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Storage study of garden cress (*Lepidium sativum* L.) seed oil

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Abstract
Packaging and storage conditions affect the quality of edible plant oils, including garden cress oil, with high content of unsaturated fatty acids, which are subjected to hydrolytic and oxidative deterioration during storage. Aim of this research was to investigate the effect of amber coloured glass (AGL) bottle and transparent polyethylene terephthalate (PET) bottle on storage stability of garden cress seed oil (GCO). The bottles were filled with GCO and stored at room and refrigerated condition. Peroxide value (PV), acid value (AV) and iodine value (IV) of oil were analyzed for every 30 day for 90 days. Results revealed the storage period had significant effect of stability of GCO. Storage temperature affected the stability of oil more than the packaging material. Lowest PV, AV and highest IV was observed for AGL bottle at every stage during the storage whereas highest PV, AV and lowest IV was observed for oil stored in PET bottle at room temperature

Keywords: Garden cress oil, storage, packaging material, storage temperature, peroxide value

1. Introduction
Vegetable oil demand has increased due to increasing domestic and industrial uses. Nutritionally, vegetable oil provides calories, vitamins, and EFA in the human diet in an easily digested form, and at relatively low cost Akinoso and Oni (2012) [3]. It contains good amount of lignans and antioxidants. Garden cress (*Lepidium sativum* L.) seeds contain 24% oil in which 32–34% is α-linolenic acid (ALA). Garden cress oil (GCO) has very high amount of tocopherols (1699 mg/kg), compared to other oils (Diwakar *et al.* 2010) [6]. In recent years, human dietary lipids intake has shifted more towards PUFAs due to their cholesterol lowering effect compared to saturated lipids. The increased consumption of vegetable oils (sunflower, corn oil, safflower, soybean oil) rich in n-6 PUFA has shifted the n-6 to n-3 PUFA ratio to 50:1 instead of a recommended ratio of 10:1 or 2:1. Garden cress oil has Linoleic acid: Linolenic acid (LA: ALA) ratio in the range of 1:4–2:3, which could give it nutritional advantages over the currently available ALA-rich plant oils in altering the n- 6/n-3 ratio in vivo.

Oxidation of unsaturated fatty acids is the main reaction responsible for the degradation of lipids (Muik *et. al.,* 2005; Morales *et al.*, 2011) [17, 16]. Indeed, the oxidation level of oil and fat is an important quality characteristic for food industry. Under mild conditions, molecular oxygen reacts with the double bonds following a free radical mechanism, and so-called auto-oxidation reaction takes place. The oxidation of fats primarily means deterioration of their quality and safety for consumption, and as a result economic loss can be expected. In addition, oxygen species that have carcinogenic effects are created during the oxidation, and they can lead to distortion of the cardiovascular system and can decrease the safety of oil for consumption (Pezzuto and Park, 2002) [20]. Oxidation, and the formation of peroxides, occurs during oil extraction and processing and can continue during storage. Peroxides are intermediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible for the deterioration of oil flavour (Pristouri *et al.* 2010) [21]. The amount of oxygen dissolved in oil is sufficient to oxidize the oil to a peroxide value of approximately 10 $meq.kg^{-1}$ in the dark (Przybylski and Eskin, 1988) [22]. Light, oxygen, humidity and temperature are some of the external factors that adversely affect the composition and quality characteristics of fats and oils during and after processing. Light is the cause and initiator of the reactions that lead to the spoilage of fats and oils. Although fat does not absorb visible light spectrum, the oxidation can be induced by light which was absorbed by the oil impurities (for example, the pigment chlorophyll). It is generally accepted interpretation that auto-oxidation of fat includes formation of free radicals (Grujic *et al.* 2011) [8].

