid molecule may be capable of capturing outside source of energy, such as heat and light, to reach a critical excitation level [10]. At this stage the double bond may break thus giving rise to a free radical species, which may in turn generate more free radicals which produce reactive oxygen species (ROS) that may attack cellular components, causing damage to lipids, proteins and DNA etc. This can initiate a chain of events which result in the onset of diseases, such as carcinogenesis, inflammation, early aging and cardiovascular disorders [11-13].

Antioxidants are the principal ingredients, which protect food quality by retarding oxidative breakdown of lipids. Radical scavenging is the main mechanism by which antioxidants protect foodstuffs [14]. Free radical scavengers such as vitamin C, anthocyanins and phenolics are most effective against auto-oxidation, whereas singlet oxygen quenchers, such as flavonoids, β-carotene and tocopherols are the most effective against photo-oxidation [15]. The Beta carotenes, along with vitamin C and E are collectively known as biological antioxidants are part of the body's natural defense against lipid peroxidation and its subsequent damage.These natural antioxidants deactivate free radicals, which can otherwise damage other healthy molecules [16]. Carotenoids have also been reported in some crude vegetable oils, but their levels are generally much lower, *i.e.* < less than 100 ppm [17]. The oil from palm is the richest known source of biologically active carotenoids. Red palm oil is considered the world richest source of carotenoids, β -carotene 500-700 ppm and Vitamin E 800ppm [18]. Apart from nutritional implications, carotenoids (α, β and lycopene) have significant antioxidant properties, to act as effective quenchers singlet oxygen and are hypothesized to play a protective role in cellular aging, atherosclerosis and cancer [19, 20].

*To whom all correspondence should be addressed.

Pakistan has a diverse environmental conditions (hot and humid). The retailers and wholesale dealers store their product in open air and natural or artificial light for publicity or lack of storage space. This problem is becoming increasingly serious due to the high intensities of lighting used during food display which causes quality deterioration and such product expires before their mentioned expiry date. It was considered important to conduct study on quality of various commercial fats oxidized by the action of light simulating the prevailing market environments.

Results and Discussion

Oils and fats generally consist of saturated and unsaturated fatty acids. RPO and VG contain a majority of saturated fatty acids whereas SFO contain mainly unsaturated fatty acids (Table-1). Linoleic acid was present in small amount in RPO, while SFO contained about 66.44% of this acid.

Among the saturated fatty acids, palmitic and stearic were the only ones, present to an appreciable extent in all of the three samples. The results show generally that under all the storage conditions, there were decreases in linoleic acid (C18:2), whereas palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) were increased. The results revealed that the decrease in C18:2 and the increase in C16:0, C18:0 and C18:1 among all the test conditions was higher in samples kept under sunlight. The data also indicated that this trend was higher in SFO as compared to other oils, so the oxidation process progresses more rapidly in SFO than VG and RPO.

In the current study the level of PUFA tended to decrease, whereas that of saturated fatty acids increased. In Table-1, it could be noticed that the concentration of total saturated fatty acids increased due to the increase in palmitic acid and stearic acid content. This increase was corresponding with reduction in total unsaturated fatty acids. On the other hand, the ratio of linoleic acid to palmitic acid tended to decrease and maximum decrease was found in SFO, while in RPO and VG, the drop in C18:2/C16:0 ratio was lower to a great extent than that of SFO. Results indicated that RPO was found more stable than SFO and VG with regards to changes in the fatty acid composition due to the fact that unsaturated fatty acid oxidized more by action of environmental factors.Peroxide value is a widely used to measure the amount of peroxides, (primary oxidation products) formed in fats and oils during oxidation [21]. Effects of storage conditions and time on POV showed that it increased almost linearly with the increase of storage time. The RPO remained stable as compared to VG and SFO. Data also revealed that stability of the oils was greatly affected by sunlight. It is clear from the results that under the influence of sunlight the highest POV was noted in case of SFO (25.67 meq/kg) followed by VG (22.15 meq/kg). While the RPO was found stable (19.73 meq/kg) as compared to other oils. Similar increasing pattern was observed in samples stored under artificial light. The rates of peroxidation after storage of 8 months under different conditions as well as the ratio of unsaturated to saturated acids together with ratios of 18:2 to 18:1 and 18:3 to 18:2 are presented in Table-2.

