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Physicochemical properties of olive oil and its stability at different storage temperatures

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Abstract
The study was undertaken to evaluate the physical and chemical characteristics of olive oils and its stability on storage. The olive oil samples were examined for physical and chemical properties viz., pH, free fatty acid, peroxide value, anisidine value, specific absorption coefficients K_{232} and K_{270}, iodine value, fatty acids composition and total phenol content. The physicochemical properties of olive oil exhibited significant changes during one month of storage at different temperatures. At the end of 30 days storage at room temperature, the oleic acid, palmitic acid, linoleic acid, stearic acid and linolenic acid content of olive oil were 76.68, 11.64, 6.11, 3.51 and 0.88 respectively and when stored at refrigerated temperature, the values obtained were 76.77, 11.59, 6.14, 3.52 and 0.89 respectively. The total phenol content showed a significant decrease to 137.22 mg/kg from 168.53 mg/kg at room temperature and at refrigerated storage, the change was 168.53 to 165.78 mg/kg.

Keywords: Olive oil, peroxide value, iodine value, phenol content

Introduction
Olive oil is obtained by mechanical extraction from the fruit of the *Olea europaea* L. tree which belongs to the Olive family and thrives in temperate and tropical climates, especially in the Mediterranean region (Liphschitz *et al.* 1994) [33]. Olive oil rich in nutritional and pleasant flavour which has to an increase in consumption of olive oil, different climatic conditions and different agronomic practices may alter olive quality (Patumi *et al*. 2002) [36].

Olive oils possess numerous nutritional benefits which are primarily related to the fatty acid composition, mainly due to both the high content of oleic acid and the balanced ratio of saturated and polyunsaturated fatty acids. Olive oil is rich in monounsaturated fatty acids and low in saturated fatty acids. In addition, olive oil contains considerable amounts of natural antioxidants and is considered important in the prevention of many diseases (Bouaziz *et al.* 2010) [10].

International Olive Council (IOC, 2006) [31] and the European Commission (EEC, 1991) [19] have defined the quality of olive oil based on parameters that include free acidity, peroxide value (PV), UV specific extinction coefficients (K_{232} and K_{270}) and sensory score. In particular, the quantity of free acidity is an important factor for classifying olive oil into commercial grades (Boskou, 1996 [9]; Rossell, 1986) [37].

One of the adverse factors causing of olive oil quality deterioration is auto-oxidation, which is a spontaneous reaction of oxygen with fat molecules that precedes the formation of radicals. The reaction rate determines the shelf life of oil (Gomez-Alonso *et al.* 2007) [24]. Oxidation that occurs in edible oils leads to the loss of minor components and formation of new compounds, causing nutritional loss as well as the development of rancid and other off flavours in olive oil (Velasco *et al*. 2003; [44] Sun-Waterhouse *et al*. 2011; Cicerale *et al*. 2013) [15]. The types and amounts of particular oxidation products depend on the fat type comprised in them, antioxidants and storage conditions including light, oxygen and temperature (Ching Man Cheung, 2007; [14] Siger, 2005) [40].

The stability of virgin olive oils is due to their natural phenolic compounds, since these compounds are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (Shahidi and Wanasundara, 1992) [39].

The effects of phenols and tocopherols on the oxidative stability in extra-virgin olive oil during storage have been extensively evaluated (Aparicio *et al*. 1999; [4] Baldioli *et al*. 1996; [8] Gordon *et al.* 2001; [25] Tsimidou *et al.* 1992) [42].

It has been demonstrated that phenolic compounds are more effective than tocopherols in enhancing the stability of olive oil against oxidation.The application of the vortex-assisted MSPD method to the analysis of real samples showed TCS in some fish liver and fish gill samples at trace levels.
Sun *et al.* [14] developed a sensitive and efficient analytical method for TCS determination in water, which involved enrichment with bamboo-activated charcoal and detection with HPLC-ESI-MS. They invest the characteristic aroma of olive oil, taste, colour and nutritive properties distinguish it from other edible vegetable oils. It is therefore a matter of great concern for the olive oil to preserve its product without loss of these positive attributes.
With this background, the research was undertaken with main objective to study the physical and chemical properties of olive oil and its stability when stored at room and refrigerated temperature.

