

PHSICO-CHEMICAL CHARACTERISTICS OF TRADITIONAL BUTTER OIL MADE FROM SPONTANEOUS FERMENTATION OF GOATS' MILK

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ABSTRACT

Chemical and physical characteristics of goats' butter oil prepared from naturally fermented goats' milk, and stored under accelerated oxidation conditions at $63\pm 0.5^{\circ}\text{C}$ for 30 days were investigated. Different concentrations of α -tocopherol (2.5-10 mg/100g fat) and 1% of milk phospholipids (PL) were added to goat's butter oil. Increasing the concentration of α -tocopherol and autoxidation process time significantly ($P<0.05$) influenced the properties of the butter oil properties. All α -tocopherol concentrations protected butter oil against oxidation to some extent and the protection factor depended on the concentration of α -tocopherol used. Increasing the concentration of α -tocopherol added did not affect the fatty acids composition in the butter. Butter oil treated with 10 mg α -tocopherol /100g fat was characterized with the highest unsaturated fatty acids (highly protected against oxidation), iodine number, saponification number, acid value and free fatty acids till the end of autoxidation process. Also, slip melting point decreased ($p \leq 0.05$) with increasing the concentration of α -tocopherol added, being the lowest with the highest α -tocopherol concentration. On the other side, the specific gravity and refractive index showed an opposite trend under accelerated oxidation storage. All textural characteristics (hardness, gumminess, springiness, cohesiveness and chewiness) and dynamic viscosity values significantly decreased ($p\leq 0.05$) with extending the accelerated oxidation time, and by increasing the concentration of α -tocopherol used, being the lowest with 10 mg α -tocopherol /100g fat till the 30th day of storage. Generally, direct addition of different concentrations of α -tocopherol and milk phospholipids significantly ($p\leq 0.05$) protected butter oil (longer shelf-life of this fat) during the stages of accelerated autoxidation.

Keywords: α -tocopherol, goats' butter oil, chemical characteristics, physical and textural properties.

INTRODUCTION

Goat milk is widely used for home consumption and played an important role in the nutritional and economic well-being of people in the developing countries particularly, where goats is reared in large numbers because of desert climate favoring goats, compared with cattle (Eissa *et al.*, 2011). This importance is also reflected in the largest animal number increase for goats during the last 20 years (FAO, 2010) and the largest increase in goat milk production tonnage compared to other mammalian farm animals (Haenlein, 2004). Special characteristics of goat milk products are usually due to their specific taste, texture and healthy image, which allow their use as profitable alternatives to cow milk products (Alferez *et al.*, 2001; Haenlein, 2004; Vargas *et al.*, 2008 and Eissa *et al.*, 2011). However, the

characteristics of goat products can be influenced by several genetic and environmental factors and processing technologies (Chandan *et al.*, 1992; Alichanidis and polychroniadou, 1996). Fermentation is one of the oldest food processing technologies in the world, which has been adopted across generations. The knowledge about traditional fermentation has not been fully documented and it is in danger of being lost since technologies are evolving and families are forgetting about traditional food preservation practices (Kebede *et al.*, 2007). Several studies have been carried on the quality and diversity of traditional fermented dairy products, including natural fermented milk (Gran *et al.*, 2002 and Benkerroum & Tamime, 2004).

In Egypt, several varieties of traditional fermented milk products prepared by rural women based on their acquired knowledge on fermentation. Two types natural fermented milk in South Sinai are widely made and consumed; the first is a liquid fermented milk called "Laben", which is rich in proteins. The second is the traditional butter which is called "Zebda Baldi". Churning is the most important step in the butter making, during which the oil-in-water emulsion is broken, leading to aqueous phase separation and formation of water-in-oil emulsion (Rousseau, 2000). The quality of stored butter is generally governed by several factors such as cream ripening, hygiene practice, manufacturing process and type of animal feeding (Fearon *et al.*, 1998). Despite the application of preventive measures (refrigeration and good hygienic practice) during its production and distribution, butter has a limited shelf life (Ozkanli & Kaya, 2005).

