"EFFECT OF STORAGE AND HUMIDIC CONDITIONS ON CHEMICAL COMPOSITION OF POMEGRANATE SEED OIL"

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ABSTRACT: Edible oils, the main constituent of daily diet, plays important role to provide energy and maintain metabolic mechanism of body. It is most important to check the chemical composition of oil because several quality problems are associated with quality of edible oils such as oxidative deterioration. Oxidation not effects the quality of oil also makes it unfit for use. Several factors are responsible for oxidation such as; storage, temperature, humidity etc. In present study, the chemical composition of Pomegranate seed oil (PSO) was monitored at different humidic conditions for 65 days by, Fourier transform infrared (FT-IR) spectrometry and conventional titrimetric methods. Oxidation of PSO was assessed by measuring peroxide value (PV) (1.93 -7.35 meq/kg), p-anisidine value (p-AV) (7.83 -36.40), free fatty acid content (FFA) (0.95 -2.19%) and total oxidation value (TOTOX) (11.75 – 51.10).Current study shows that the oxidation influences the quality characteristics of PSO. PV increased from initial day28th day of oxidation and decreased till 281.89 to 3.71 meq/kg. On very first day the PV value of PSO samples were noted. When the PV values of all three samples were compared with values of 28th day of oxidation, an increased trend was observed from day 1st to 28th day. The same increased trend was followed till last day of oxidation whereas slight decreased in PV was noticed till 42nd day. FFA level was observed continuously increased from initial day till last day, the p-AV value also enhanced from day 1st to last day and totox value was also observed in increased order during 65 days of storage at different humidic conditions. FT-IR study illustrated major effect on the CLnA band during oxidation. Furthermore. In conclusion significant variation in the chemical composition of pomegranate seed oil was observed during oxidation at different humidic conditions which also affected the chemical behavour of oil. It was also suggested that humidic condition is not suitable for quality and edible point of view of oil.

Key words: Effect, Storage, Humidic, Pomegranate, Seed oil.

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INTRODUCTION

Basically Pomegranate is the most prominent member of the Punicaceae family; it has various local names in different languages but its botanical name (Punica granatum L.).Pomegranate belongs with category of most important fruits which are mentioned in "holy Quran"'. It has great importance in folk medicine since oldest civilization. Hundreds of varieties of pomegranate were cultivated throughout the world; among them fifty varieties possess great importance due to their commercial value[1].

Due to the high antioxidant properties pomegranate is widely used for medicinal purposes since ancientcivilization[2].Compounds present in pomegranate fruit are very effective to decrease the effect of herpes and influenza viruses also reduce the spread of human breast and cancer cells and chances of bone erosion were also observed reduced after use of pomegranate extracts [3,4].

Mediterranean regions especially China, USA, Japan and Russia are most suitable for the cultivation of pomegranate fruit, including Asian countries[5]. By origin it is considered as plant of north Himalayas of India[6]. The total annual production of pomegranate is expected about 8.1 million tons although there is no precise data regarding to its production..[7].

Pomegranate seed oil has pleasing taste with yellowish appearance[8].The conjugated octadecatrienoic fatty acid which is very essentialconstituent of pomegranate seed oil (PSO) is found about 15% inside it.[9]. pomegranate seed oil also contains tocopherols, phytosterols an exclusive fatty acidin high concentration, among all punicic acid (60 to 80 %) is found in excess[10]. It is itselfan omega-5 long chain polyunsaturated fatty acid also known astrichosanic acid

the alternate name of the punicic acid is also called as trichosanic acid its structure resembles to conjugated linoleic acid (CLA) and α-linolenic acid(LAn)[11].The oil of pomegranate seed contains different types of the fatty acids but among all the fatty acids C18:3, C18:2 and C18:1are very essential type of fatty acids. [12].The fatty acid composition and oil content of pomegranate seeds is mainly affected due to several factors such as; cultivation sites, fruit genotypes; harvesting time and climatic conditions etc.[13].oils having unsaturated fatty acids in their composition are more valuable as compared tothose oils which contains saturated fatty acids in their composition, as compared to some commercial edible oils such as; sunflower oil, soybean oil and coconut oil etc., pomegranate oil has a lower proportion of saturated fatty acids(SFA) and mono unsaturated fatty acids(USFA) and higher proportion of PUFA [14].There are various essential fatty acids commonly known due to their ability to reduce plasma triacylglycerol's and considered helpful against many disease, such essential fatty acids are found in pomegranate seed oil (PSO).Punicic acid which is main ingredient of pomegranate seed oil (PSO) is best known for its ability of anticancer agent.[15]. Total cholesterol level can also be controlled by sufficient intake of PSO. Furthermore, PSO also contains the polyphenols and phytosterols which are very effective for healing of wounds, reducingswollen responses, reduces the aging indications and also decreases the irritation feelings on skin[16,17]. Besides various benefits to human health pomegranate seed oil is also beneficial against cardiac diseases and various types of cancer. [18].The present study was designed to carry out oxidative behavior of PSO at different humidic environmental conditions at different time intervals, chemical composition of fatty acids and antioxidant behavour was observed by using different instrumental and classical techniques.