Materials and Methods
Materials
Olive oil was purchased from the local super market. The samples of olive oil were packaged in sterilized polypropylene (PP) bottles and kept in a dark room.

Storage
The oil samples were stored at different storage temperatures viz, room temperature and refrigerated temperature for a period of one month. The room temperature varied from 25±4 °C and the refrigerated temperature varied from 5±1 °C throughout the period of study.

Physicochemical characterization of olive oil
The pH measurements of olive oils were obtained with a pH meter (MP-1PLUS), calibrated with two standard solutions buffered at pH=4.00 and pH=7.00.
To determine the free oil acidity, a known weight of olive oil was dissolved in a mixture of diethyl ether/ethanol (1:1 v/v). The mixture was titrated with potassium hydroxide in methanol (0.05M) in the presence of phenolphthalein as indicator (official analytical methods described in EC Regulation 2568/91 (Commission Regulation, 1991) [19].

Determination of Oxidative Stability-Peroxide Value (PV) and Anisidine Value (AV)
The analysis of the PV in samples was performed according to the AOAC, Official method of analysis described in the 15th ed. Association of Official Analytical Chemists (1998). The PV of extracted olive oil was expressed as peroxide milli equivalent per Kg oil. Anisidine value was determined by the standard 2504 IUPAC method (IUPAC, 1987) using a Shimadzu UV-2450 UV-Visible Spectrophotometer.

Specific Absorption Coefficients (K_{232} and K_{270})
Specific extinction coefficients of the olive oil samples were analysed using European Official Method of Analysis (Commission Regulation EEC N-2568/91). 250 mg of olive oil was weighed. The weighed sample was placed into a 25 ml graduated flask and diluted to 25 ml with cyclohexane. The sample was homogenized using centrifuge at 10000 rpm and vortexed for 30 seconds. Absorbance was taken at 232 nm and 270 nm.

Determination of Iodine Value (IV)
The iodine value was determined according to the AOAC (1999), method 993.20, using carbon tetrachloride as solvent. Dissolved oil sample was mixed with Wijs solution and freshly prepared potassium iodide (10%) solution. Liberated iodine was titrated with standard potassium thiosulphate (0.1 M) solution, using carbon tetrachloride as a blank and starch as an indicator.

Analysis of fatty acid profile
The analyses of fatty acids were performed according to the official method of the European Community Regulation (Commission Regulation, 1991) [19]. The olive oil samples were esterified in a methanol solution of 2N potassium hydroxide for 30 minutes at 50 ℃. The gas chromatographic analyses of fatty acid methyl esters were performed with a Perkin Elmer gas chromatograph, equipped with a flame ionisation detector (Shimadzu QP2010). The column was a fused silica capillary SE30 length 25 meters, diameter 0.25 μm. Helium was the carrier gas (6ml/min). The column temperature program was: initially isotherm at 140 ℃ for 10 min, an initial programmed rate of 1 ℃/min up to 160 ℃, then a second rate of 2℃/min up to 220℃ and a final isotherm for 15 min. Samples were injected into the split mode. The apparatus itself carried out recording and integration. The gas chromatographic peaks were identified as corresponding fatty acid methyl esters by check of the elution order on the column and compared the retention times with those of pure standards.

Determination of total phenol content
The total phenol content of the olive oil samples was determined using the Folin-Ciocalteu method (Bouaziz *et al*. 2010) [10]. Briefly, a 50 μl aliquot of the extracts was assayed with 250 μl Folin reagent and 500 μl sodium carbonate (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 5 ml. After incubation for 30 min at room temperature, the absorbance was read at 765 nm; total phenols were expressed as gallic acid equivalents (GAE) in one Kg oil.