Butter oil is made by heating butter and separating pure fat from milk serum. Deterioration (lipolysis and oxidation) of butter oil results in flavor impairment, lowers nutritional quality and limited shelf life (Lin *et al.*, 1996 (a,b); Ashes *et al.*, 1997; Focant *et al.*, 1998; Im and Marshall, 1998). Both storage time and type of treatment have highly significant effects on the changes occurred in the saturated and unsaturated fatty acids, and free fatty acids contents of butter oil (Amr, 1991; Joshi *et al.*, 1994; Muir, 1996 and Ozkanli & Kaya, 2005). Unsaturated fatty acids are oxidized to form hydroperoxides, which are very unstable. Secondary oxidation products, including saturated and unsaturated aldehydes, ketones, hydrocarbon, semialdehydes and alcohols, may be perceptible to consumer even at low concentrations (Fox and McSweeney, 1998). Also, the number of double bonds in fatty acids influences melting behavior and oxidative stability, where as distribution of the fatty acids in the triglycerides structure influences crystallization behavior, melting behavior and the nutritional aspects. (Hawke and Taylor, 1995; Kaylegian and Lindsay, 1995).

The spontaneous milk fat oxidation can be catalyzed by certain metals and light exposure (Ozkanli & Kaya, 2005). The distribution of unsaponifiable matter and the concentration of natural antioxidants are the main factors affecting the degree of stability of milk fat to oxidation. Among the constituents of the unsaponifiable matter, the anti-oxidative effect of α -tocopherol has been studied (Huang *et al.*, 1996; Havemose *et al.*, 2006). α -Tocopherol plays a crucial role in preventing or delaying fat oxidation. As food antioxidant, α -tocopherol acts by terminating free radical chain reactions by donating hydrogen or electrons to free radicals and converting

them to more stable products (Frankel, 1998). The antioxidant activity of α -tocopherol in milk fat increased markedly in the presence of phospholipids. Phospholipids act as synergists for tocopherol action and increase its activity (Nath & Murthy, 1983; Rossell, 1989 and Hamzawi, 1990). The highest inhibitory effect against oxidation was shown by the synergy between α -tocopherol and phosphatidyl ethanolamine (Bandarra *et al.*, 1999).

Although many researches have been carried out on the oxidative stability of cows' and buffaloes' milk fat (Arul *et al.*, 1988; Hamzawi, 1990 and Fatouh *et al.*, 2005), little has been done on the oxidative stability of goats' butter oil (Desouky, 2014). Therefore, the aim of the present work is to evaluate the effect of added α -tocopherol and milk phospholipids on protecting and inhibition the changes occurred on the fatty acid profile, chemical characteristics as well as textural profile and rheological properties of butter oil produced from naturally fermented (as a traditional processing method) goats' milk and stored under accelerated oxidation conditions at 63 ± 0.5 C for 30 days.

MATERIALS AND METHODS

Goats' milk samples were collected randomly from Shami goats animals at small holder folks raised in Ras Sudr area, South Sinai Governorate. Milk phospholipids (amount % of the total lipid phosphorus: phosphatidyl choline 34.5; phosphatidyl ethanolamine 31.80; sphingomyelin 25.2; phosphatidyl inositol 4.6; phosphatidyl serine 3.1; lyso phospholipids 0.8) and α -tocopherol were obtained from Sigma chemical Co. (St. Louis, MO, USA). Pure grade of n-Hexane (p.b 69 C and vapor pressure 256 mm Hg 37.7 C) was obtained from Fisher Scientific, Limited, UK.

For making Manufacture of goats' butter, goats' milk was naturally fermented by keeping at room temperature (25 ± 5 C) until complete coagulation (after ~ 18 h). The coagulated fermented milk (Rayeb) was then churned at ambient temperature (25 ± 5 C) by the traditional skin bag method as described by El-Gendy (1983). The end of churning is discerned by the sound of the butter lumps when shaking (after ~ 2 h). 10% (v/v) of warm water (40 C) was added towards the end of churning period to enhance coalesce of fat globules and to increase the butter yield.