Table-1: Changes in fatty acid composition (%) of different oils after storage (8 months) under different conditions.

Fatty acids	Storage conditions								
	SFO			VG			RPO		
	Ambient	F. light	S.light	Ambient	F. light	S.light	Ambient	F. light	S.light
C14:0	0.06	0.07	0.09	1.91	1.95	1.99	0.95	1.05	2.05
C16:0	6.12	6.58	6.72	37.38	38.1	38.7	38.8	39.9	40.7
C18:0	3.59	3.85	4.35	8.11	11.62	13.3	4.57	4.63	4.72
C18:1	23.5	24.9	25.5	35.35	34.5	36.7	40.9	42.02	42.8
C18:2	66.5	63.4	62.4	23.8	23.0	22.9	13.1	12.3	12.1
C18:3	0.18	0.12	0.12	-	-	-	0.36	0.31	0.29
Others	2.68	2.70	2.70	0.82	0.75	0.82	2.51	1.45	1.30
TSFA	9.77	10.5	11.16	47.40	51.67	52.59	44.32	45.58	47.47
TMUFA	23.5	24.9	25.5	35.35	34.5	36.7	40.9	42.02	42.8
TPUFA	66.68	63.52	62.52	23.8	23.0	22.9	13.46	12.61	12.39
S/P	0.146	0.165	0.178	1.99	2.24	2.60	3.10	3.60	3.89
C18:2/C16:0	10.86	9.5	9.28	0.636	0.60	0.59	0.358	0.308	0.28

F.light=Flourescent light, S.light=Sunlight , TSFA= Total saturated fatty acids, TMUFA=Total mono unsaturated fatty acids, TPUFA= Total poly unsaturated fatty acids, S/P= Saturated/ poly unsaturated

Table-2: Peroxide values (meq/kg) of oils and fats exposed to different storage conditions for 8 weeks and ratios of unsaturation.

Oils	POV /Storage conditions			Fatty acid ratios		
	Ambient	F. light	S.light	Unsat/Saturated	18:2/18:1	18: 3
RPO	6.7	19.73	12.78	1.24	0.27	0.02
SFO	9.92	25.67	17.11	9.23	2.85	-
VG	8.00	22.15	15.18	1.25	0.56	-

F.light=florescent light, S.light=Sunlight

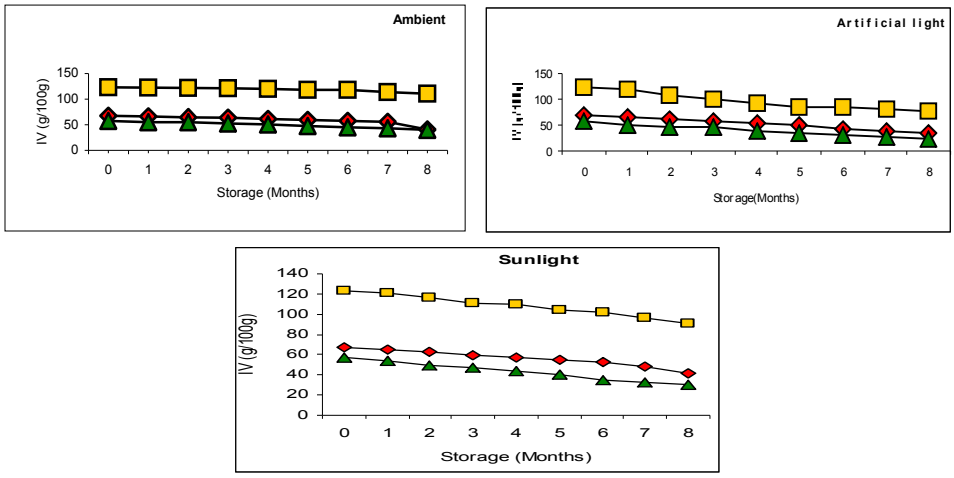

Fig. 1: Effect of Conditions on the Iodine Values of Red Palm Oil■, Sunflower Oil■ and Vegetable Ghee ▲.

