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Extending the Shelf Life of Ghee Using Garden Cress and Jojoba Oils as Alternatives of Synthetic Antioxidants

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Abstract

The goal of this study was to assess the efficacy of garden cress (GCO) and jojoba (JO) oils compared to butylated hydroxyl anisole (BHA) to extend ghee shelf-life under accelerated conditions (80°C). Both oils were added individually to ghee at concentrations of 100, 200, and 300 ppm while BHA was added at 200 ppm. Oils were examined for some chemical properties as well as their phenolic components while ghee samples either incorporated with BHA or both oils were examined for their free fatty acids, peroxide value, thiobarbituric acid, conjugated dienes, and radical scavenging activity (%) during storage at 80°C. JO had a high content of vanillin, benzoic acid, cinnamic acid, and kaempferol compared to GCO. Both oils exhibited varying antioxidant potential. Statistically, ghee incorporated with BHA had the highest significant differences in antioxidant activity followed by JO (200 - 300 ppm) and GCO (300 ppm) which have proven to be effective in delaying the undesirable changes in the ghee. Overall, jojoba oil followed by garden cress oil offer a promising option as alternatives of synthetic antioxidants to extend ghee shelf-life under accelerated conditions.

Key words: Ghee; shelf-life; garden cress oil; jojoba oil; BHA; antioxidant activity

1. Introduction

Ghee is the fatty dairy product most widely used in the Middle East and Indian diets. Compared to other dairy products, it is distinguished by its pleasant flavor and long shelf-life under good preservation conditions. Oxidative rancidity is the most important cause of ghee deterioration that limits its shelf life [1]. It affects its nutritional and economic values in addition to the formation of toxic and harmful compounds [2] which cause negative health effects [3]. A variety of synthetic antioxidants like butylated hydroxy anisol (BHA), butylated hydroxytoluene (BHT), tertbutylhydroquinone (TBHQ), and propyl gallate (PG) are often used to retard fat oxidation and prolong the shelf life of foods [4] but they have health hazards and toxic effect [5] and responsible for hepatic damage and carcinogenesis [6, 7]. Therefore, natural antioxidants have greater application potential in the food industry and researches focused on the use of natural antioxidants as an alternative to synthetic ones to suppress lipid oxidation [8-11]. Polyphenols or phenolic compounds are the most important phytochemicals in human nutrition. They are secondary plant metabolites found in all plant tissues and are primarily used to protect plants against insects, ultraviolet radiation, and microbial infections, and to attract pollinators [12]. According to the chemical structures, polyphenols are categorized as flavonoids, phenolic acids, lignans, and stilbenes [13]. They are potent antioxidants with high ability in free radical scavenging [14]. They are effective protective agents against many degenerative diseases, protecting body tissues against oxidative stress.

Jojoba (*Simmondsia chinensis*) is unique among plants; its seeds contain around 40-60% by weight oil [15]. It has medicinal, antimicrobial, antiproliferative, antifungal, and antioxidant properties [16-18]. Jojoba oil has been used in the cosmetics and skincare industry [19]. Evaluation of selected quality features of creams with addition of jojoba oil designed for dry skin. Polish J Cosmetol. 18(2):132–137]. It has some medicinal properties such as the relief of headaches and throat inflammation, wounds healing [20], anti-inflammatory activity, as well as antimicrobial [21], and antifungal/insecticidal properties [22]. Garden cress (*Lepidium sativum*) is an edible annual herb, belongs to the family of Cruciferae (Brassica). Garden cress seeds contain 18–24% fat of which ~32-

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34% of total fatty acids is alpha-linolenic acid (ALA) [23, 24]. Garden cress is a vital source of protein, fat, carbohydrates, fibers, vitamins, and minerals, so, it can be used as a functional food ingredient [25]. Garden cress oil (GCO) has a very high tocopherol content compared to other oils [26, 27], which act as biological scavengers of free radicals that inhibit oil oxidation. Tocopherols also help in preventing diseases, besides possessing an important nutritional function for human beings as a source of vitamin E. GCO 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 [24]. Garden cress seeds have many pharmacological activities including anti-inflammatory, bone fracture healing, hepato-protective, antihypertensive, antimicrobial, anti-diabetic, chemoprotective, and laxative impact [28]. Currently, no sufficient studies on the use of jojoba and garden cress oils to preserve ghee, so the objective of this research was to evaluate ability of both oils as synthetic antioxidant alternatives to prolong ghee's shelf life.

2. Materials and Methods

2.1 Materials

Cow butter was obtained from Dairy Unit, Fac. Agric., Cairo Univ. Cold-pressed Garden cress and Jojoba oils were purchased from EL Hawag for Natural Oil Co., EL Nasr City, Cairo, Egypt. Butylated hydroxyl anisole (BHA) was obtained from Sigma Georgia, (USA). 2,2-Diphenyle-1-picrylhydrazyl (DPPH) was purchased from Merck, Darmstadt, Germany. Thiobarbituric acid (TBA) was obtained from Cymann, Michigan, USA. All the other chemicals were of analytical grade.

2.2 Experimental procedure

Ghee was prepared from cow butter using a heat process according to Fahmi [29]. The resultant ghee was divided into 4 equal portions. The first one served as a control (without antioxidants) while to the second one, 200 ppm of butylated hydroxyanisole (BHA) was added. The third and fourth portions were divided into 3 equal portions containing 100, 200, and 300 ppm garden cress and jojoba oils separately. All samples were filled in sterile test tubes (constant equal volumes sufficient for all the testes used) and incubated at 80°C ± 1 °C until deterioration to accelerate the autoxidation of ghee and periodically analyzed.