Required Materials and Methods:

Reagents and Collection of sample: During study all the chemicals which used were from E-Merk (Darmstadt, Germany).these all chemicals includes; ethyl alcohol, sodium hydroxide, like, sodium chloride, n-hexane, iso-octane, p-anisidine methyl alcohol, anhydrous sodium sulphate, acetic acid, magnesium chloride and potassium hydroxide.

For the purpose of study pomegranate seeds were collected from fruit juice extractors of Karachi city. Collected seeds were washed and dried properly finally grinned into powder and stored for further study.

Extraction of Oil: The Oil from powdered pomegranate seeds was extracted by following, AOCS Aa 4- 38 (AOCS 2013) method.

The Soxhlet extraction method was used to extract oil from pomegranate seeds.Seed powder was enfolded into cellulose filter paper and placed in a Soxhlet cartridge.

Oxidation of Pomegranate Seed Oil (PSO) samples: The oxidation of pomegranate seed oil (PSO) samples was carried out at room temperature (RT) and various relative humidic conditions (RH33% and RH75%). For this purpose exactly 250g of PSO sample was taken in three beakers.For desired humidic conditions two solutions were prepared in two desiccators, the solution having relative humidic value 75% was prepared from NaCl and other solution having relative humidic value 33% was prepared from $MgCl_2$. Two beakers of PSO samples were placed into desiccators of desired humidic value and third beaker was kept at room temperature. To check the oxidative behavior, FT-IR spectra of all three samples was recorded three times in a week followed by other chemical parameters related to oxidative behavour of sample.

Determination of free fatty acid (FFA) value: For purpose to check the free fatty acid formation in oxidized PSO samples standard AOCS method Aa 6-38 (AOCS 2013) was followed. By applying this method ethanol was taken in conical flask and few drops of indicator were added (here the indicator phenolphthalein was used)andsame was neutralized for neutralization purpose 0.01N solution of KOH was prepared and used. Neutralization was confirmed by the appearance of pink colour. The neutralized solution was lightly heated after that exactly 2g of oxidized oil samples were added and was titrated till the appearance ofpink colour.

Following mentioned formula was used to calculate the FFA % in oxidized oil:

$$\text{FFA\%} = \frac{28.2 \times \text{mL (KOH)} \times \text{N(KOH)}}{\text{Weight of sample(g)}}$$

Determination of peroxide (PV) value: AOCS method was used to find the peroxide value of pomegranate seed oil. Following this method, two solutions were prepared one solution of oil and chloroform, in which 2g of oil sample and 10ml of chloroform were mixed and dissolved, another solution of acetic acid and potassium iodide was prepared here we mixed 15ml of acetic acid and 1ml of potassium iodide. For 5minutes oil sample solution was kept at room temperature in darkness.Few drops of indicator and 75ml of de ionized water were also added in oil solution after 5 minutes; here the starch solution indicator was used. The sample solution was neutralized until thecomplete disappearance of colour.
Peroxide value (PV) value was calculated by using following formula.

$$\text{PV (mEq/kg of oil)} = \frac{\text{V (mL)} \times \text{N of sodium thiosulphate}}{\text{Weight of sample(g)}} \times 1000.$$

p-anisidine(p-AV) valuedetermination: AOCS standard method was followed to find p- anisidine value of

pomegranate oil. Following this method 2g of sample was taken and diluted with isooctane. Light absorbance of Sample solution was measuredwith spectrophotometer while isooctane solvent was used as blank. After taking absorbance, another absorbance of oil solution was taken after mixing of 5ml of oil solutionand 1ml of p- anisidine solution. In the blank reference cuvette p- anisidine solution was used.

p- anisidine value was calculated by using following formula: (p-AV)

$p\text{-}AV = 25 \times (1.2A_2 - A_1) / W$

A_2 = absorbance of oil solution prepared with p- anisidine solution

A_1 = absorbance of solution prepared with isooctane

W = weight of oil sample

Determination oftotal oxidative (TOTOX) value: As total oxidative value is sum of primery and secondary oxidation values here we always take the peroxide value (PV) and p-anisidine value (p-AV).

Following simple formula was followed to find the TOTOX value:

Totox value = 2PV + p-AV

ATR FT-IR analysis: FT-IR (Thermo Nicolet 5700) with deuteratedtriglycine sulfate as detector (DTGS) was used to record the infrared spectra of sample. While for data OMINIC software (7.2version) was used. The instrumental programming was followed as: resolution 4 cm^{-1}, range 4000-650cm^{-1}, scans 32, SB-ATR accessory with diamond crystal. For obtaining the spectra of pomegranate oil sample about 60µl of oil sample was poured onto the diamond crystal. Before recording the sample spectra a background spectrum was recorded first.