The results revealed that samples with high unsaturation exhibit correspondingly high rates of oxidation. Comparison of these ratios with the POV also showed that the rate of oxidation depend entirely on the degree of unsaturation. Most lipids contain fatty acids that are attached to the lipid molecule through carboxylic ester linkages, hydrolysis of these linkages liberate free fatty acids. The FFA represents the extent to which the glycosides in the oil sample have been decomposed by lipase in to hydrolytic products. Formation of free fatty acids might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture [22]. FFA increased linearly with the increase of storage time. Result revealed that storage under the given set of conditions significantly ($P<0.05$) increase the production of FFA. The initial value of FFA was 0.027 %, 0.112 % and 0.125% for RPO, SFO and VG. The results revealed that the production of FFA in SFO was higher than other test oils because it contains appreciable amount of unsaturates and is thus particularly susceptible to rancidity. On the other hand the RPO was found very resistant towards oxidative deterioration, as it is partially refined and contains substantial quantities of natural antioxidants. Our results are in agreement with the results of some earlier researchers who observed that linoleic acid oxidizes 64 times faster than oleic acid, so in our case due to the presence of higher amount of polyunsaturated fatty acids (66.44 %) in SFO, it oxidized more rapidly [23]. It is also reported that in fat deterioration, the first initiating step is the formation of free fatty radicals, which are susceptible to oxygen attack in the presence of light, resulting in formation of many organic compounds and free fatty acids [24].

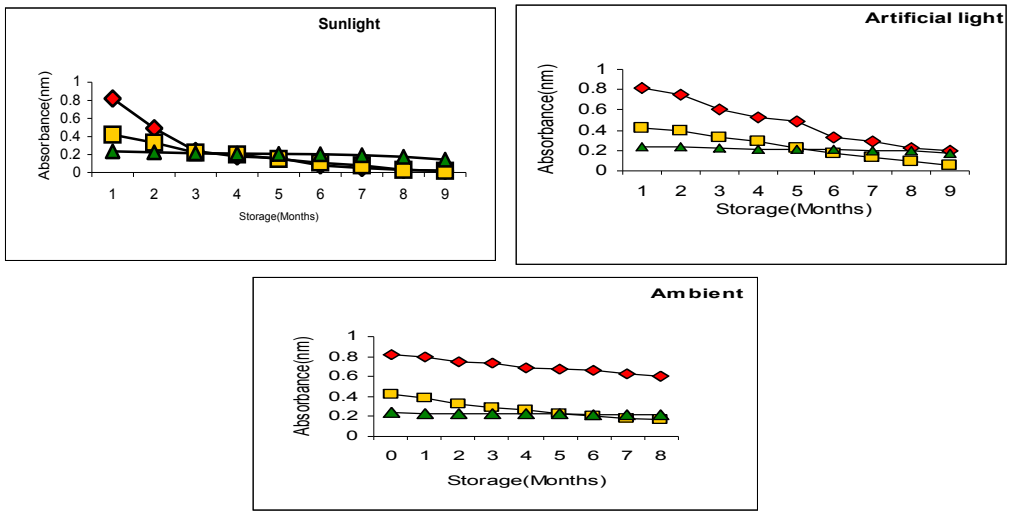

Fig. 2: Effect of storage Conditions on color (absorbance) of Red Palm Oil■ Sunflower Oil■ and Vegetable Ghee ▲.