Statistical analysis
Statistical analysis of the data was done by analysis of variance (ANOVA) using SPSS software. Significance was accepted at 5% level probability and Duncan's Multiple Range Test was used to compare mean variance. Data were expressed as mean values± standard deviation derived from triplicate determination.

Results and Discussion
Physicochemical characteristics
The physical and chemical characteristics of olive oil samples stored under different temperatures for a period of one month are shown in the Table 1 and 2. There were notable differences in chemical values viz, pH, free fatty acid, peroxide value and anisidine value during storage depending on the initial quality of the olive oil.

PH and Free Fatty Acids
The pH of olive oil stored under room temperature and refrigerated temperature decreased from 5.17 to 4.49 and 5.17 to 5.09 respectively. Free oil acidity (% oleic acid) of the olive oil samples stored at room temperature and refrigerated temperature increased from 0.28 to 0.63% and 0.28 to 0.34% respectively. The olive oil pH has decreased during room temperature storage and is shown in Fig.1. According to the International Olive Oils Council (I. O. O. C, 2007), olive oils should have acidity (%) ≤3.3%. The acidity contents of olive

oil stored at different temperatures were not higher than the prescribed limit. There were significant differences in pH and acidity of olive oil stored at room temperature while there were no significant differences in pH and acidity of olive oil stored at refrigerated temperature. Maximum acidity of 0.63% was obtained after the end of 30 days of storage at room temperature.

Oxidative Stability Peroxide Value (PV) and Anisidine Value (AV)

The peroxide values of the stored olive oil samples showed significant increase from 4.50 to 8.40 milli equivalents (meq) O_2/Kg at room temperature and non-significant increase from 4.50 to 5.30 meq O_2/Kg under refrigerated temperature and is depicted in Fig.3 and 4. The observed results were in accordance with the study performed by Mendez and Falque (2007) [34, 35] during 3 and 6 months that confirmed the variation of PV in olive oil over time period.

According to Baiano *et al*. (2005) [7], when there was an initial increase in hydro peroxides (odourless, flavourless compounds, produced during the primary step of oxidation), they successively broke down into aldehydes and ketones, which were responsible for off-flavours (secondary oxidation) in oils. Thus, a rapid hydro peroxide formation demonstrated the initiation of the oxidative reactions that precede rancidity (Elez-Martinez *et al*. 2007) [20].

However, the peroxide values of all olive oil samples were under the value of 20 meq O_2/Kg of olive oil, which is the maximum established by the Council for International Olive Oil and Algerian Official Journal. Anisidine value of olive oil for samples stored in room temperature and refrigerated temperature ranged from 2.20 to 4.30 and from 2.20 to 2.80 respectively.

Specific Absorption Coefficients

Specific absorption coefficient K_{232}, with absorbance at 232 nm, is indicative of the formation of conjugated di-enes. Similarly, K_{270}, with absorbance at 270 nm, is indicative of the conjugation of tri-enes and of the presence of carbonylic compounds. The values for absorbance at 232 and 270 nm that were recorded during one month of room and refrigerated temperature storage are shown in Table 1 and 2. The value of K_{232} ranged from 1.14 to 1.56 and K_{270} is 0.12 to 0.24 at room temperature and at refrigerated temperature K_{232} ranged from 1.14 to 1.21 and K_{270} from 0.12 to 0.18 and is shown in Fig. 5 and 6.

Vekiari *et al.* (2002) reported that the values of K_{232} in samples of Greek olive oils stored in dark showed an increase during the first 7 months and the K_{232} values of these samples did not exceed the upper limit value for K_{232}.

Gutierrez and Fernandez, (2002) [26] pointed out that in olive oils stored at 2 °C and in dark for 6 months, K_{232} values remained practically constant or very slightly increased. Specific extinction at 232 nm ranged from 2.86 to 3.45 and 270 nm ranged from 0.32 to 0.62, which were not exceeding permitted limits according to (IOOC, 2007).