RESULTS AND DISCUSSION

Chemical parameters of oxidized samples of pomegranate seed oil at room temperature and different humidic environments: Chemical properties are essential quality parameters of any oil. These parameters are most important for oil regarding to its industrial as well as edible point of view.

The results of quality characteristics such as FFA, PV,p-AV,andTOTOX value of oxidized samples of pomegranate seed oil (PSO) are tabularized in table: 01

Formation of free fatty acids (FFA) is essential factor which decides the quality of oil. Here the formation of FFA was monitored during storage; it was observed that FFA value was increased during complete study. Before the storage the fresh FFA value of PSOsamples was observed0.95 it was noticed that PSO sample stored at RT showed greater change in FFA, followed by RH75% and RH33%. Minor change in FFA value was observed during 1st week; further major change in FFA value was noticed till last day of oxidation. Peroxide value (PV) which is the best indicator to measure the primary oxidation. Before oxidation of oil sample peroxide value of fresh PSO samples was determined this was in range of 1.93 meq/kg.When after the oxidation PVs of all three PSO sampleswere compared with the initial values, increase in values was observed till 28th day of oxidation, after that till 42nd day decrease in PV values was noticed; same was followed by an increased in PVs of all three PSO samples till last day. Overall the peroxide value of all samples was observed increased throughout the study, PV of PSO sample stored at RH75% was more thansamples stored at RH33% and at RT. This clearly shows that the oxidative stability decreases by increasing RH value. Secondary oxidation of pomegranate seed oil samples was confirmed by the formation of carbonyl compounds by p-anisidine test. As during initial days the chances of secondary oxidation were very les therefore slightly change in p-AV was noticed. However after 28th day significant change in p-AV was observed. The initial p-AVs of PSO samples were found 7.98 and increase in p-AV was noticed till last day of study. Finally the total oxidation of all samples of PSO was determined by adding the primery and secondary oxidation products and it is represent totox value. When the totox value of all three samples of PSO were compared all the samples showed the increase in totox value from day first to day 65 (last day of oxidation). It was also noticed that the totox value increased by increasing the humidic condition, as shown in table 01.

FT-IR characterization of pomegranate seed oil (PSO) stored at RT, RH33% and RH75%: In this study on of the important wave number (cm^{-1}) was studied, which was highly responsible for change in the FT-IR spectra of PSO samples during storage. A significant difference between fresh (0 days) and oxidized PSO spectra is shown in Figure 1.1, 1.2 and1.3.the FT-IR spectra shown in figures 1.4, 1.5 and 1.6 shows the difference between PSO spectra at day first and last day of the oxidation at RT, RH33% and RH75%.As the oxidation results the formation of saturated aldehydes secondary oxidation products, these all are determined by the monitoring the changes in the peaks of the regions between the wavenumbers of 988 and 933 cm^{-1}. –HC=CH– (trans) bending results the peak at 988 cm^{-1} and–HC=CH– (cis) vibration indicated by the peak at 937cm^{-1}. It was observed that the peak intensity of band 988 $cm^{-1}$was increasing continuously and the peak intensity of band 937cm^{-1} and 759 cm^{-1} were decreased continuously.

Table: 01 Chemical properties of PSO during oxidation at RT, RH (33%) and RH (75%).