Researchers have worked on the factors such as packaging material (Mendez and Falque, 2007; Kucuk and Caner, 2005; Pristouri *et al*, 2010; Tiwari *et al.*, 2012) [15, 13, 21, 26], oxygen and light transmittance (Kucuk and Caner, 2005 and Pristouri *et al*, 2010) [21], temperature (Diwakar *et al,* 2010) [13, 6,] and storage time (Ogbonnaya and Yahaya 2008) [19] on oxidation and quality degradation of oil. Finding suitable packaging material and storage condition to protect the oil from oxidation and quality degradation is important.

Materials and Methods

Garden cress seed were procured from local market. Seeds were ground and oil was extracted by soxhlet extraction method using petroleum ether. Oil was filled in amber colour glass (AGL) and polyethylene terephthalate glass (PET) bottle and stored at room as well as refrigerated temperature. Oil sample was analyzed for peroxide value (PV), acid value (AV) and iodine value (IV) at an interval of 30 days for period of 90 days.

2.1 Peroxide value

The peroxide value (PV) was determined by iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the fat sample. A 2.0 g sample of oil was dissolved in 30 mL mixture of glacial acetic and chloroform (30:70 v/v). Then 0.5 mL saturated potassium iodide solution was added. After 1 min under darkness, 30 mL H_2O purified was immediately added and titrated with 0.01 N sodium thiosulphate ($Na_2S_2O_3$). The liberated I_2 was titrated with 0.01 N $Na_2S_2O_3$ using a starch solution (1%) as an indicator, until the solution became colorless Kolanowski *et al*. (2004).

2.2 Acid value

The free fatty acid in oil was estimated by titrating it against KOH in presence of phenolphthalein indicator. The acid number is defined as mg of KOH required to neutralize the free fatty acids present in 1 g of sample. Acid values of garden cress seed oil was determined by titration method (AOAC, 1990) [1].

2.3 Iodine value

The iodine value is a measure of the degree of unsaturation of fatty acids and is used to characterize oils and fats. The garden cress seed oil contains both saturated and unsaturated fatty acids. Halogens add across the double bonds of unsaturated fatty acids to form additional compounds. Iodine monochloride (ICL) is allowed to react with the fat in the dark. Iodine gets incorporated into the fatty acids chain wherever the double bond exists. The amount of iodine consumed is then determined by titrating the iodine released (after adding KI) with standard thiosulphate and comparing with a blank in which the fat is omitted. Hence, the measure of iodine absorbed by an oil or fat gives the degree of unsaturation. Iodine value of garden cress seed oil was determined by titration method (AOAC, 1990) [1].

3. Results and Discussion

3.1 Effect of storage on peroxide value (PV) of garden cress seed oil

Irrespective of packaging material peroxide value increased with storage period. Storage temperature highly affected the peroxide value of garden cress oil than exposure to light. Peroxide value was highest for the oil stored in PET bottle at room temperature than stored at refrigerated condition. Similar trend was observed for garden cress seed oil stored in glass amber bottle which resists the exposure to light. It is important to point out that the relatively high peroxide values were measured in garden cress seed oil stored in PET bottles after 60 days of storage (14.724 meq.kg^{-1}) and 90 days (25.760 meq.kg^{-1}), as demonstrated in Table 1. It could be established that the entry of light accelerates the oxidative processes, resulting in oil spoilage, what was in agreement with the results obtained by Abramovich and Adam, 2005; Raza *et al*., 2009 [23]. At room temperature peroxide value increased from initial 2.591 to 25.760 meq.kg^{-1} after 90 days of storage in PET bottle whereas it increased from 2.591 to 10.843 meq.kg^{-1} for oil stored in amber colour glass bottle. Results reveled that, PET bottle provide less oil protection from destructive effect of the light during storage, in comparison with amber glass bottle protection, and this findings confirmed the results of the other researchers (Grujic *et al.*, 2011 and Manzocco *et al.,* 2011) [8, 14]. Amber or green coloured glass bottles provide better protection for edible oils. Abramovich and Adam (2005) indicated that the peroxide number is not used for examination of the early stages of fat oxidation and its accuracy is questionable, but it is used in a numerous tests during examination of oil sustainability. They used the monitoring of changes in peroxide value of oil kept under different conditions, as good and helpful indicator for comparing the effect of different storage conditions on rate of oxidative development process in oil. Increase in peroxide value of different oils, oil blends and processed oily products have been reported (Yang *et al.* 2005; Ogbonnaya and Yahaya, 2008; Krichene *et al.* 2010) [27, 19, 12]. The changes in these values may be due to oxidation which increases the peroxide value (Thakkar and Parikh, 2014) [24].