Iodine value is a measure of overall unsaturation and is used to characterize oils and fats. The results revealed that IV decreased almost linearly in all the samples stored under different conditions and with storage time Fig. 1. The data indicated a significant ($P<0.05$) effect of storage conditions and time was observed for different oils. Maximum decrease in IV was noted in SFO (123.23-78.33 g/100g) followed by VG (67.58-23.98g/100g) and RPO (57.58 -35.23g/100g) kept under sunlight at the end of 8^{th} month storage period. Oxidative rancidity affects first the more unsaturated fatty acids and as it progresses, polyunsaturated fatty acids polymerize, or breakdown to smaller molecules with fewer double bonds and iodine values tend to fall. It was observed that the storage under sunlight and artificial light drastically accelerated oxidation of the test oils and the Iodine values linearly decreased with storage time. The decrease in IV is an indication of increased saturation and is an indicator of lipid oxidation [25].

The colour of oil is one of the important quality parameter. The results revealed a gradual decrease in colour of all the test oils stored under photo oxidative stresses (Fig. 2). The intensity of the colour significantly ($P< 0.05$) affected by the storage conditions. Maximum colour loss (0.42-0.02 nm) was observed in case of SFO samples kept under sunlight and artificial light (0.42-0.05), while in case of RPO the less (0.82-0.42) colour bleaching was observed, owing to the presence of greater number of β-carotene and α- tocopherol, which has stabilizing effect on oil colour. The rate of colour loss is significantly affected by the photo-oxidation reaction [26]. The presence of β- carotene was considered to serve as filter for light of low wavelength. Some other workers also, suggested that quenching of singlet oxygen by β-carotene is an important mechanism against photodynamic damage [27, 28]. The carotenoids in crude palm oil appear to offer some protection against oxidation by themselves being oxidized first prior to the oxidative attack on the triglycerol [16].

Coefficients of correlation among different quality parameters for VG, SFO and RPO under different conditions were calculated. It was noted that the POV in case of SFO was significantly ($P< 0.01$) correlated with FFA ($r =0.987$) and AV ($r =0.995$), when stored under sunlight, whereas a negative significant correlation was observed between POV and IV ($r=-0.989$) and colour ($r =-0.968$) at this particular storage condition. Colour was negatively correlated with POV, FFA and AV but positively with IV. It was observed that in case of RPO, although all the quality parameters were affected by the test conditions, however, non significant correlation was noted among various parameters like POV and FFA ($r=0.687$) and AV($r=0.567$) for this oil stored under sunlight. In samples stored under sunlight condition, it was observed that the correlation matrices of all the three oils showed the same pattern, *i.e.* they are strongly positively or negatively correlated. The correlation of POV with AV and OD seems alike in SFO and VG but this correlation decreased in RPO, it means that the correlation becomes weak for this oil.

The findings are in agreement with our results who stated that the action of major antioxidants in RPO (tocopherols, tocotrienols and carotenoids), are through their free radical scavenging as well as their singlet oxygen quenching activities. These components rendering ineffective the factors, which promote oxidation by interfering with one or more steps, involved in oxidation also found that crude palm oil contains tocopherols and tocotrienols, which impart oxidative stability to the oil [29, 30]. In some relevant studies, rapid oxidation of commercial extra virgin olive oil stored under fluorescent light was found and reported that natural pigments such as chlorophyll played a major role in photo oxidative deterioration [31]. It was stated by other workers that carotenoids like β-carotene or lycopene are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals generated during photooxidation. In the stability study, it was observed that crude palm oil is one of the richest and safest source of important phytonutrients, carotenoids and tocopherols.These components are well retained for up to 12 months of storage under 3 simulated storage conditions prevalent in Malaysia namely super market (20 °C), Kitchen (30 °C) and warehouse (40 °C) [32, 33].

Impact of Storage on Antiradical Performance of Vegetable Oils

Radical scavenging activity measurement might be the possible alternative method. The model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods [34]. Fig. 3 show that the antiradical performance of Fresh and photo oxidized oil. The results revealed that RPO has the highest RSA followed by VG and SFO. After 08 months, 88% of DPPH radicals were quenched by RPO, while VG and SFO were able to quench 75% and 71%, respectively. The significantly stronger RSA of RPO compared to VG and SFO may be due

to (i) the differences in content of bioactive compounds and antioxidants (ii) the diversity in structural characteristics of potential antioxidants present in oils (iii) a synergism of antioxidants with other components present and (iv) different kinetic behaviors of potential antioxidants. All these factors may contribute to the radical quenching efficiency of vegetable oils [35].