Days		FFA (%)	PV(meq/kg)	p-AV	Totox
0	RT	0.95±0.03	1.93±0.01	7.89±0.02	11.75
	RH (33%)	0.95±0.04	1.93±0.03	7.89±0.03	11.75
	RH (75%)	0.95±0.01	1.93±0.02	7.89±0.02	11.75
3	RT	0.97±0.02	1.94±0.04	8.31±0.01	12.19
	RH (33%)	0.96±0.03	1.95±0.05	9.01±0.05	12.91
	RH (75%)	0.98±0.05	1.99±0.03	10.26±0.03	14.24
7	RT	0.99±0.01	1.99±0.01	10.01±0.04	13.99
	RH (33%)	0.98±0.02	2.01±0.02	10.95±0.02	14.97
	RH (75%)	1.01±0.04	2.09±0.04	11.85±0.01	16.03
10	RT	1.03±0.01	2.02±0.05	12.16±0.05	16.20
	RH (33%)	1.02±0.04	2.08±0.03	12.87±0.01	17.03
	RH (75 %)	1.09±0.05	2.16±0.05	13.01±0.03	17.33
14	RT	1.23±0.02	2.07±0.03	13.05±0.03	17.19
	RH (33%)	1.15±0.03	2.15±0.01	13.97±0.02	18.27
	RH (75%)	1.37±0.01	2.59±0.02	14.97±0.05	20.15
17	RT	1.15±0.02	2.09±0.03	13.92±0.02	18.10
	RH (33%)	1.13±0.03	2.39±0.05	14.62±0.01	19.40
	RH (75%)	1.53±0.01	2.99±0.04	15.89±0.04	21.87
21	RT	1.62±0.02	2.35±0.01	14.89±0.04	19.59
	RH (33%)	1.43±0.05	2.97±0.05	15.09±0.01	21.03
	RH (75%)	1.61±0.01	3.17±0.02	16.91±0.03	23.11
24	RT	1.71±0.02	2.56±0.01	14.91±0.02	20.03
	RH (33%)	1.52±0.04	3.01±0.04	15.93±0.05	21.95
	RH (75%)	1.69±0.05	3.69±0.02	17.80±0.03	25.18
28	RT	1.79±0.01	2.65±0.03	15.06±0.01	20.36
	RH (33%)	1.59±0.03	3.06±0.01	16.71±0.03	22.83
	RH (75%)	1.72±0.01	3.78±0.04	17.19±0.04	24.75
31	RT	1.81±0.03	2.01±0.03	15.95±0.01	19.97
	RH (33%)	1.62±0.05	2.99±0.02	17.51±0.04	23.49
	RH (75%)	1.78±0.02	3.10±0.04	18.39±0.02	24.59
35	RT	1.97±0.01	1.98±0.01	16.03±0.01	19.99
	RH (33%)	1.73±0.03	2.05±0.03	18.01±0.03	22.11
	RH (75%)	1.83±0.04	2.97±0.04	19.96±0.01	25.90
38	RT	1.95±0.01	1.58±0.05	17.06±0.03	20.22
	RH (33%)	1.79±0.02	1.96±0.01	18.09±0.02	22.01
	RH (75%)	1.91±0.04	2.10±0.05	20.16±0.01	24.36
42	RT	1.99±0.05	2.17±0.02	19.31±0.04	23.65
	RH (33%)	1.78±0.01	2.25±0.01	20.51±0.05	25.01
	RH (75%)	1.93±0.03	2.95±0.03	22.74±0.02	26.94
45	RT	2.01±0.01	2.99±0.02	20.63±0.01	26.61
	RH (33%)	1.83±0.05	3.32±0.01	21.97±0.03	28.65
	RH (75%)	1.97±0.02	3.56±0.04	23.91±0.04	33.11
49	RT	2.05±0.04	3.10±0.05	21.10±0.05	27.30
	RH (33%)	1.93±0.05	3.69±0.02	22.43±0.03	29.81
	RH (75%)	1.99±0.04	3.97±0.03	25.29±0.01	33.86
52	RT	2.01±0.04	3.35±0.04	22.10±0.04	28.80
	RH (33%)	1.99±0.01	4.01±0.01	23.15±0.02	31.17
	RH (75%)	2.03±0.03	4.65±0.05	27.31±0.01	36.61
56	RT	2.09±0.01	3.99±0.02	23.56±0.04	31.54
	RH (33%)	2.04±0.04	4.89±0.01	25.16±0.01	34.94
	RH (75%)	2.06±0.05	5.10±0.05	29.25±0.04	39.45
59	RT	2.10±0.02	4.38±0.01	23.96±0.02	32.72
	RH (33%)	2.05±0.03	5.61±0.03	26.69±0.01	37.91

	RH (75%)	2.09±0.04	5.99±0.05	30.18±0.04	42.16
	RT	2.13±0.01	5.10±0.01	24.35±0.02	34.55
63	RH (33%)	2.07±0.04	6.01±0.02	27.75±0.01	39.77
	RH (75%)	2.1±0.05	6.78±0.03	32.95±0.03	46.15
	RT	2.19±0.02	5.97±0.01	25.32±0.02	37.26
65	RH (33%)	2.09±0.01	6.88±0.03	29.19±0.01	43.67
	RH (75%)	2.16±0.03	7.35±0.04	36.40±0.05	51.10