2.3 Methods of analysis

Garden cress and jojoba oils were analyzed for iodine (IV), saponification (SV), and peroxide values (PV as % meq. peroxide/ kg oil) and free fatty acids according to AOAC [30]. Identification of phenolic components of both oils was measured using Agilent1260 infinity HPLC Series (Agilent Application Note, Publication number 5991-3801EN, 2014) as follows: A 2.0 g amount of oil was accurately weighed into a 15 mL tube. A 1 mL amount of the internal standard solution (syringic acid 0.015 mg/mL in methanol/water 80/20 (v/v)) was transferred to the previously weighed sample. The sealed sample tube was vortexed for 30 seconds. After adding 5 mL of methanol/water 80/20 (v/v) extraction solution, it was again vortexed for exactly 1 minute before further extraction in the ultrasonic bath for 15 minutes at room temperature. Afterwards, the sample was centrifuged at 5,000 rpm for 25 minutes. An aliquot of the supernatant phase was filtered through a 1 mL plastic syringe with Captiva Premium Syringe Filters Regenerated Cellulose, 4 mm, 0.45 µm (p/n 5190-5107) before injection into the HPLC system. Retention time and peak area were used for calculation of phenolic compounds concentration by the data analysis of Agilent Software.

Ghee samples were analyzed for free fatty acids, peroxide, and thiobarbituric acid (TBA) values according to Patton and Kurtz [31]. The antioxidant activity % of the different concentrations of both oils was determined using DPPH assay according to Karamać et al [32]. Conjugated dienes (%) were measured according to AOAC [33].

2.4 Statistical analysis

A randomized complete block design with two factors was used for analysis of all data with three replications for each parameter. The treatment means were compared by the least significant difference (L.S.D.) test as given by Snedecor and Cochran [34] using assistant program [35].

3. Results and Discussion

3.1 Some chemical properties of GCO and JO

Chemical constants of fats or oils are common quality factors that provide information about the fat composition and quality, as well as changes that occur during storage. It also acts as a method of detecting and distinguishing fat adulteration. The acid value (AV), saponification number (SV), iodine value (IV), and peroxide value (PV) are included.

Data in Table (1) show some chemical properties of GCO and JO. Non- significant differences were observed between GCO and JO in both free fatty acids, peroxide and iodine values. Both oils had almost the same content of free fatty acids (0.22 to

0.25%) which are in agreement with Diwakar et al. [27] and Youssef et al. [36] who reported that free fatty acid (% oleic) of cold-pressed GCO was 0.28 and 0.29% respectively. IV values were 87.15 ± 0.85 and 85.20 ± 1.25 for GCO and JO resp, while PV of GCO was 0.80 ± 0.15 and 0.73 ± 0.20 for JO. GCO has higher SV as compared to JO (106.76 ±1.31for GCO and 88.02 ±1.00 for JO with significant differences). Diwakar et al. [27] reported that PV, FFA (% Oleic), SV, IV of GCO were 0.70, 0.28, 178.85, and 122 respectively. While Yenge et al. [37] mentioned that PV, AV, and IV of GCO were 0.83, 0.61and 97.27 resp. Zia-Ul-Haq [38] stated that the acid value and saponification value of GCO were 1.04 and 179.03 respectively. Araiza-Lizarde et al. [15] reported that iodine index, acid index, and peroxide index (meq/kg de sample) of JO were 80.85- 83.11, 0.35- 0.39, and 2, respectively. Spencer and List [39] reported that acid value, peroxide value, saponification number, and iodine value of JO were 0.7, 5.0, 90-95, and 80-85 respectively. El-Kinawy [40] mentioned that acid value, iodine value, saponification value, and peroxide value of JO were: 0.74, 82, 89, and 0.0, respectively.

Table 1. Some chemical properties of garden cress and jojoba oils

Properties	Garden cress oil	Jojoba oil
Free Fatty acids (% oleic)	0.25±0.05 ^{A}	0.22±0.08 ^{A}
Peroxide value (% meq. peroxide/ kg oil)	0.80±0.15 ^{A}	0.73±0.20 ^{A}
Iodine value (g of I_2 absorbed/100 g)	87.15±0.85 ^{A}	85.20±1.25 ^{A}
Saponification value (mg /100 gm)	106.76±1.31^{A}	88.02±1.00^{B}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts letters (A,B, C……) do not differ significantly (P<0.05).

Table 2. Phenolic components (mg/L) of garden cress and jojoba oils

Components	Garden cress oil	Jojoba oil
Vanillin	2.25640	10.78609
Ferulic acid	9.40973	2.99601
Benzoic acid	117.07075	122.68422
o- Coumaric acid	2.37647	1.70139
Salicylic acid	ND	4.23248
Myricetin	ND	171.99053
Cinnamic acid	2.62612	4.45474
Quercitin	74.36312	27.94300
Rosemarinic	116.65729	25.61829
Neringein	295.44807	113.70829
Kampherol	44.17204	308.95476
p- Coumaric acid	8.42168	ND
Rutin	38.46441	ND

ND: not detected

Concerning the phenolic components of GCO and JO, data in Table (2) show that GCO had higher levels of ferulic acid, o- coumaric acid, quercitin, rosemarinic, and naringenin than JO. GCO contained p- coumaric acid and rutin while they were not detected in JO. On the other hand, JO had a higher content of vanillin benzoic acid, cinnamic acid, and kaempferol as compared to GCO. JO contained salicylic acid and myricetin which were not found in GCO. Doke and Guha [41] identified the phenolic components of GC seeds and reported that it contained coumaric acid, vanillic acid, quercetin, and kaemferol. All the identified components are characterized by their high antioxidant activity [42]. in Table 2 and Fig. 2.