Iodine Value

Iodine value of olive oil samples stored for one month at room temperature and refrigerated temperature ranged from 85.60 to 74.02 g of iodine/100 g oil and 85.60 to 84.00 g of iodine/100 g oil respectively and the decreasing pattern is projected in Fig.7.

Fatty Acid Composition

The fatty acid compositions of the olive oil samples during room and refrigerated temperature were determined by gas chromatography and the results are shown in Table 3. No differences in fatty acid composition were observed in olive oil samples when stored at different temperatures. The concentration of oleic acid ranged from 76.81 to 76.68% and 76.81 to 76.77% when stored at room and refrigerated temperature respectively. It was followed by palmitic acid, linoleic acid, stearic acid, linolenic acid and palmitoleic acid which ranged from 11.55 to 11.64%, 6.15 to 6.11%, 3.53 to 3.51%, 0.91 to 0.88% and 0.28% to 0.29% respectively under room temperature and from 11.55 to 11.59%, 6.15 to 6.14%, 3.53 to 3.52%, 0.91 to 0.89% and 0.28 to 0.28% respectively under refrigerated temperature.

According to Gutierrez and Fernandez (2002) [26] extra virgin olive oil Stored for 6 months in a container had no effect on the fatty acids under room and refrigerated storage conditions. The fatty acids profiles were slightly modified throughout the storage period.

The stability of the fatty acid composition during 3 months of storage at different conditions is in concordance with those of a previous report (Frega *et al*. 1999). The observed values of individual fatty acids appear to decrease when stored for one month at different temperatures.

Total phenolic composition

Phenolic compounds are naturally present in olive oils and the phenolic compounds are major responsible for the stability of the oil during storage. The phenol content of olive oil samples stored at different temperatures is shown in Table 4. Total phenol contents of stored samples expressed as gallic acid of olive oils values ranged from 168.53 to 137.22 mg/Kg at room temperature and from 168.53 to 165.78 mg/Kg at refrigerated temperature. It can be observed that the phenolic compounds in olive oils stored at room temperature and refrigerated temperature were showing a decreasing trend as given in Fig. 8. The phenol concentration of olive oil decreased drastically at room temperature storage. This fact of phenolic fraction decreasing is attributed to the joint action of light and the PP container to the oxygen that catalyzes the oxidation reaction (Vekiari *et al*. 2007; [43] Bouaziz *et al*. 2008) [11].

This finding is similar to other research works showing that during storage, phenols undergo qualitative and quantitative modifications due to decomposition and oxidation reactions (Esti *et al*. 2009; [21] Dabbou *et al*. 2011) [17].

Table 1: Physicochemical properties of olive oil stored at room temperature

Storage in days	pH	FFA (% Oleic Acid)	PV (meq O_2/kg)	Anisidine Value	K_{232}	K_{270}	Iodine Value
0	5.17^{a*}±0.05**	0.28^a±0.01	4.50^a±0.09	2.20^a±0.14	1.14^a±0.03	0.12^a±0.01	85.60^a±0.23
5	4.98^b±0.01	0.31^b±0.02	4.80^b±0.09	2.50^b±0.14	1.17^a±0.01	0.15^b±0.03	84.80^b±0.25
10	4.87^c±0.03	0.35^c±0.01	5.30^c±0.14	2.60^b±0.18	1.21^b±0.05	$0.16^{b,c}$±0.01	82.70^c±0.26
15	4.75^d±0.02	0.39^d±0.01	6.10^d±0.02	3.30^c±0.06	1.27^c±0.02	$0.18^{c,d}$±0.03	81.08^d±0.05
20	4.63^e±0.02	0.43^e±0.02	7.40^e±0.08	3.50^d±0.02	1.35^d±0.01	$0.19^{d,e}$±0.02	80.15^e±0.01

25	4.54^{f}±0.03	0.54^{f}±0.04	7.90^{f}±0.14	3.90^{e}±0.09	1.41^{e}±0.04	0.21^{e}±0.01	78.60^{f}±0.14
30	4.49^{g}±0.02	0.63^{g}±0.03	8.40^{g}±0.14	4.30^{f}±0.14	1.56^{f}±0.02	0.24^{f}±0.02	74.02^{g}±0.06

Means along the column followed by different letters are significantly different at $p \leq 0.05$
Values are expressed as Mean ± S. D.