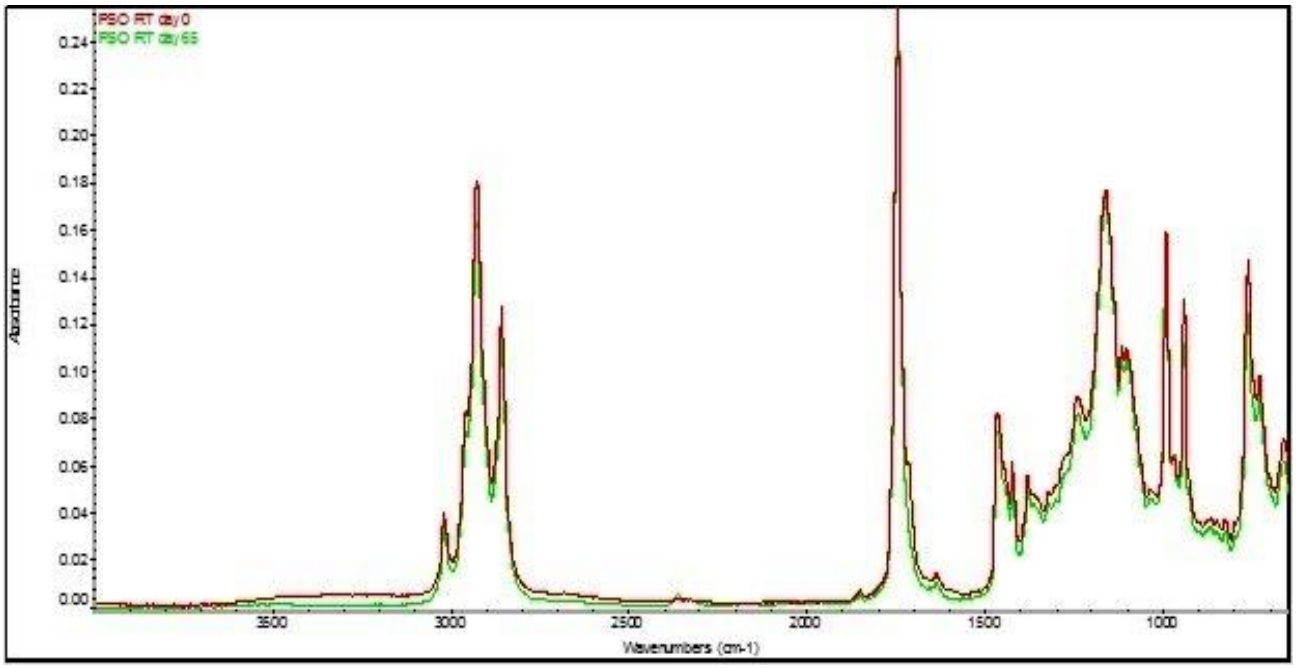

Figure:1.1 Difference between fresh and oxidized PSO spectra at room temperature (RT)

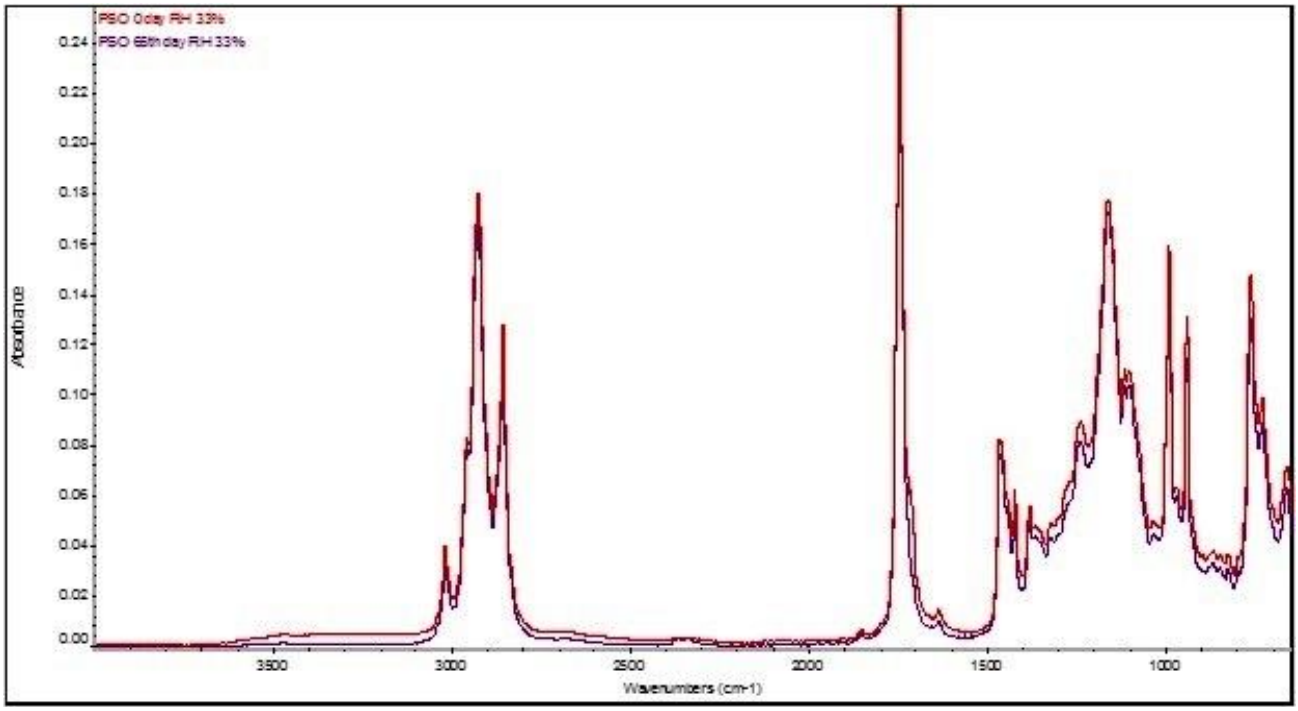

Figure: 1.2 Difference between fresh and oxidized PSO spectra at relative humidity (RH) 33%