3.2 Effect of storage on acid value (AV) of garden cress seed oil

Data related to acid value of garden cress seed oil during storage is presented in Table 2. Acid value was found to be increasing with the storage period. Increase in acid value was marginal upto 30 days of storage, thereafter the steady and higher increase in acid value was observed. Oil stored in refrigerated condition showed lower acid value than that of stored at room temperature. At the same time, oil stored in PET bottle, exposed to light have higher acid values than stored in amber glass bottle, which prevents the entry of oxygen as well as light. Increase in acid value have been correlated with the presence of enzyme lipase (Ogbonnaya and Yahaya, 2008) [19]. Changes in acid values in analyzed garden cress seed oil samples were statistically significant, showing the phenomenon of lipid oxidation (Tian, *et al.*, 2000; Anwar *et al*., 2007) [25]. Grujic *et al.*, 2011 [8] found the increase in acid value of sunflower oil during storage. Increase in acid value during storage was also reported by Gaye, (2009) [7]; Gulla and Waghray (2012) [9]; Tiwari *et al.* (2012) [26] and Thakkar and Parikh, (2014) [24].

3.3 Effect of storage on iodine value (IV) of garden cress seed oil

Iodine value is a measure of the degree of unsaturation in oil, reduction in IV suggests a decrease in number of double bond in the oils (Henn Lu and Tan, 2009) [10]. Iodine value of the garden cress seed oil stored in different packaging material and storage conditions was observed to be decreasing with the advancement in the storage days (Table 3). Decrease in iodine value is an indicator of lipid oxidation (Naz *et al.,* 2004) [18]. As expected, unsaturated fatty acids declined during storage period. Decreasing of unsaturated fatty acids was observed for all storage period. After 90 days of storage, highest iodine value (128.791) was observed in the amber glass bottle stored in refrigerated storage whereas lowest iodine value (122.182) was observed in the PET bottle stored at room temperature.

Table 1: Effect of storage and packaging material on peroxide value (meq.kg^{-1}) of garden cress seed oil

Storage days	Packaging material	Storage condition		Mean
		T1	**T2**	
S_1 (0)	P1	2.591	2.591	2.591
	P2	2.591	2.591	2.591
	Mean	2.591	2.591	2.591
S_2 (30)	P1	7.641	4.567	6.104
	P2	7.082	4.001	5.542
	Mean	7.362	4.284	5.823
S_3 (60)	P1	14.724	7.969	11.347
	P2	11.772	7.402	9.587
	Mean	13.248	7.686	10.467
S_4 (90)	P1	25.760	12.075	18.918
	P2	20.123	10.843	15.483
	Mean	22.942	11.459	17.200
		T1	T2	Mean
	P1	12.679	6.801	9.740
	P2	10.392	6.209	8.301
	Mean	11.536	6.505	9.020
Source		Sem(±)	CD @5%	CD @1%
Storage days (S), Months		0.091	0.263	0.353
Storage temperature (T), °C		0.065	0.186	0.250
Packaging material (P)		0.065	0.186	0.250
SxP		0.129	0.367	0.490
SxT		0.129	0.372	0.500
P x T		0.091	0.263	0.353
SxP x T		0.183	0.526	0.707
C.V. (%)		7.515		

Table 2: Effect of storage and packaging material on acid value (mg KOH.g^{-1}) of garden cress seed oil