For the Preparation of goats' butter oil (BO) butter oil (BO) was prepared as described by Amer *et al.*, (1985). Butter was melted at 60 C and the top fat layer was separated, filtered through four layers of cheese cloth to obtain clear butter oil. The resultant BO contained approximately 99.5 % milk fat. Various concentrations of α -tocopherol (2.5-10 mg/100g fat) and 1% of milk phospholipids (PL) were dissolved in 10 ml of n-hexane according to Black (2007) and briefly vortex mixed for 5min before their addition in butter oil samples, and then continuous agitation by vortex for 1 min. The solvent was removed by evaporation under vacuum at 40 C/ 30 min.

Goats' milk and butter samples were analysed for total solids, fat by using Gerber method, total nitrogen by using micro-Kjeldahl method, and

ash by using thermolyne,type 1500 muffle furnace contents according to AOAC (2007), and total carbohydrates were calculated by the difference and pH values were measured by using digital pH meter (Inolad model 720, Germany) according to AOAC (2007). α -Tocopherol content of butter was estimated according to Havemose *et al.*, (2004). Results are presented in Table 1.

Table (1): Chemical properties of fresh bulk goats' milk and traditional goats' butter (Mean \pm Standard deviation)

Constituents	Goat milk*	Butter
Ph	6.64 \pm 0.01	5.8 \pm 0.10
Fat (%)	3.96 \pm 0.06	83.28 \pm 0.23
Protein (%)	3.64 \pm 0.01	0.57 \pm 0.12
Ash (%)	0.74 \pm 0.02	0.82 \pm 0.07
Total carbohydrates (%)	4.64 \pm 0.02	0.57 \pm 0.06
Total solids (%)	12.98 \pm 0.07	85.42 \pm 0.29
α -tocopherol (mg/100g fat)	Not detected	0.04 \pm 0.01

Data represented average of 3 separate trials. *:bulk milk sample
 Protein%= T.N % \times 6.38 Total carbohydrates %: Calculated by the difference

The fatty acid profile of goat milk fat was determined after conversion of the fatty acids into the corresponding methylesters as described by Amer *et al.*, (1985). HP5980 Series II gas chromatography (GC) (Hewlett – Packard, San Fernando, CA, USA) equipped with a flame-ionization detector and on-column injector. The fatty acid profile was based on the peak area integrated by using computer software interface HP 3365 Series II Chem Station. Fatty acid methylester peaks were identified by comparing the retention times with those of a standard mixture of fatty acid methylesters (NU-Check-Prep, Elysian MN, USA).

Chemical characteristics of BO (iodine number, saponification number, acid value and free fatty acid) were measured in goats' butter oil as described in AOAC (2007). Physical characteristics of BO (slip melting point, specific gravity (by means of bottle pycnometer) and refractive index at 40 C) were determined by the method of AOAC (2000).

Rheological data of butter oil was measured using a Brook Brookfield digital viscometer (Middleboro, MA 02346, U.S.A) with B 92 spindle size 3 ml that rotated at different rpm ranged from (5-100) at shear rates ranging from 8.73 to 260.73 cm⁻¹. Data were collected using Brookfield Engineering Laboratory soft ware, Middleboro, U.S.A. Viscosity was monitored at 5°C during accelerated oxidation storage for all butter oil treatments, as formerly described by Scott *et al.*, (2003).

Textural properties were performed using texture analyzer (Stable Micro System Ltd., Godalming, UK) loaded with Dimension software (SMS, stable Micro System Program). Universal testing machine (model 1100) with an auto compression load cell was used to measure texture profile analysis (TPA). A 55mm diameter plunger was attached to the moving crosshead. The speed of the crosshead was set at 10 mm/min. Penetration of the

plunger into the sample was set for 20 mm. An auto scale load was used and the consecutive bites were taken. The resulting curves obtained were similar to the generalized TPA curves from the Instron Universal Testing Machine and five textural parameters were obtained from the analysis of these force distance curves. The machine used in this experimental was located in a temperature controlled room ($20\pm 2^\circ\text{C}$). All samples were allowed to equilibrate to the controlled room temperature for 30 min prior to testing.