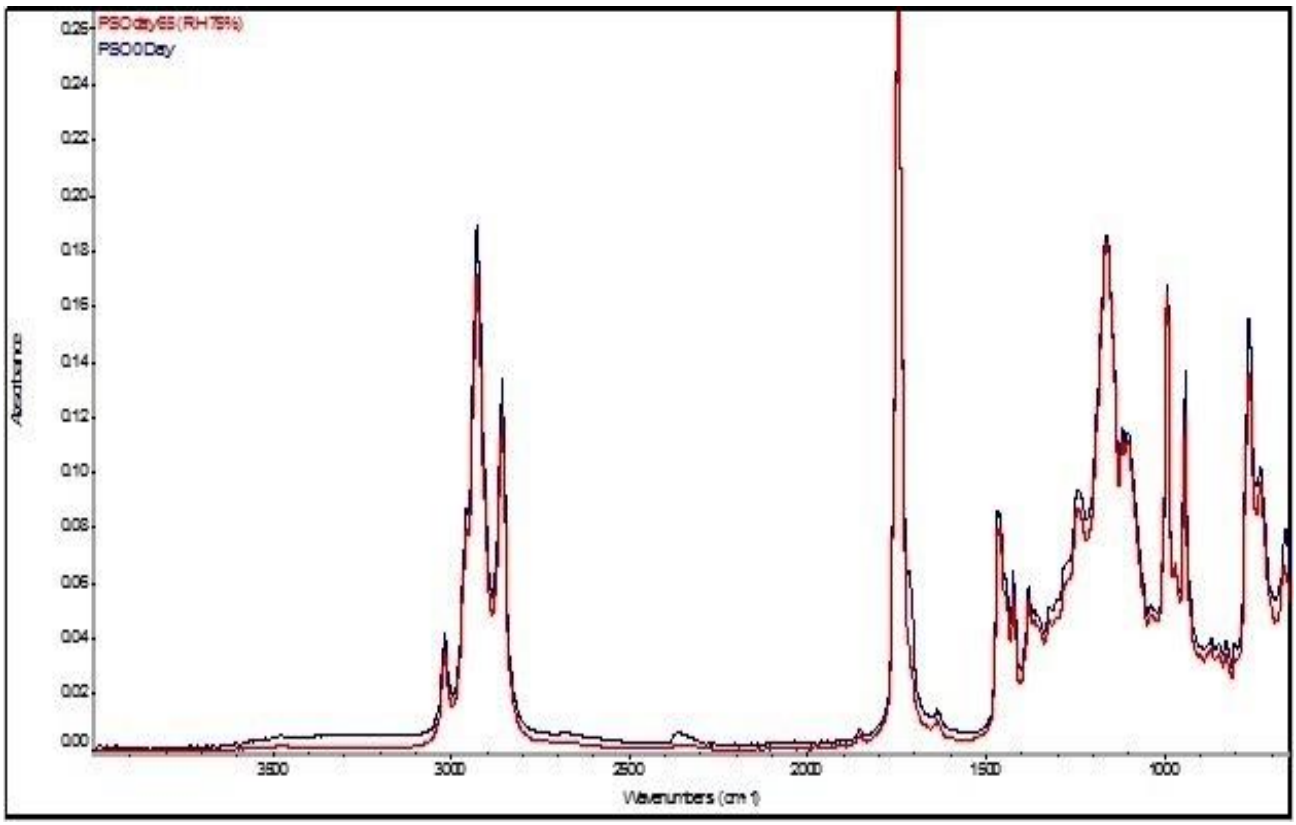

Figure: 1.3 Difference between fresh and oxidized PSO spectra at relative humidity (RH) 75%.