3.2 Effect of GCO and JO on some chemical properties of ghee stored at 80 °C

FFA content is regarded as a key factor linked with the quality as well as the economic value of edible fats and oils. Generally, FFAs are the hydrolysis products of oil and fat oxidation during long-term storage or processing at elevated temperatures during heating or frying [43]. The FFA value is expressed as a percentage of a fatty acid predominant in the product being tested. For butter fat, tallow, or soybean oil, values are often expressed as percent of oleic acid. Fresh ghee has acidity ~ 0.2% oleic acid [44].

Data in Table (3) demonstrate the changes in the free fatty acid content of ghee samples during storage at 80 °C as influenced by GCO or JO compared with BHA. All fresh ghee samples had FFA % ranged from 0.13-0.16% with non-significant differences. As the storage period progressed, it increased significantly in all treatments with different rates. The formation of FFA in control ghee was more pronounced and significantly higher relative to other treatments. BHA was found to be significantly more effective as compared with both GCO and JO which is in line with Shende et al. [45]. It is also obvious that increasing the concentration of the used oils reduces the rate of FFA release. As compared to GCO, the effect of JO is more efficient. These results are in agreement with Asha et al. [44] and Nadeem et al. [46].

Peroxide value (PV) is an indicator of the extent of primary oxidation products in oils and lipids [47, 48]. It represents the quantity (mg) of active oxygen contained in 1 g of lipid. Table (4) shows the formation of peroxides in control and treated ghee samples during storage at 80 °C. In all fresh samples, the PV is ranged from 0.49 to 0.51 millimoles /g fat. During storage at 80 °C, its values in control ghee are rapidly and significantly increased while the addition of BHA possesses a slight increment and higher activity as an antioxidant as compared to both oils. PV in treated ghee with either GCO or JO is gradually and significantly increased. The rate of increase was the highest in control and the lowest in the case of BHA which agreed with the findings of Gandhi et al. [49] and Shende et al. [45]. The highest

arise in peroxides in the control may be attributed to the production of more hydroperoxides (initial products of oxidation) in the absence of antioxidant compounds. The inhibitory effect of the used oils on PV was concentration dependent. The ability of jojoba oil to lower the peroxide value is better than garden cress oil. JO (200 ppm) is more effective than GCO (300 ppm).

Table 3. Changes in free fatty acids (FFA %) of ghee during storage at 80 °C

Storage period (days)	Treatments*/ Concentration (ppm)							
	Control	BHA	GCO			JO		
			100	200	300	100	200	300
0	0.15 ± 0.01^{lm}	0.16 ± 0.01^{lm}	0.14 ± 0.01^{m}	0.14 ± 0.03^{m}	0.13 ± 0.02^{m}	0.14 ± 0.01^{m}	0.14 ± 0.02^{m}	0.16 ± 0.02^{lm}
3	0.27 ± 0.02^{hi}	0.23 ± 0.03^{jk}	0.26 ± 0.01^{hij}	0.23 ± 0.01^{jk}	0.22 ± 0.01^{jk}	0.25 ± 0.01^{ijk}	0.21 ± 0.03^{kl}	0.23 ± 0.02^{jk}
6	0.42 ± 0.01^{de}	0.36 ± 0.01^{efg}	0.40 ± 0.03^{ef}	0.27 ± 0.03^{hi}	0.25 ± 0.00^{ijk}	0.29 ± 0.01^{hi}	0.27 ± 0.01^{hi}	0.26 ± 0.01^{hij}
9	0.70 ± 0.02^{TUV}	0.47 ± 0.03^{cd}	0.55 ± 0.05^{YZa}	0.54 ± 0.01^{za}	0.42 ± 0.01^{de}	0.36 ± 0.01^{efg}	0.30 ± 0.02^{gh}	0.29 ± 0.01^{hi}
12	0.84 ± 0.04^{PQ}	0.59 ± 0.03^{XYZ}	0.67 ± 0.03^{VW}	0.62 ± 0.01^{WX}	0.53 ± 0.02^{za}	0.47 ± 0.03^{cd}	0.37 ± 0.02^{efg}	0.32 ± 0.01^{gh}
15	1.83 ± 0.04^{C}	0.67 ± 0.03^{VW}	0.78 ± 0.01^{RS}	0.68 ± 0.02^{UV}	0.60 ± 0.02^{D}	0.57 ± 0.01^{XYZa}	0.48 ± 0.04^{bc}	0.40 ± 0.01^{ef}
18	1.59 ± 0.09^{ef}	0.77 ± 0.03^{RS}	0.90 ± 0.08^{O}	0.80 ± 0.03^{QR}	0.68 ± 0.01^{UV}	0.66 ± 0.03^{D}	0.56 ± 0.02^{YZa}	0.55 ± 0.04^{YZa}
21	1.74 ± 0.02^{D}	0.88 ± 0.04^{OP}	1.20 ± 0.07^{K}	1.07 ± 0.21^{LM}	0.74 ± 0.02^{ST}	0.73 ± 0.02^{ST}	0.85 ± 0.05^{C}	0.77 ± 0.02^{RS}
24	2.00 ± 0.21^{B}	0.97 ± 0.03^{N}	1.52 ± 0.01^{G}	1.34 ± 0.06^{I}	1.05 ± 0.14^{B}	1.10 ± 0.04^{B}	1.04 ± 0.04^{M}	1.05 ± 0.03^{LM}
27	2.33 ± 0.17^{A}	1.04 ± 0.02^{M}	1.68 ± 0.01^{E}	1.54 ± 0.02^{FG}	1.46 ± 0.04^{H}	1.55 ± 0.03^{FG}	1.34 ± 0.04^{I}	1.27 ± 0.02^{J}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts capital or small letters do not differ significantly (P<0.05).
Treatments*: Control: ghee without addition of antioxidant; BHA: ghee samples contain 200 ppm of butylated hydroxyanisole (BHA), GCO100: ghee samples contain 100 ppm of garden cress oil, GCO200: ghee samples contain 200 ppm garden cress oil, GCO300: ghee samples contain 300 ppm garden cress oil, JO 100: ghee samples contain 100 ppm of jojoba oil, JO 200: ghee samples contain 200 ppm of jojoba oil, JO 300: ghee samples contain 300 ppm of jojoba oil.