All experiments were done in triplicate, and all of the obtained data are expressed as averages. Statistical analyses were carried out using the General Linear Models procedure of the SPSS 16.0 Syntax Reference Guide (SPSS, 2007) The results were expressed as least squares means with standard errors of the mean. Statistically different groups were determined by the LSD (least significant difference) test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Changes in the fatty acids composition of traditional goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) during the accelerated ($63\pm 0.5^\circ\text{C}/30\text{days}$) oxidation are presented in Table (2). The fatty acids content influenced ($p \leq 0.05$) by the concentration of α -tocopherol used, and the extending of the storage under acceleration ($63\pm 0.5^\circ\text{C}/30\text{ days}$) of oxidation storage. It could be observed that total saturated fatty acids (TSFAs) gradually increased and total unsaturated fatty acids (USFAs) gradually decreased in all butter oil treatments and the control during the stages of autoxidation process. In addition, the most predominant SFAs present in all treatments including the control was palmitic acid ($\text{C}_{16:0}$) followed by myristic acid ($\text{C}_{14:0}$) then stearic acid ($\text{C}_{18:0}$). While, the most predominant USFAs present in all treatments was oleic acid ($\text{C}_{18:1}$) followed by linoleic ($\text{C}_{18:2}$) (Table, 2). Same observations found till 30th day of autoxidation. These results are in agreement with previous studies on goats' butter oil (Arora and Rai, 1998; Sanz Sampelayo *et al.*, 2002; Ward *et al.*, 2002; Sagdic *et al.*, 2004; Ozkanli and Kaya 2005). It is quite clear from Table, 2 that significant ($p \leq 0.05$) differences were found in the fatty acids percent between goat BO containing different mixtures of the antioxidant used and the control during the stages of autoxidation. There were increases of USFAs associated with decreasing in SFAs with increasing the concentration of α -tocopherol used. It could be due to that, α -tocopherol exhibited milk fat oxidation. Frankel, (1998) and Hurtaud *et al.*, (2010) reported that, α -tocopherol play a crucial role in preventing or delaying oxidation by terminating free radical chain reactions by donating hydrogen or electrons to free radicals and converting them to more stable products. Also, recording to the method of manufacture goats' butter, ca used spontaneous lipolysis and affected its chemical composition as well as influenced its susceptibility towards hydrolysis and oxidation by decreasing the amount of USFAs in the control butter oil. Among all treatments, butter oil treated with 10 mg α -tocopherol /100g fat was characterized by the highest USFAs (highly protected against oxidation or

less oxidation rate) and as well as lowest SFAs. It could be attributed to the specific characteristics of the antioxidant effect on the fatty acids distribution (Luna *et al.*, 2008 a,b). α -tocopherol was capable of reducing the extent of milk fat USFAs depression Focant *et al.*, (1998) and Bell *et al.*, (2006). Moreover, the use of different concentrations of α -tocopherol affected ($p \leq 0.05$) the pattern fatty acids during accelerated oxidation process. These changes could be attributed to the degradation of the fat matter under heating oxidation. Ozkanli and Kaya (2005) reported that fatty acid composition of butter oil made from ewes' milk was varied under heat treatment due to the effect of temperature on lipolytic activity. Finally, increasing the concentration of α -tocopherol added helped butter to keep its fatty acids composition, depending on the concentration used or by another words α -tocopherol exhibited milk fat oxidation (Hamzawi, 1990 and Desouky, 2014).

Table (2): Fatty acids profile (mean \pm standard deviation) of goats' butter oil treated with mixtures containing of 1% PL and different concentrations of α tocopherol (2.5-10 mg/100g fat) during the acceleration of storage under accelerated oxidation conditions ($63 \pm 0.5^\circ \text{C}/30$ days).