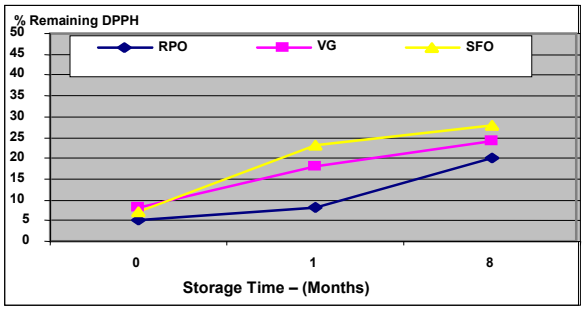

Fig. 3: Scavenging effect of different oils during photo-oxidation on DPPH radical as measured by changes in absorbance values at 515 nm.

Chemiluminescence (CL) method was employed for estimation of peroxide content in RPO, SFO and VG, generated by photo oxidative stresses. The CL intensity of RPO showed low level of peroxide formation as compared to other test oils (Table-3). This is due to presence of carotenoids and tocopherols. Carotenoids have antioxidant properties as radical scavengers and singlet oxygen quenchers in lipid oxidation [36].

Table-3: Correlation coefficient of CL values and different stress conditions.

F.light=Flourscent light, S.light=Sunlight

Treatments	RPO	SFO	VG
S. light	0.9789	0.9336	0.9712
F. light	0.9309	0.9056	0.9510

The presence of natural antioxidants in RPO could inhibit further decomposition of hydroperoxide by reacting with alkoxyl radicals (RO·), which are mainly responsible for the generation of volatile compounds, contributing to rancidity [16]. Recently Chemiluminescence (CL) method was employed for estimation of peroxide content in RPO, SFO and VG, generated by radiolytic stress [37]. The detection of low levels of lipids hydroperoxides is very important to estimate the progress of oxidation. Some researchers reported that Chemiluminescence is sensitive and convenient method can be applied to the detection of low levels of lipid peroxides. The CL method has been applied for estimating the deterioration of edible oils [38-40]. It was thought worthwhile to further see whether a significant regression of the increase of peroxide value and CL intensity exist under influence of 100 ft-c of artificial light and sun light the regression lines of peroxide value of the test samples were drawn (Fig. 4 and 5).

The CL intensities of RPO, SFO and VG showed a proportional increase with POV and the relationship was expressed by equation indicated in the Figures, where x is POV (meq/kg) and y is the amount of CL value in mv. The increase in CL was found positively correlated to the POV as evident from R-values.

Antioxidants present in food commodities provide protection against oxidative attack by intercepting singlet oxygen, decreasing the oxygen concentration, preventing first chain initiation by scavenging initial radicals, binding metal ion catalyst, decomposing primary products of oxidation to nonradical compounds and chain-breaking substances to prevent continuous hydrogen abstraction from substrate [341]. The stability of RPO towards photo oxidation is due to the presence of natural antioxidants *i.e.*, carotenoids, which act as radical scavengers and singlet oxygen quenchers in lipid oxidation [36], which could inhibit the decomposition of hydro peroxide by reacting with alkoxyl radicals RO·, mainly responsible for the generation of volatile compounds, contributing to rancidity [16]. Our results are quite identical to the findings of some recent workers who reported that the oxidative deterioration level of the photo-oxidized SFO was more as compared to that of auto- oxidation [42].