Table 4. Changes in peroxide value (millimoles /g of fat) of ghee during storage at 80 °C

Storage period (days)	Treatments*/ Concentration (ppm)							
	Control	BHA	GCO			JO		
			100	200	300	100	200	300
0	0.49 ± 0.05^{F}	0.51 ± 0.07^{F}	0.51 ± 0.03^{F}	0.51 ± 0.03^{F}	0.52 ± 0.05^{F}	0.51 ± 0.04^{F}	0.50 ± 0.06^{F}	0.49 ± 0.05^{F}
3	2.18 ± 0.04^{bcd}	0.68 ± 0.04^{j}	1.90 ± 0.06^{cde}	1.45 ± 0.04^{efgh}	1.22 ± 0.10^{ghi}	1.37 ± 0.08^{fgh}	1.13 ± 0.11^{ghi}	0.91 ± 0.08^{ij}
6	5.01 ± 0.12^{E}	1.01 ± 0.09^{EF}	3.21 ± 0.13^{Y}	2.77 ± 0.08^{za}	2.23 ± 0.11^{bc}	1.52 ± 0.01^{efg}	1.32 ± 0.05^{ghi}	1.03 ± 0.03^{hij}
9	7.32 ± 0.05^{PQ}	1.18 ± 0.04^{ghi}	4.60 ± 0.03^{VW}	3.39 ± 0.05^{Y}	2.42 ± 0.09^{ab}	2.20 ± 0.07^{bcd}	1.77 ± 0.06^{def}	1.48 ± 0.04^{efg}
12	11.20 ± 0.21^{KL}	1.81 ± 0.08^{cde}	7.72 ± 0.41^{P}	5.94 ± 0.12^{TU}	3.94 ± 0.26^{X}	3.10 ± 0.21^{YZ}	2.40 ± 0.07^{ab}	1.82 ± 0.04^{cde}
15	13.44 ± 0.30^{I}	2.22 ± 0.05^{bcd}	11.10 ± 0.21^{kL}	8.52 ± 0.37^{O}	6.37 ± 0.23^{ST}	4.63 ± 0.17^{V}	3.44 ± 0.40^{Y}	2.43 ± 0.11^{ab}
18	14.23 ± 0.40^{H}	2.46 ± 0.15^{ab}	15.24 ± 1.53^{efg}	10.65 ± 0.18^{M}	7.03 ± 0.25^{QR}	6.85 ± 0.14^{R}	4.60 ± 0.47^{VW}	3.35 ± 0.57^{Y}
21	21.83 ± 0.45^{C}	2.77 ± 0.22^{za}	17.19 ± 0.48^{F}	11.09 ± 0.30^{KLM}	8.37 ± 0.30^{O}	8.98 ± 0.33^{DC}	6.21 ± 0.08^{TU}	5.89 ± 0.05^{U}
24	24.41 ± 1.78^{B}	3.34 ± 0.26^{Y}	19.04 ± 0.45^{E}	12.57 ± 0.37^{J}	9.18 ± 0.24^{N}	11.29 ± 0.33^{K}	7.47 ± 0.06^{PQ}	6.79 ± 0.08^{RS}
27	29.32 ± 1.13^{A}	4.19 ± 0.47^{WX}	20.77 ± 0.83^{D}	13.3 ± 00.02^{I}	10.84 ± 0.30^{LM}	13.13 ± 0.54^{I}	9.41 ± 0.22^{N}	8.29 ± 0.52^{O}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts capital or small letters do not differ significantly (P<0.05).
Treatments*: Control: ghee without addition of antioxidant; BHA: ghee samples contain 200 ppm of butylated hydroxyanisole (BHA), GCO100: ghee samples contain 100 ppm of garden cress oil, GCO200: ghee samples contain 200 ppm garden cress oil, GCO300: ghee samples contain 300 ppm garden cress oil, JO 100: ghee samples contain 100 ppm of jojoba oil, JO 200: ghee samples contain 200 ppm of jojoba oil, JO 300: ghee samples contain 300 ppm of jojoba oil.

Thiobarbituric Acid (TBA) indicates the quantity of malondialdehyde (in mg) present in 1 kg of sample. TBA test measures the secondary products of lipid oxidation. It involves reacting thiobarbituric acid with malondialdehyde formed by lipid hydroperoxide decomposition to form a red chromophore with a peak absorbance at 532 nm.

During storage, changes in the TBA value as affected by the addition of GCO and JO are shown in Table (5). It can be noted that all fresh ghee treatments had low TBA values being 0.06-0.07 (as absorbance at 532 nm) with non-significant differences between treatments. During storage at 80 °C, its values were significantly and progressively increased in control and are higher than treated ghee samples which are in line with Shende et al. [45] and Asha et al. [44]. TBA value is gradually and significantly increased in BHA and oils treated samples and were significantly lower as compared to control. BHA was more effective in reducing the rise in TBA than both GGO and JO even at the high concentration. The obtained data suggested that JO (300ppm) is more effective than GCO at the same concentration.