Storage days	Packaging material	Storage condition		Mean
		T1	**T2**	
S_1 (0)	P1	0.78	0.78	0.78
	P2	0.78	0.78	0.78
	Mean	0.78	0.78	0.78
S_2 (30)	P1	2.63	2.18	2.41
	P2	2.47	1.73	2.10
	Mean	2.55	1.96	2.25
S_3 (60)	P1	5.28	2.78	4.03
	P2	3.93	2.04	2.99
	Mean	4.61	2.41	3.51
S_4 (90)	P1	7.53	5.17	6.35
	P2	6.48	4.63	5.55
	Mean	7.01	4.90	5.95
		T1	T2	Mean
	P1	4.06	2.73	3.39
	P2	3.42	2.30	2.86
	Mean	3.74	2.51	3.12
Source		SEm(±)	CD @5%	CD @1%
Storage days (S), Months		0.031	0.089	0.120
Storage temperature (T), °C		0.022	0.063	0.085
Packaging material (P)		0.022	0.063	0.085
SxP		0.044	0.124	0.166
SxT		0.044	0.126	0.169
P x T		0.031	0.089	0.120
SxP x T		0.062	0.178	0.239
C.V. (%)		7.056		

Table 3: Effect of storage and packaging material on iodine value (I_2.100 g^{-1}) of garden cress seed oil.

Storage days	Packaging material	Storage condition		Mean
		T1	**T2**	
S_1 (0)	P1	131.70	131.70	131.70
	P2	131.70	131.70	131.70
	Mean	131.70	131.70	131.70
S_2 (30)	P1	128.41	130.41	129.41
	P2	129.97	130.83	130.40
	Mean	129.19	130.62	129.90
S_3 (60)	P1	126.03	128.03	127.03
	P2	127.18	129.30	128.24
	Mean	126.61	128.67	127.64
S_4 (90)	P1	122.18	127.72	124.95
	P2	126.82	128.79	127.81
	Mean	124.50	128.26	126.38
		T1	T2	Mean
	P1	127.08	129.47	128.27
	P2	128.92	130.16	129.54
	Mean	128.00	129.81	128.90
Source		SEm(±)	CD @5%	CD @1%
Storage days (S), Months		0.167	0.480	0.645
Storage temperature (T), °C		0.118	0.339	0.456
Packaging material (P)		0.118	0.339	0.456
SxP		0.236	0.670	0.895
SxT		0.236	0.679	0.912
P x T		0.167	0.480	0.645
SxP x T		0.333	0.960	1.290
C.V. (%)		0.441		

Mendez and Falque (2007) [15] reported a slight decrease in iodine value of oil after 3 months storage in all the containers used for experiment. Sharper decrease in iodine value with respect to the initial value was observed in plastic (normal and opaque), followed by glass and tin containers. Gaye, (2009) [7] observed that the decrease in iodine value of the extra virgin olive oil samples during storage. Grujic *et al*., (2011) [8] reported the drop in iodine value of sunflower oil during storage, which may be the result of light impact and creation of radicals on unsaturated hydrocarbon chain, which links appear connecting the complex compounds. They also observed the faster changes of iodine number occur in sunflower oil packed in transparent PET packaging, which supported the results obtained in present study. Tiwari *et al.* (2012) [26] reported that decrease in iodine value is due to the oxidative and hydrolytic rancidity which increased the FFA and decreased IV. IV does not take into account the structural differences present in different fatty acids such as the nature, quantity and position of the unsaturated bonds in the chain available for oxidation (Bouaid *et al*. 2007) [5]. Therefore, IV is not considered as the best way to evaluate the oxidative stability of oil.

4. Conclusion
Garden cress oil is susceptible to the oxidation during storage. Coloured glass bottles protects oil better than the polyethylene terephthalate bottle. Refrigerated storage prevents the oxidation oil better than the room temperature. Highest PV of GCO was 25.76 meq.kg^{-1}, AV was 7.53 **(**mg KOH.g^{-1}) and IV was 131.70 I_2.100 g^{-1}.

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