It is concluded from the study that dietary fats may be protected from natural and artificial lights to avoid deterioration. The oil having a balanced fatty acid profile possesses beneficial micronutrients and natural antioxidants showed greater stability than other test oils under all photo stress conditions. These results were also proven by estimation of changes in quality indices using old as well as by applying some relatively advanced techniques. There are number of shopkeepers and wholesale dealers in Pakistan store their oils and fat supplies in open air and light for publicity and also for the lack of storage space. Under such conditions process of lipid oxidation may be more pronounced. The results show that photo oxidation is more pronounced than auto-oxidation. Lipid oxidation products make the oil unfit for human health, therefore in order to minimize the oxidation the oils may not be placed under direct sunlight/artificial light and may be stored in proper packaging in cold dark conditions.

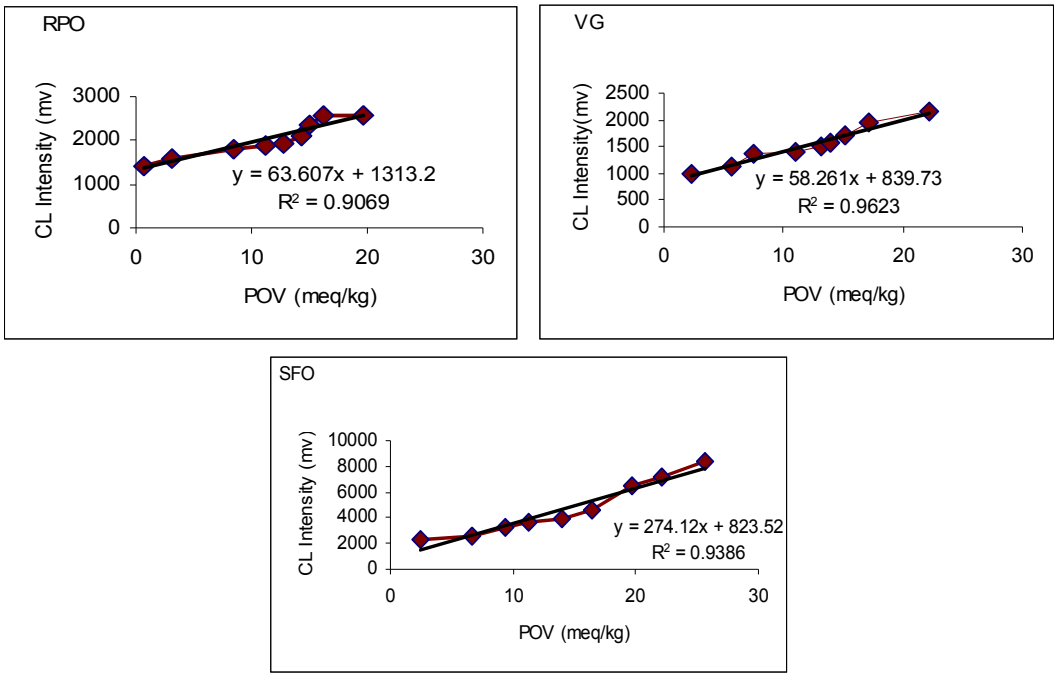

Fig. 4: Relationship between POV and CL intensity under Artificial light.