Data in Table (6) show that all fresh ghee samples had low and non-significant CD values being 0.48-

0.52 %. During storage at 80 °C, its values are gradually and significantly increased in control ghee to reach 1.92% while BHA was the most capable to reduce the formation of conjugated dienes with significant differences compared to other treatments to reach 0.87% at the end of storage. The lowering effect of the used oils is concentration dependent. At the higher concentration of both oils, JO significantly reduced the formation of dienes to 1.1% while in case of GCO, the value reached 1.33%. The ability of jojoba oil to lower the formation of CD is better than garden cress oil. JO (200 ppm) is more effective than GCO (300 ppm). These results are in line with Pawar et al. [50].

Table 5. Changes in TBA value (OD at 532) of ghee during storage at 80 °C

Storage period (days)	Treatments*/ Concentration (ppm)							
	Control	BHA	GCO			JO		
			100	200	300	100	200	300
0	0.06 ± 0.01^{d}	0.06 ± 0.01^{d}	0.07 ± 0.02^{bc}	0.07 ± 0.01^{bc}	0.06 ± 0.01^{d}	0.07 ± 0.01^{bc}	0.07 ± 0.01^{bc}	0.07 ± 0.01^{bc}
3	0.18 ± 0.04^{Y}	0.09 ± 0.03^{bc}	0.20 ± 0.01^{Y}	0.20 ± 0.01^{Y}	0.18 ± 0.01^{Y}	0.16 ± 0.01^{YZa}	0.15 ± 0.01^{a}	0.10 ± 0.01^{b}
6	0.35 ± 0.05^{RS}	0.15 ± 0.01^{a}	0.29 ± 0.01^{U}	0.26 ± 0.01^{VW}	0.19 ± 0.01^{E}	0.20 ± 0.03^{Y}	0.17 ± 0.01^{YZ}	0.16 ± 0.01^{YZa}
9	0.47 ± 0.06^{MN}	0.20 ± 0.01^{Y}	0.39 ± 0.01^{PQ}	0.32 ± 0.01^{T}	0.27 ± 0.02^{UV}	0.34 ± 0.03^{RS}	0.19 ± 0.01^{E}	0.19 ± 0.01^{E}
12	0.57 ± 0.08^{JK}	0.22 ± 0.01^{X}	0.48 ± 0.01^{M}	0.39 ± 0.04^{PQ}	0.38 ± 0.01^{PQ}	0.42 ± 0.01^{O}	0.24 ± 0.03^{WX}	0.23 ± 0.03^{X}
15	0.72 ± 0.04^{EF}	0.27 ± 0.03^{UV}	0.58 ± 0.04^{HIJ}	0.57 ± 0.02^{C}	0.47 ± 0.02^{MN}	0.50 ± 0.01^{C}	0.40 ± 0.04^{OP}	0.32 ± 0.01^{T}
18	0.85 ± 0.05^{C}	0.32 ± 0.01^{T}	0.76 ± 0.03^{D}	0.70 ± 0.01^{FG}	0.58 ± 0.04^{HIJ}	0.57 ± 0.04^{C}	0.56 ± 0.010^{KL}	0.46 ± 0.01^{N}
21	0.99 ± 0.06^{A}	0.37 ± 0.03^{QR}	0.88 ± 0.01^{B}	0.76 ± 0.01^{D}	0.70 ± 0.02^{FG}	0.73 ± 0.01^{E}	0.60 ± 0.01^{HI}	0.58 ± 0.01^{HIJ}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts capital or small letters do not differ significantly (P<0.05).
Treatments*: Control: ghee without addition of antioxidant; BHA: ghee samples contain 200 ppm of butylated hydroxyanisole (BHA), GCO100: ghee samples contain 100 ppm of garden cress oil, GCO200: ghee samples contain 200 ppm garden cress oil, GCO300: ghee samples contain 300 ppm garden cress oil, JO 100: ghee samples contain 100 ppm of jojoba oil, JO 200: ghee samples contain 200 ppm of jojoba oil, JO 300: ghee samples contain 300 ppm of jojoba oil.