NO. of carbon	% Fatty acids														
	Initial					Middle (15 days)					Final(30 days)				
	control	T1	T2	T3	T4	control	T1	T2	T3	T4	control	T1	T2	T3	T4
C _{4:0}	5.86	5.88	5.87	5.88	5.87	4.71	5.22	5.76	5.84	5.86	3.42	4.49	5.27	5.42	5.66
C _{6:0}	6.73	6.72	6.71	6.7	6.7	5.27	5.84	5.95	6.09	6.45	4.62	5.72	5.78	5.86	6.22
C _{8:0}	3.35	3.34	3.34	3.35	3.34	3.23	3.28	3.31	3.32	3.33	3.14	3.24	3.26	3.29	3.31
C _{10:0}	13.02	13.02	13.03	13.03	13.02	13.24	13.21	13.18	13.15	13.11	13.54	13.27	13.23	13.19	13.14
C _{12:0}	4.43	4.42	4.43	4.42	4.44	5.23	5.12	4.49	4.47	4.47	6.21	5.18	5.11	4.97	4.78
C _{14:0}	12.44	12.52	12.54	12.54	12.54	13.12	12.73	12.69	12.67	12.6	14.41	12.83	12.72	12.66	12.64
C _{14:1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C _{16:0}	22.44	22.37	22.38	22.37	22.38	23.12	22.71	22.68	22.59	22.4	25.11	23.06	22.74	22.71	22.49
C _{16:1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C _{18:0}	10.42	10.41	10.41	10.4	10.4	11.11	10.72	10.68	10.59	10.47	12.19	11.24	10.88	10.69	10.51
C _{18:1}	19.25	19.25	19.23	19.25	19.25	18.95	19.13	19.21	19.23	19.25	15.57	18.96	18.99	19.16	19.19
C _{18:2}	2.04	2.05	2.05	2.04	2.04	1.99	2.01	2.02	2.03	2.03	1.76	1.98	2	2.01	2.02
C _{18:3}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C _{20:0}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSC	15.94	15.94	15.92	15.93	15.91	13.21	14.34	15.02	15.25	15.64	11.18	13.45	14.31	14.57	15.19
TLC	84.04	84.04	84.07	84.05	84.07	86.76	85.63	84.95	84.73	84.33	88.79	86.52	85.67	85.39	84.77
USFA	21.29	21.29	21.28	21.29	21.29	20.94	21.14	21.23	21.26	21.28	17.33	20.94	20.99	21.17	21.21
SFA	78.69	78.69	78.71	78.69	78.69	79.03	78.83	78.74	78.72	78.69	82.64	79.03	78.99	78.79	78.75

Control: butter oil without α -tocopherol added

T1: 2.5mg α -tocopherol /100g fat

T2: 5mg α -tocopherol /100g fat

T3: 7.5 mg α -tocopherol /100g fat

T4: 10mg α -tocopherol /100g fat

TSC: total short chain C_{4:0} – C_{8:0}

TLC: total long chain C_{10:0} – C_{20:0}

USFA: unsaturated fatty acids C_{16:1} – C_{18:3}

SFA: saturated fatty acids C_{4:0} – C_{20:0}

The changes in chemical properties of traditional goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) during the accelerated ($63 \pm 0.5^\circ \text{C}/30$ days) oxidation are presented in Table (3).

Table (3): Chemical properties (mean \pm standard deviation) of traditional goats' BO treated with mixtures containing of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) during the acceleration (63 ± 0.5 C/30days) of oxidation.