Table 2: Physicochemical properties of olive oil stored at refrigerated temperature

Storage in days	pH	FFA (% Oleic Acid)	PV (meq O_2/kg)	Anisidine Value	K_{232}	K_{270}	Iodine Value
0	5.17^{a*}±0.02**	0.28^{a}±0.02	4.50^{a}±0.14	2.20^{a}±0.25	1.14^{a}±0.02	0.12^{a}±0.01	85.60^{a}±0.23
5	5.15^{a,b}±0.03	0.29^{a}±0.01	4.50^{a}±0.14	2.20^{a}±0.09	1.15^{a,b}±0.03	0.13^{a,b}±0.01	85.30^{b}±0.08
10	5.13^{b,c}±0.02	0.29^{a}±0.02	4.60^{a,b}±0.14	2.30^{a,b}±0.06	1.17^{b,c}±0.01	0.14^{a,b,c}±0.02	85.10^{c}±0.02
15	5.12^{c,d}±0.01	0.30^{a,b}±0.02	4.70^{b}±0.02	2.40^{b,c}±0.20	1.18^{c}±0.01	0.14^{a,b,c}±0.03	84.80^{d}±0.05
20	5.12^{c,d}±0.02	0.32^{b,c}±0.02	4.90^{c}±0.22	2.50^{c}±0.07	1.18^{c}±0.03	0.15^{b,c}±0.02	84.50^{e}±0.08
25	5.10^{d,e}±0.02	0.32^{b,c}±0.02	5.10^{d}±0.03	2.70^{d}±0.14	1.19^{c,d}±0.01	0.16^{c,d}±0.02	84.20^{f}±0.08
30	5.09^{e}±0.01	0.34^{c}±0.03	5.30^{e}±0.02	2.80^{d}±0.14	1.21^{d}±0.03	0.18^{d}±0.02	84.00^{g}±0.03

Means along the column followed by different letters are significantly different at $p \leq 0.05$
Values are expressed as Mean ± S. D

Table 3: Fatty acid compositions of olive oil stored at different temperatures

Fatty acids	Stored at room temperature in days							Stored at refrigerated temperature in days						
	0	**5**	**10**	**15**	**20**	**25**	**30**	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Oleic	76.81	76.81	76.81	76.80	76.78	76.72	76.68	76.81	76.81	76.81	76.81	76.79	76.78	76.77
Palmitic	11.55	11.57	11.56	11.58	11.61	11.62	11.64	11.55	11.55	11.55	11.56	11.57	11.58	11.59
Linoleic	6.15	6.15	6.14	6.14	6.12	6.12	6.11	6.15	6.15	6.15	6.15	6.14	6.14	6.14
Stearic	3.53	3.53	3.54	3.53	3.52	3.51	3.51	3.53	3.53	3.53	3.53	3.52	3.52	3.52
Linolenic	0.91	0.91	0.91	0.90	0.89	0.89	0.88	0.91	0.91	0.91	0.90	0.90	0.90	0.89
Palmitoleic	0.78	0.76	0.77	0.78	0.81	0.86	0.89	0.78	0.78	0.78	0.79	0.79	0.80	0.81
Others	0.28	0.28	0.28	0.28	0.28	0.29	0.29	0.28	0.28	0.28	0.29	0.28	0.28	0.28