Table 6. Changes in the conjugated dienes (CD %) of ghee during storage at 80 °C

Storage period (days)	Treatments*/ Concentration (ppm)							
	Control	BHA	GCO			JO		
			100	200	300	100	200	300
0	0.48 ± 0.04^{i}	0.49 ± 0.03^{i}	0.50 ± 0.01^{i}	0.51 ± 0.01^{hi}	0.50 ± 0.01^{i}	0.49 ± 0.01^{i}	0.52 ± 0.01^{ghi}	0.50 ± 0.01^{i}
3	0.74 ± 0.02^{UVW}	0.57 ± 0.02^{D}	0.67 ± 0.02^{YZa}	0.64 ± 0.02^{Zab}	0.56 ± 0.01^{def}	0.63 ± 0.02^{abc}	0.57 ± 0.02^{def}	0.55 ± 0.01^{fgh}
6	0.94 ± 0.03^{M}	0.65 ± 0.02^{Zab}	0.76 ± 0.01^{TUV}	0.72 ± 0.01^{UVW}	0.69 ± 0.03^{XYZ}	0.72 ± 0.01^{UVW}	0.61 ± 0.01^{bcd}	0.60 ± 0.01^{cde}
9	1.25 ± 0.05^{I}	0.69 ± 0.01^{XYZ}	0.83 ± 0.03^{PQR}	0.79 ± 0.01^{RST}	0.77 ± 0.02^{STU}	0.78 ± 0.02^{RST}	0.66 ± 0.01^{YZa}	0.65 ± 0.01^{Zab}
12	1.46 ± 0.05^{D}	0.76 ± 0.03^{TUV}	0.93 ± 0.03^{MN}	0.84 ± 0.01^{PQ}	0.83 ± 0.01^{PQR}	0.86 ± 0.02^{OP}	0.78 ± 0.02^{RST}	0.70 ± 0.01^{WXY}
15	1.60 ± 0.04^{C}	0.81 ± 0.01^{QRS}	1.24 ± 0.02^{I}	1.20 ± 0.09^{I}	0.92 ± 0.024^{MN}	0.92 ± 0.09^{MN}	0.87 ± 0.02^{OP}	0.81 ± 0.02^{QRS}
18	1.78 ± 0.08^{B}	0.85 ± 0.01^{OP}	1.33 ± 0.03^{GH}	1.30 ± 0.02^{H}	1.17 ± 0.02^{J}	1.30 ± 0.02^{H}	1.00 ± 0.01^{L}	0.87 ± 0.01^{OP}
21	1.92 ± 0.09^{A}	0.87 ± 0.01^{OP}	1.50 ± 0.01^{B}	1.39 ± 0.01^{EF}	1.33 ± 0.07^{GH}	1.41 ± 0.01^{E}	1.15 ± 0.07^{JK}	1.10 ± 0.08^{K}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts capital or small letters do not differ significantly (P<0.05).
Treatments*: Control: ghee without addition of antioxidant; BHA: ghee samples contain 200 ppm of butylated hydroxyanisole (BHA), GCO100: ghee samples contain 100 ppm of garden cress oil, GCO200: ghee samples contain 200 ppm garden cress oil, GCO300: ghee samples contain 300 ppm garden cress oil, JO 100: ghee samples contain 100 ppm of jojoba oil, JO 200: ghee samples contain 200 ppm of jojoba oil, JO 300: ghee samples contain 300 ppm of jojoba oil.

Data in Table (7) show the antioxidant activity% (as radical-scavenging activity using DPPH assay) of ghee samples as affected by the addition of antioxidants. It is clear that on zero-day (prior oxidation), the radical scavenging activity% of control was significantly much lower as compared to the treated samples which may be attributed to the absence of antioxidant compounds in control being 21.10% while, the radical scavenging activity of ghee containing BHA was found to be the highest being 85.14% and was significantly better as compared with both of GCO or JO even at the end of storage.

The radical scavenging activity % of ghee incorporated with GC and JO was found to be 31.56 - 41.71 and 52.02- 62.29 % respectively. The presence of antioxidant compounds in ghee incorporated with BHA, GCO, or JO exhibited stronger radical scavenging activity. This effect is concentration dependent. At the end of storage at 80 °C, the radical scavenging activity % was significantly and progressively decreased in control ghee to reach 3.49% whereas the extent of reduction in the other treatments (that contained antioxidant compounds) was much lower. The radical scavenging activity % of ghee incorporated with BHA was 73.29% while it was 17.36 – 29.34 % for GCO while it reached 35.83- 47.87% for JO. It is also noticeable that the antioxidant activity of JO even at its lower concentration (100 ppm) is more effective than GCO (300 ppm). These results are in agreement with Gandhi et al. [49] and El-Shourbagy and El-Zahar [51].

Table 7. Radical scavenging activity (%) of ghee during storage at 80 °C

Storage period (days)	Treatments*/ Concentration (ppm)							
	Control	BHA	GCO			JO		
			100	200	300	100	200	300
0	21.10±0.92^{GHI}	85.14±0.33^{A}	31.56±0.83^{FG}	37.16±0.50^{EF}	41.71±0.50^{DE}	52.02±0.98^{D}	60.35±0.04^{C}	62.29±1.30^{C}
27	3.49±0.03^{K}	73.29±2.74^{B}	17.36±0.18^{IJ}	22.58±0.04^{GH}	29.34±1.15^{GH}	35.83±0.45^{F}	42.10±1.48^{DE}	47.87±0.44^{DE}

Values are Means ± Standard deviation of three replicates.
Means with the same superscripts letters (A,B, C ……) do not differ significantly ($P < 0.05$).
Treatments*: Control: ghee without addition of antioxidant; BHA: ghee samples contain 200 ppm of butylated hydroxyanisole (BHA), GCO100: ghee samples contain 100 ppm of garden cress oil, GCO200: ghee samples contain 200 ppm garden cress oil, GCO300: ghee samples contain 300 ppm garden cress oil, JO 100: ghee samples contain 100 ppm of jojoba oil, JO 200: ghee samples contain 200 ppm of jojoba oil, JO 300: ghee samples contain 300 ppm of jojoba oil.

4. Conclusion

From a dietary point of view, plant oils rich in bioactive compounds have become an important solution for consumers to minimize the risk of a particular disease or health issue. These are also important for utilization of plant oils as alternatives to synthetic antioxidants. From the foregoing results, it can be inferred that ghee incorporated with BHA showed stronger antioxidant activity compared to JO and GCO. From a health point of view, ghee incorporated with JO and GCO exhibited better radical scavenging activity as compared to control due to their polyphenols content and have proven to be successful in delaying the undesirable changes in the ghee, and accordingly, it is recommended to use JO (200-300 ppm) or GCO (300 ppm), in maintaining and prolonging the shelf life of ghee.

References

[1] Mehta, B., Ragi, (Eleusine coracana L.) – A natural antioxidant for ghee (butter oil). International Journal of Food Science and Technology, 41(s1), 86–89(2006).