Treatments	Characteristics			
	*Initial			
	Iodine Number ¹	Saponification Number ²	Acid Number ³	Free Fatty Acids ⁴
control	31.29 ^A \pm 0.48	228.49 ^B \pm 0.94	0.32 ^A \pm 0.01	0.24 ^B \pm 0.02
T1	31.29 ^A \pm 0.44	228.53 ^B \pm 1.02	0.32 ^A \pm 0.01	0.25 ^A \pm 0.03
T2	31.29 ^A \pm 0.37	228.49 ^B \pm 0.84	0.33 ^A \pm 0.02	0.24 ^B \pm 0.01
T3	31.28 ^A \pm 0.45	228.51 ^B \pm 0.90	0.33 ^A \pm 0.04	0.24 ^B \pm 0.02
T4	31.28 ^A \pm 0.26	229.52 ^A \pm 0.74	0.33 ^A \pm 0.02	0.25 ^A \pm 0.03
Mean	31.29 ^a \pm 0.50	228.71 ^a \pm 0.88	0.33 ^a \pm 0.01	0.24 ^a \pm 0.01
Middle (15 days)				
control	27.21 ^{cd} \pm 0.31	224.26 ^{df} \pm 0.94	0.27 ^{ce} \pm 0.02	0.20 ^{cf} \pm 0.02
T1	28.75 ^{cc} \pm 0.40	226.87 ^{cd} \pm 0.87	0.29 ^{bd} \pm 0.02	0.21 ^{ce} \pm 0.03
T2	29.55 ^{bcb} \pm 0.36	227.95 ^{bc} \pm 0.76	0.30 ^{bc} \pm 0.04	0.22 ^{bcd} \pm 0.03
T3	30.12 ^{ab} \pm 0.43	228.18 ^a \pm 1.03	0.31 ^{ab} \pm 0.03	0.23 ^{abc} \pm 0.04
T4	30.58 ^{ab} \pm 0.38	229.13 ^{aa} \pm 0.93	0.32 ^{aa} \pm 0.01	0.24 ^{ab} \pm 0.01
Mean	29.24 ^b \pm 0.42	227.28 ^b \pm 0.70	0.30 ^b \pm 0.02	0.22 ^b \pm 0.01
Final (30 days)				
Control	24.52 ^{df} \pm 0.26	221.33 ^{dg} \pm 1.05	0.25 ^{df} \pm 0.01	0.17 ^{ch} \pm 0.04
T1	26.21 ^{ce} \pm 0.37	225.26 ^{ce} \pm 1.02	0.27 ^{ce} \pm 0.01	0.19 ^{bc} \pm 0.01
T2	27.36 ^{bd} \pm 0.45	226.21 ^{cd} \pm 0.96	0.29 ^{bd} \pm 0.01	0.20 ^{bf} \pm 0.02
T3	28.72 ^{abc} \pm 0.39	227.33 ^{bc} \pm 0.80	0.30 ^{abc} \pm 0.03	0.22 ^{ab} \pm 0.02
T4	29.65 ^{ab} \pm 0.25	228.51 ^{ab} \pm 0.74	0.31 ^{ab} \pm 0.04	0.23 ^{ac} \pm 0.01
Mean	27.29 ^c \pm 0.46	225.73 ^c \pm 0.85	0.28 ^c \pm 0.03	0.20 ^c \pm 0.03

Means in the column with the same small (^{a,b}), and in row with capital (^{A,B}) superscript letter indicate no significant ($P\leq 0.05$) Differences between α -tocopherol treatments and storage time, respectively.

Data represented average of 3 separate trials. Control: butter oil without α -tocopherol added; T1: 2.5mg α -tocopherol /100g fat;

T2:5mg α -tocopherol /100g fat; T3: 7.5 mg α -tocopherol /100g fat; T4:10mg α -tocopherol /100g fat.

¹: g I₂ adsorbed /g fat; ²: mg KOH /g fat; ³: mg KOH / g fat; ⁴: % Oleic acid

All milk fat constants (iodine number, saponification number, acid value) and free fatty acids influenced ($p\leq 0.05$) by the concentration of α -tocopherol used and extending the accelerated (63 ± 0.5 C/30 days) oxidation time. It is well known that, iodine number (IN) is a measure of unsaturation in fat; the higher proportion of USFAs the higher IN. (Fatouh *et al.*, 2005; Bindal &Wadhwa 1993; Arora and Rai, (1998 & 1999 and Ozkanli and Kaya (2005).). IN of all BO treatments including the control significantly ($p\leq 0.05$) decreased up to the end of oxidation time. This is due to the gradually decreased in the USFAs (Table, 2), which is attributed to the degradation of the fat matter under heating oxidation. IN of goat BO at the beginning and the end of accelerated oxidation period was much lower than that of α -tocopherol treatments, indicating faster oxidation rate (lower USFAs concentration as compared to other α - tocopherol treatments, Table 2). A substantial alteration in the fatty acid composition of the original BO through