Table 4: Total phenol content of olive oil stored at different temperatures

Storage in days	Total phenol / Gallic acid equivalent (GAE) (mg/Kg)	
	Room Temperature	**Refrigerated Temperature**
0	168.53^{a}±0.014	168.53^{a}±0.014
5	167.49^{b}±0.021	168.47^{b}±0.028
10	158.13^{c}±0.018	168.32^{c}±0.020
15	152.28^{d}±0.030	168.15^{d}±0.026
20	147.36^{e}±0.025	165.88^{e}±0.034
25	141.64^{f}±0.022	165.84^{f}±0.026
30	137.22^{g}±0.020	165.78^{g}±0.022

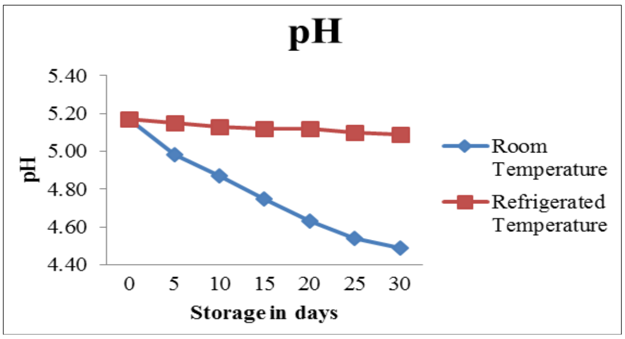

Fig 1: pH of olive oil stored at different temperatures

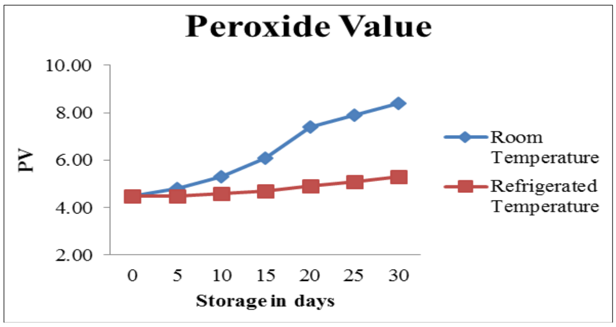

Fig 3: Peroxide Value of olive oil stored at different temperatures

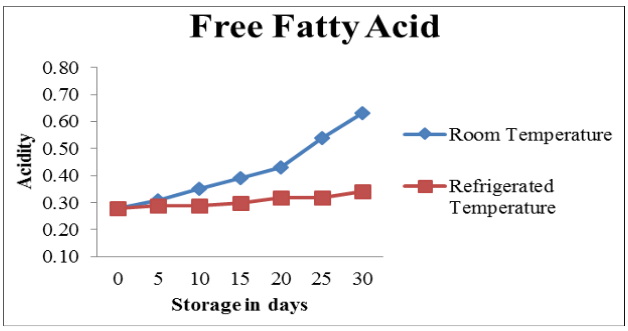

Fig 2: Acidity of olive oil stored at different temperatures

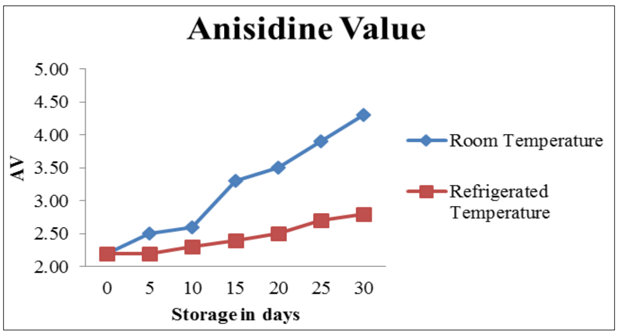

Fig 4: Anisidine of olive oil stored at different temperatures

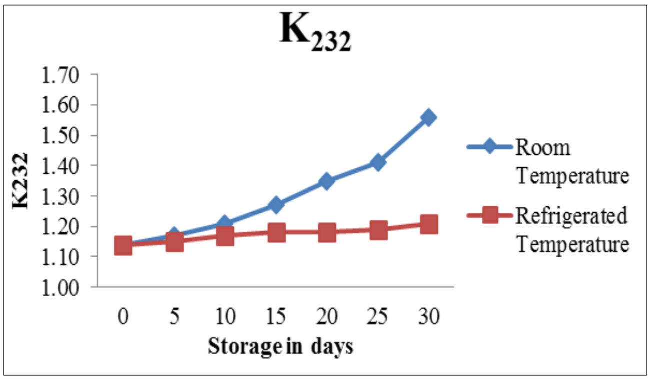

Fig 5: K_{232} of olive oil stored at different temperatures

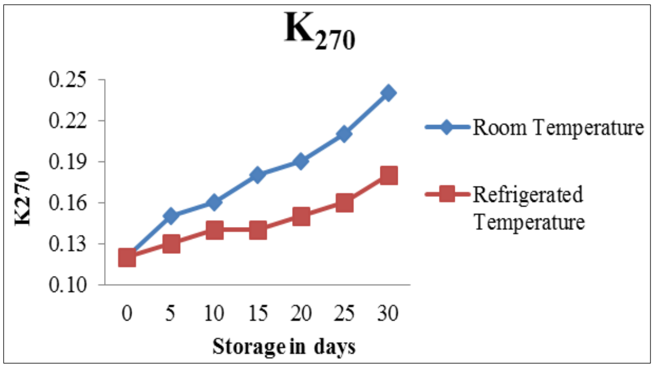