[2] Mehta, B. M., Aparnathi, K. D., and Darji, V. B., Comparison of different methods of monitoring the secondary stage of oxidation of ghee. *International Journal of Dairy Technology*, **68**(4), 589–594(2015).

[3] Sanders, T. A. B.. Nutritional aspects of rancidity. In J. C. Allen and R. J. Hamilton (Eds.): Rancidity in foods (pp. 125–130). *London: Elsevier Applied Science Publishers* (1989).

[4] Sherwin, E. R., Antioxidants. In: Food Additives; Branen, A.L.; Davidson, P.M.; Salminen, S.; Eds.; *Marcel Dekker: New York*, 139–193(1990).

[5] Kahl, R., and Kappus, H., Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Zeitschrift fur Lebensmittel-Untersuchung und –Forschung*, **196** (4), 329–338(1993).

[6] Grice, H.C.. Safety evaluation of butylated hydroxyl toluene (BHT) in the liver, lung, and gastrointestinal tract. *Food and Chemical Toxicology*, **24**, 1127–1130(1986).

[7] Wichi, H. P., Enhanced tumor development by butylated hydroxyl anisole (BHA) from the perspective of effect on the fore stomach and oesophageal squamous epithelium. *Food and Chemical Toxicology*, **26**, 717–723(1988).

[8] Pokorny, J., and Korczak, J., Preparation of Natural Antioxidants. Antioxidants in Food–Practical Application, *Woodhead Publishing Ltd.: Cambridge*, 311–330(2001).

[9] Kapadiya, D. B., Dabhi B. K., and Aparnathi, K. D.. Spices and herbs as a source of natural antioxidants for food. *International Journal of Current Microbiology and Applied Sciences*, **5** (7), 280- 288 (2016).

[10] Lodh, J. and Khamrui, K.. Evaluation of physico-chemical changes in Curcumin fortified buffalo Ghee during storage at 30±1°C. *International Journal of Chemical Studies*, **5**(2), 141-144(2017).

[11] Kapadiya, D. B., and Aparnathi, K. D.. Evaluation of commonly used herbs to enhance shelf life of ghee against oxidative deterioration. Journal of Food Processing and Preservation, **42**(7), e13658 (2018).

[12] Del Rio, D., Rodriguez-Mateos, A., Spencer J.P.E., Tognolini, M., Borges, G., and Crozier, A., Dietary (Poly) phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, **18** (14), 1818-1892 (2013).

[13] Manach, C.. Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**, 727-747(2004).

[14] Visioli, F., Bellomo, G. and Galli, C., Free radical scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* **247**, 60–64(1998).

[15] Araiza-Lizarde, N., Alcaraz-Meléndez, L., Angulo-Escalante, M. A., Reynoso-Granados, T., Cruz-Hernández, P., and Calderón-Vázquez, C. L., Physicochemical composition of seed oil of wild jojoba populations in northwestern Mexico. Journal of Food Nutrition Research, **5**, 443-450 (2017).

[16] Al Obaidi, J., Halabi, M.F., AlKhalifah,N.S. , Asanar, S., Soqeer, A.A.A., and Attia M. F., A review on plant importance, biotechnological aspects, and cultivation challenges of jojoba plant. *Biological Research*, **50**, 25-34 (2017).

[17] AL-Qizwini, H., AL-Khateeb E., Mhaidat, N.M., and Maraqa, A., Antioxidant and antimicrobial activities of jordanian simmondsia chinensis (link) c.k. schneid. *European Scientific Journal*, **10**, No.27: 1857 – 7881(2014).

[18] Sobhy, H. M., Mansour, M. K., Amal, A. Zaki and Maha, M. Elkholy. Hepatoprotective effect of jojoba oil on dna damage and antioxidant enzymes induced by cadmium in rats. *Egyptian Journal of Chemistry and Environmental Health, 1 (1)*, 94-112 (2015).

[19] Sandha, G., and Swami V., Jojoba oil as an organic, shelf stable standard oil phase base for cosmetic industry. *Rasayan Journal Chemistry* , **2** (2), 300–6 (2009).

[20] Ranzato, E., Martinotti, S., and Burlando, B., Wound healing properties of jojoba liquid wax: an in vitro study. *Journal of ethnopharmacology*, **134**(2), 443-449(2011).

[21] Habashy, R. R, Abdel-Naim A. B, Khalifa, A. E,and Al-Azizi M. M., Anti-inflammatory effects of jojoba liquid wax in experimental models. *Pharmacol Research.*, **51** (2), 95–105(2005).

[22] Abdel-Mageed, W.M., Bayoumi, S.A.L., Al-wahaibi, L.H., Li, L., Sayed, H.M., Abdelkader, M.S.A., El-Gamal, A.A., Liu, M., Zhang, J., Zhang, L., and Liu, X.., Noncyanogenic cyanoglucoside cyclooxygenase inhibitors from Simmondsia chinensis. *Organic Letters*, **18** (8),1728–31(2016).

[23] Sumangala, S. G., Malleshi, N. G.,and Guo, M., Chemical composition of garden cress (lepidium sativum) seeds and its fractions and use of bran as a functional ingredient, *Plant Foods for Human Nutrition*, **59**, 105–111(2004),

[24] Shetty, U. S., and Akhilender, N. K., Garden cress (Lepidium sativum L.) Seed Oil: Alternative Source for ALA. The FASEB Journal, 31, 971-12 (2017).

[25] Gaafar, M. A., Morsi, A. A., and Elghamry, E. H., Chemical, nutritional and biochemical studies of garden cress protein isolate. *Nature and Science*, **11**, 8-13(2013).