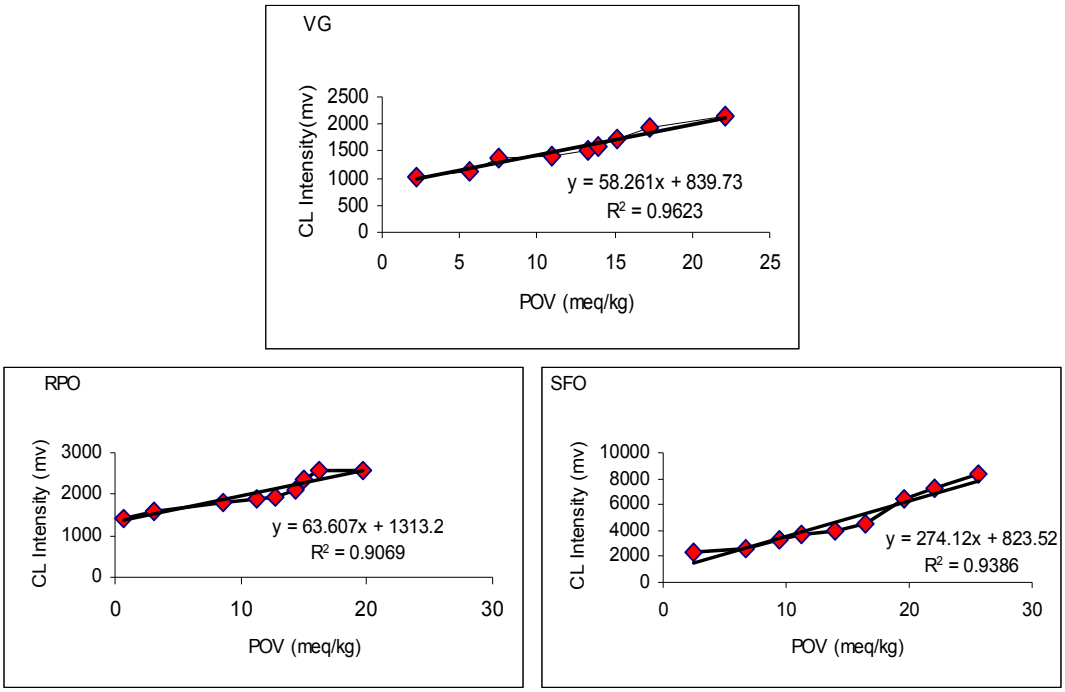

Fig. 5: Relationship between POV and CL intensity under sunlight light.

Experimental

Sample Collection and Storage

Sample of RPO was supplied by Malaysian Palm Oil Board (MPOB), Kuala Lumpur, Malaysia, through their regional office at Karachi, Pakistan while SFO and VG were obtained from open market. One set of test samples were kept under fluorescent light (100 ft candle) while other set of same samples was exposed to open sun light for 08 months in order to determine the storage stability. The sample kept at ambient condition was considered as control.

Physico-chemical Quality Constant Study

The quality constants i.e peroxide value (POV), free fatty acid (FFA), anisidine value (AV), iodine value (IV) and color index was determined initially followed by monthly analysis using A.O.C.S (1972) methods [43], while colour index was determined by method of Mancini et al 1986 [44].

Fatty Acid Profile

Prior to the gas chromatographic analysis the oils were converted into corresponding methyl esters by esterification according to the method reported by Shehata *et al.* [45]. The gas chromatographic analysis was performed using Perkin-Elmer gas chromatograph model 3920.

Chemiluminescence (CL)

CL response of photo oxidized and control samples were 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.

Radical Scavenging Activity (RSA)

RSA of the oils and fats was assayed with DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radicals dissolved in toluene according to the method of earlier workers [35]. For evaluation, 10 mg of oil sample (in 100 μL toluene at room temperature) was mixed with 390 μL toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 60 min of mixing using UV-160 visible recording spectrophotometer (Shimadzu,).RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample test}}{\text{Absorbance of control}} \times 100$$

Statistical analysis of the data wherever necessary was conducted for each of the measured traits by analysis of variance (ANOVA- using CRD factorial design) and the means were separated by Duncan's Multiple Range test (DMR) using MStat-C software [46].

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Nomenclature

AOCS	= Association of Oil Chemical Society
AV	= Anisidine Value
CL	=Chemiluminescence
DMR	= Duncan's multiple range test
DPPH	=2, 2-Diphenyl-1-picrylhydrazyl
FFA	= Free Fatty Acid
Ft-c	= Foot Candle
GC	= Gas Chromatography
IV	= Iodine Value
Meq/kg	= Milliequivalent per Kilogram
MPOB	= Malaysian Palm Oil Board
MUFA	= Mono unsaturated fatty acid
NIFA	= Nuclear Institute for Food & Agriculture
OD	= Optical Density
POV	= Peroxide Value
PUFA	= Poly unsaturated fatty acid
RPO	= Red Palm Oil
RSA	=Radical Scavenging Activity
SAFA	= Saturated fatty acid
SFO	= Sunflower Oil
VG	= Vegetable Ghee
B	= Beta

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