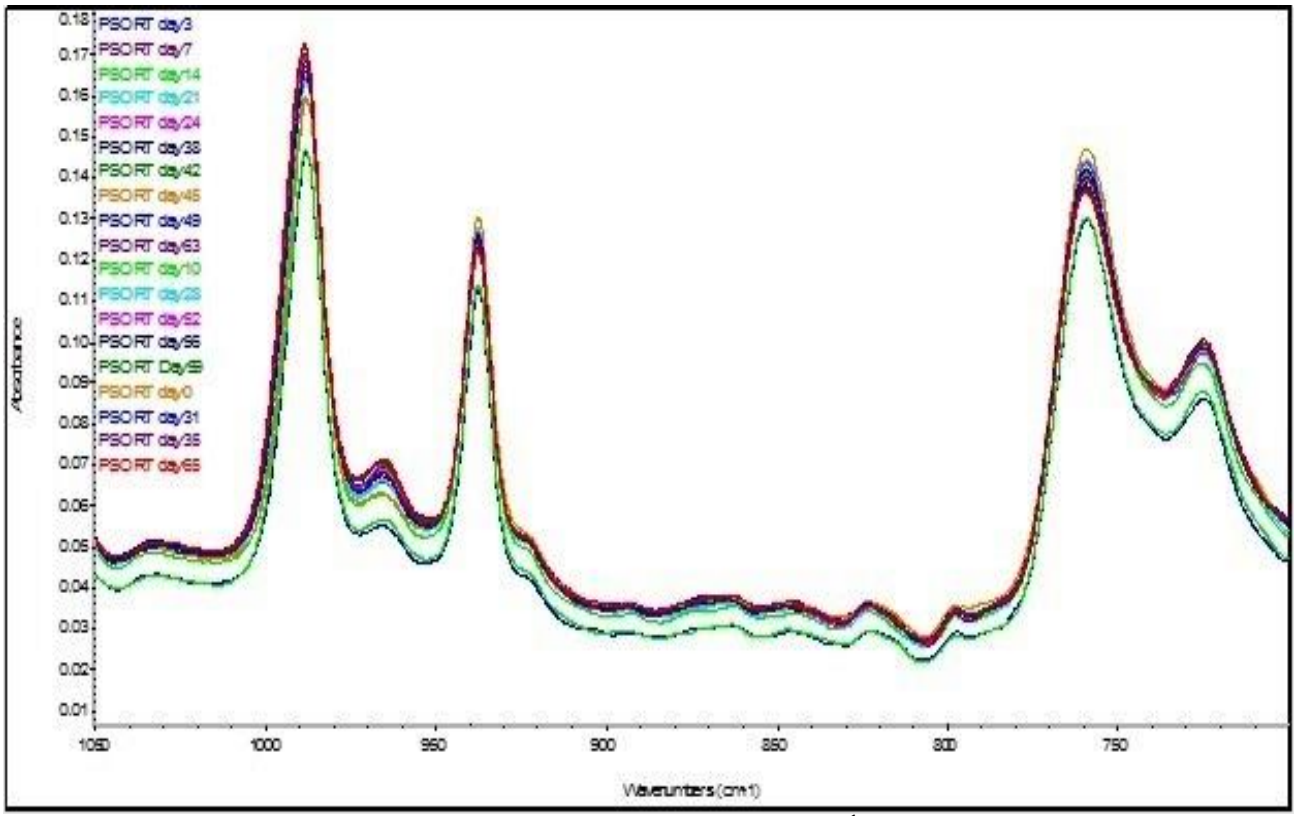

Figure 1.4 Change in FTIR group spectra of PSO in region of 1050-700cm^{-1} stored at room temperature (RT).