the time of accelerated oxidation is responsible of the decreasing in free fatty acid content and USFAs, which is responsible of decreasing the IN. The same finding was reported by Arora & Rai (1998) and Fatouh *et al.*, (2005). On the other side, all α -tocopherol concentrations protected butter oil against oxidation to some extent and the protection factor (PF) depended upon the concentration of α -tocopherol used. This increase in the PF could be attributed to the synergistic effect of PL with α -tocopherol as antioxidant (Hamzawi, 1990 and Desouky, 2014). Also, the compositional properties of milk fat and the concentration of antioxidants are the main factor affecting the degree of stability to oxidation (Samet-Bali *et al.*, 2009). Frankel, (1998) reported that, α -tocopherol play a crucial role in preventing or delaying oxidation by terminating free radical chain reactions by donating hydrogen or electrons to free radicals and converting them to more stable products. The BO treated with 10 mg α -tocopherol /100g fat was the highest IN (highest USFAs). It is also confirm the higher stability against oxidation and longer shelf-life of this fat, even compared to other treatments or the control. Same findings reported by Pruthi, (1984); Lakshiminarayana & RamaMurthy (1986); Samet-Bali *et al.*, (2009) and Baliolfa *et al.*, (2010).

The saponification number (SN) differed ($p \leq 0.05$) between all the α -tocopherol treated treatments in one side and the control on the other side, depending on the antioxidant concentration and the accelerated ($63 \pm 0.5^\circ \text{C}/30$ days) oxidation time as presented in Table, 3. It is evident from the data that, non treated butter oil had lower SN than all treated α -tocopherol treatments stored under accelerated oxidation conditions, which might be due its high levels of saturated long chain fatty acids (SLCFAs) and low levels of saturated short chain fatty acids (SCFAs) as previously shown in Table 2. These results are in agreement with those reported by Sonntag (1982); Abd EL-Rahman *et al.*, (1998) Arora and Rai, (1998); Fatouh *et al.*, (2005). Among all treatments, butter oil containing 10 mg α -tocopherol had the highest SN after 15 and 30 days of the accelerated at oxidation $63 \pm 0.5^\circ \text{C}$. It could be explained by the lower levels of SLCFA than that of SCFAs (Table, 2). The trend found was similar to other studies (Abd EL-Rahman *et al.*, 1998 and Fatouh *et al.*, 2005). As the oxidation proceeded towards 30th day, SN significantly ($p \leq 0.05$) decreased in all butter oil treatments. This could be also explained by increasing the saturated LCFA vice versa the decreasing of SCFA (Table, 2).

Changes of acid value (AV) among various BO treatments and the control upon the stability to oxidation (at $63 \pm 0.5^\circ \text{C}/30$ days) are also shown in Table, 3. AV increased ($p \leq 0.05$) with increasing the concentration of α -tocopherol till the end of autoxidation period. Chemical composition of butter oil has a great influence on their fatty acids hydrolyzed from triacylglycerols, in addition free fatty acids can also contribute the acidity. The higher USFAs content the higher acid value (Sagdic *et al.*, 2004; Fatouh *et al.*, 2005 and Amer *et al.*, 1985). The original butter oil characterized with the lowest values comparing with other treatments during the oxidation process. It could be explained by the highest level of saturated long chain fatty acids and lowest USFA, indicating faster oxidation rate (Table, 2). This trend concurs with Fatouh *et al.*, (2005) and Abd EL-Rahman *et al.*, (1998). On the other side,

BO treated with 10 mg α -tocopherol /100g fat had the highest AV (highest USFAs) which is also confirm the higher stability to oxidation and longer shelf-life of this fat, even compared to other treatments or the control. Possible explanation could be due to that, α -tocopherol play a crucial role in delaying oxidation by terminating free radical chain reactions by donating hydrogen or electrons to free radicals and converting them to more stable products. Same findings reported by Pruthi, (1984); Lakshiminarayana & RamaMurthy (1986); Hamzawi (1990); Samet-Bali *et al.*, (2009); Baliolfa *et al.*, (2010) and Desouky, (2014).