[26] Moser, B. R, Shah, S. N, Winkler-Moser, J. K, Vaughn, S. F, and Evangelista, R. L., Composition and physical properties of cress (Lepidium sativum L.) and field pennycress (*Thlaspi arvense* L.) oils. *Industrial Crops and Products*, **30**, 199–205(2009).

[27] Diwakar, B.T., Dutta, P.K, Lokesh, B.R., and Naidu, K. A., Physicochemical properties garden cress (Lepidium sativum) seed oil. *Journal of American Oil. Chemistry society*, **87**, 539-548 (2010).

[28] Shail, M. D., Neeraj, K., and Gupta, L. N., Nutritional importance of Lepidium sativum L., (Garden cress/Chandrashoor): A review. *Int J. Pharm Anal Res*, **5**(1), 152-60(2016).

[29] Fahmi, A. H., Ghee. New Commercial publisher. *Arabic book.* (1961)

[30] AOAC., Official Methods of Analysis. 18th Edition, *Association of Official Analytical chemists*, *Gaithersburg*. (2007)

[31] Patton, S. and Kurtz, G. W., 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. *Journal of Dairy Science*, **34**, 669–674(1951).

[32] Karamać, M., Kosińska, A., Pegg, R. B., Comparison of radical-scavenging activities for selected phenolic acids. *Polish Journal of Food and Nutrition Science*, **14**,165–169(2005).

[33] AOAC., Official methods of analysis 16th Ed. *Association of official analytical chemists. Washington DC, USA.* (1995)

[34] Snedecor, G.W., and Cochran, W.G., Statistical Methods. 9th Ed., *Iowa State Univ. Press, Ames, Iowa, USA.* (1994)

[35] Silva, F., de, A. S. E., and Azevedo, C. A. V. de., Principal Components Analysis in the Software Assistat-Statistical Attendance. In: World Congress on Computers. In Agriculture, 7, Reno-Nv-Usa: *American Society Of Agricultural And Biological Engineers* (2009).

[36] Youssef, G. M., Heba, E., El-Ghamery and Hayam, A. E., Study the physico-chemical properties and antihyperlipidemic activities of garden cress seed oil. *Journal of American Science*,10 (12), 324- 330 (2014).

[37] Yenge, G. B., More H.G., Kenghe, R. N., Kanawade, V. L., Nimbalkar C. A. and Patil A.P., Effect of different extraction methods on yield and physico-chemical properties of garden cress (Lepidium sativam L.) oil. *Journal of Oilseed Brassica*, **8** (2),138 -142(2017).

[38] Zia-Ul-Haq, M., Shakeel, A., Luca, C., Teresa, M., Daniele, D. R., and Nicoletta P.. Compositional study and antioxidant potential of ipomoea hederacea jacq.

And lepidium sativum l. Seed. *Molecules.* **17**, 10306-10321(2012).

[39] Spencer, G. P., and List, G. R., Specifications, physical properties and methods of analysis for jojoba oil. Proc. 7th Int. Conf. *on Jojoba and Its Uses*, 173-189 (1988)

[40] EL kinawy, O. S. N., Comparison between Jojoba oil and other vegetable oils as a substitute to petroleum. *Energy Sources*, **26**, 639–645 (2004).

[41] Doke, S., and Guha, M., Garden cress (Lepidium sativum L.) Seed - an important medicinal source: A Review. *Journal of Natural Product and Plant Resources*, **4** (1), 69-80 (2014) .

[42] Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., and Bahorun, T., Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation Research/Fundamental and Molecular mechanisms of mutagenesis, **579**(1-2), 200-213(2005).

[43] O'Brien, R. D. Fats and Oils: Formulating and Processing for Applications, *Technomic Publishing Company, Lancaster, M.P.A.* (2008).

[44] Asha, A., Manjunatha, M., Rekha, R. M., Surendranath, B., Heartwin1, P., Rao, J., Magdaline1, E., and Chitranayak, S., Antioxidant activities of orange peel extract in ghee (butter oil) stored at different storage temperatures. *Journal of Food Science and Technology*, **52** (12), 8220–8227 (2015).

[45] Shende, S., Patel, S., Arora, S., and Sharma, V., Oxidative stability of ghee incorporated with clove extracts and BHA at elevated temperatures. *International Journal of Food Properties*, **17**, 1599–1611(2014).

[46] Nadeem, M., Abdullah, M. and Imtiaz, H., Improvement of the oxidative stability of butter oil by blending with moringa oleifera oil. *Journal of Food Processing and Preservation*, **38** , 1491–1500 (2012).

[47] Chatha, S. A. S., Anwar, F., Manzoor, M., and Bajwa, J. R., Evaluation of the antioxidant activity of the rice bran extracts using different antioxidant assays. *Grasas y Aceites*, **57**, 328–335(2006).

[48] Anwar, F., Siddiq, A., Iqbal, S., and Asi, M. R., Stabilization of sunflower oil with Moringa oleifera leaves under ambient storage. *Journal of Food lipid*s, **14**, 35–49 (2007)

[49] Gandhi K., Arora, S., Pawar N., and Kumar A., Effect of vidarik and (extracts) on oxidative stability of ghee: a comparative study. *Journal of Dairy Science Technology*, **2** (1), 1 – 11(2013).

[50] Pawar, N., Gandhi, K., Purohit, A., Arora, S., and Singh, R. R. B.. Effect of added herb extracts on oxidative stability of ghee (butter oil) during accelerated oxidation condition. *Journal of Food Science and Technology*, 51(10), 2727-2733 (2014).

[51] El-Shourbagy, G. A., and El-Zahar, K. M., Oxidative stability of ghee as affected by natural antioxidants extracted from food processing wastes. *Annals of Agricultural Science*, 59(2), 213–220 (2014).