Fig 6: K_{270} of olive oil stored at different temperatures

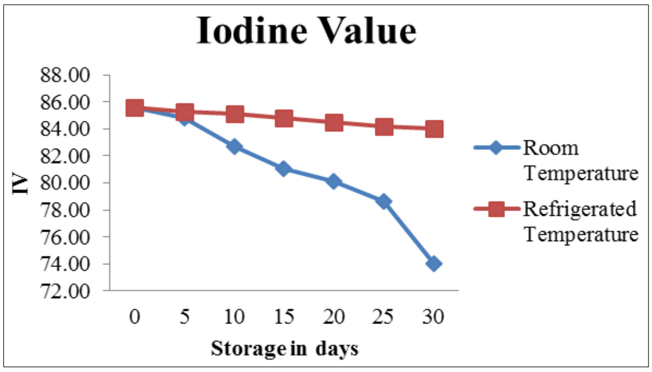

Fig 7: Iodine Value of olive oil stored at different temperatures

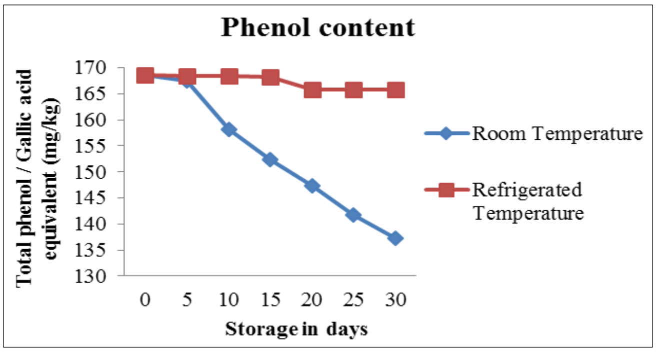

Fig 8: Total phenol content of oilve oil stored at different temperature

Conclusion

The olive oil stored at different temperatures viz. room and refrigerated showed better stability at refrigerated storage against oxidation and preserved their quality parameters for 30 days at 5 °C. The acidity and peroxide values of the samples tested were within the limits prescribed by the International Olive Oils Council. The physical and chemical characteristics of olive oil samples showed significant differences at different storage temperature but the oleic acid level was within the limit up to 30 days. The results of the present study indicate the important parameters that determine the quality of olive oil during storage.

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