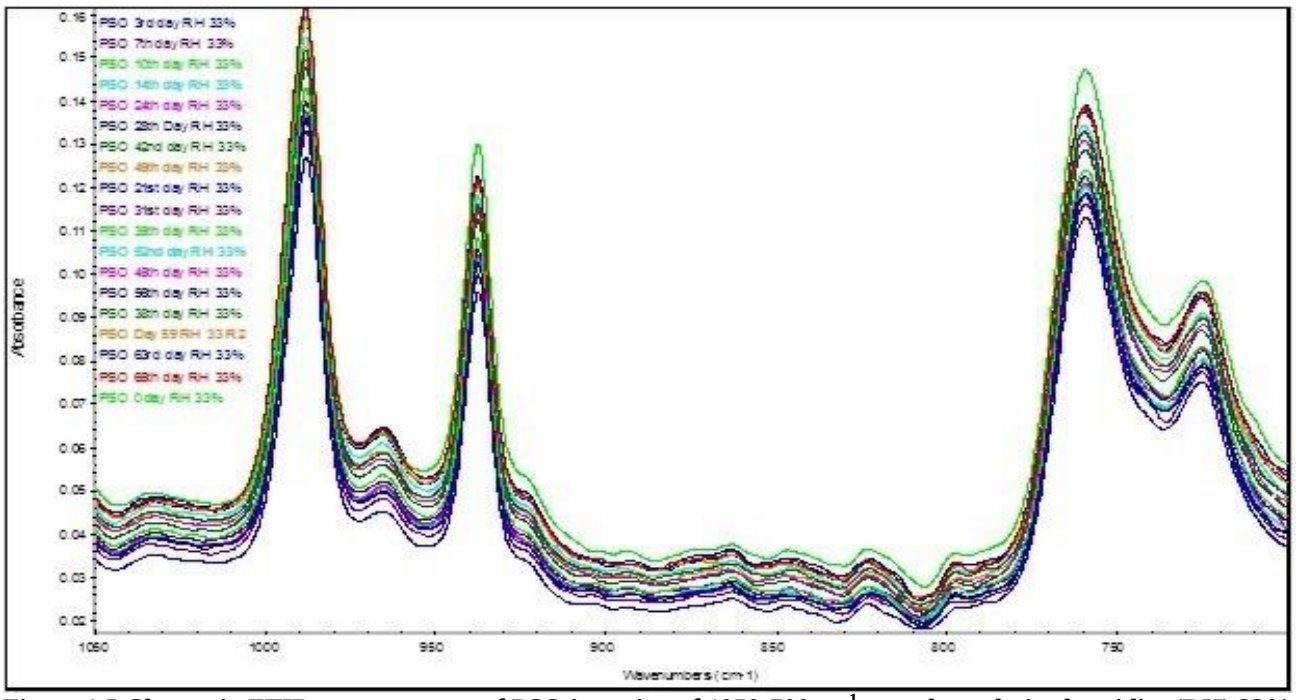

Figure 1.5 Change in FTIR group spectra of PSO in region of 1050-700cm^{-1} stored at relative humidity (RH) 33%.

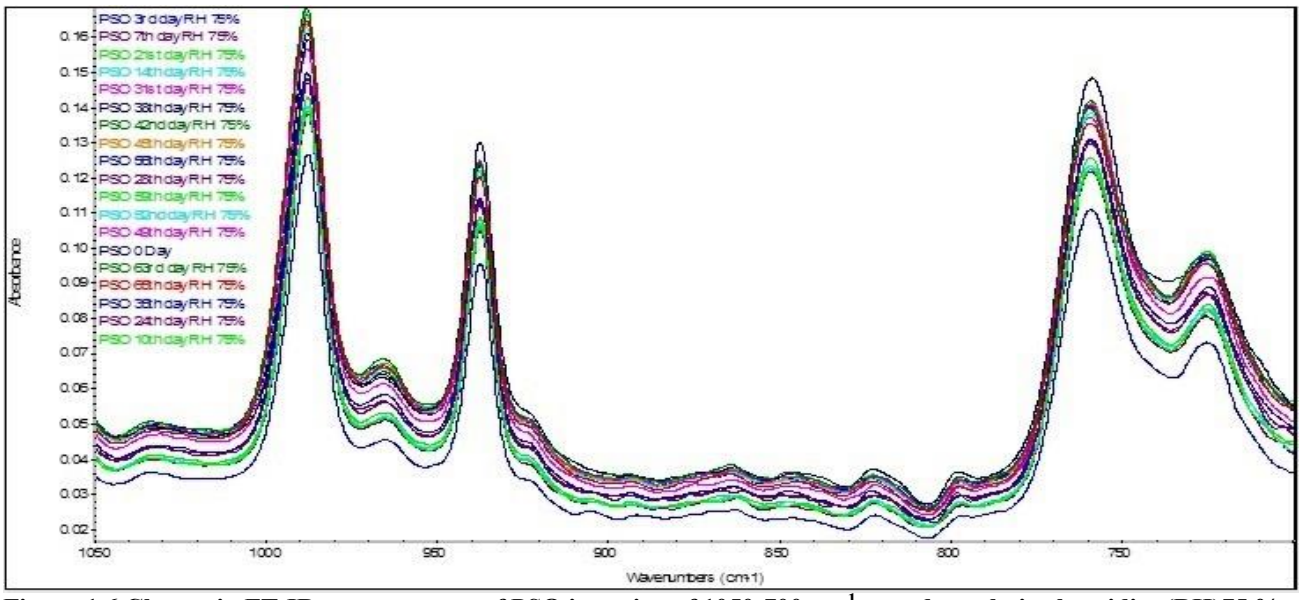

Figure:1.6 Change in FT-IR group spectra of PSO in region of 1050-700 cm^{-1} stored at relative humidity (RH)75 %.

Conclusion: As oilsare commonly considered as key compofhffbbbh6cnent of our diet, are very helpful to transport fat soluble vitamins within body, supplies energy and source of essential fatty acids. Due to important role of oil, it is most important to monitor the chemical changes taking place in their composition due to several factors. Among them oxidation is also most important factor which denatures the oil and makes it unfit to use. The oxidation of oils and fats can have a detrimental effect on their quality and marketability.

The present study was designed to carry out oxidative behavior of PSO at different Humidic environments with different time intervals for 65 days. For this purpose different instrumental and classical techniques were used. Chemical properties are essential quality parameters of any oil. These parameters are most important for oil regarding to its industrial as well as edible point of view.The quality characteristics parameters related to oxidation were compared before and after oxidation.The recorded FT-IR spectra of all the

tree samples of PSO stored at various humidic conditions were also compared. As the oxidation results the formation of saturated aldehydes secondary oxidation products, these all are determined by the monitoring the changes in the peaks of the regions between the wavenumbers of 988 and 933 cm^{-1}The peak at 988 cm^{-1} shows–HC=CH– (trans) bending and the peak at 937cm^{-1} shows -HC=CH– (cis) vibration.A continuous increase in the peak intensity of band 988 cm^{-1} and a continuous decrease in the peak intensity of band 937cm^{-1} and 759 cm^{-1} were determined.

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