There were significant differences ($p \leq 0.05$) in the free fatty acids (FFAs) content among BO treatments and control stored under accelerated oxidation /30days (Table, 3). The higher the degree of unsaturation, the higher FFAs content (Lakshiminarayana & RamaMurthy, 1986; Fatouh *et al.*, 2005 Arora & Rai 1998 and Arul *et al.*, 1988). With extending the oxidation period till the 30th day, the FFAs significantly ($p \leq 0.05$) decreased in all treatments including the control. It could be due to the decreasing in the USFAs. The same findings reported by Fatouh *et al.*, (2005); AbdEL-Rahman *et al.*, (1998); Samet-Bali *et al.*, (2009) & Baliolfa *et al.*, (2010). Among all treatments the highest concentration of α -tocopherol was characterized by the highest FFAs. It could be due to the greatest amount of USFAs which is due to the role of α -tocopherol in delaying oxidation. In addition, goat milk fat (control) had lowest FFAs than all treatments. It could be explained recording to the method of manufacture butter which caused spontaneous lipolysis, and affected its chemical composition and influenced its susceptibility towards hydrolysis and oxidation by decreasing the amount of USFAs.

The flow behavior (shear stress / shear rate curves) of traditional goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5 -10 mg/100g fat) stored under accelerated ($63 \pm 0.5^\circ \text{C}/30$ days) oxidation conditions are illustrated in Figs. 1, 2 and 3. There were significant differences ($p \leq 0.05$) between shear stress values of butter oil treated treatments in one side and the original butter oil on the other side. As seen, the increase in shear stress response was not linear with the increase in the applied shear rate values. Also, the decrease in viscosity with increase shear rate was typical of non-Newtonian and exhibiting pseudoplastic behaviors for all butter oil treatments (Hurtaud *et al.*, 2010). The original butter oil was showed higher upward shifting of the flow curve and characterized with the higher viscosity values than other treated butter oil treatments during the exposed storage ($63 \pm 0.5^\circ \text{C}/30$ days). It could be due to; the lowest USFAs (highest oxidation rate) and highest SFAs content which make butter more strong structure. Also, higher concentration (10 mg α -tocopherol / 100g fat) of the antioxidant used was resulted in the downward shifting of the flow curve as compared with other treatments and the control (Samet-Bali *et al.*, 2009). This decrease in flow curve indicated that there was decrease in the viscosity values.

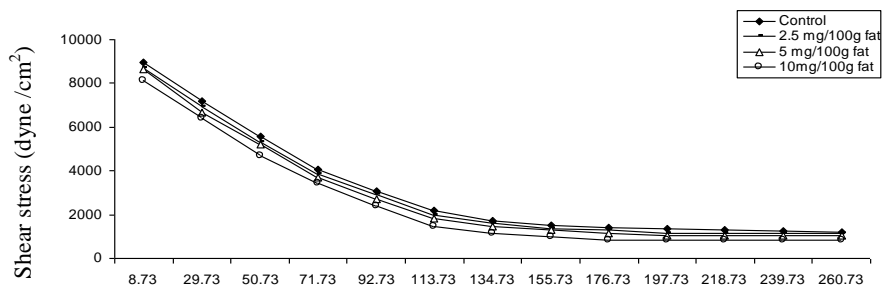


Fig.1: Flow behavior of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) at the beginning of accelerated ($63\pm 0.5^\circ\text{C}$) oxidation.

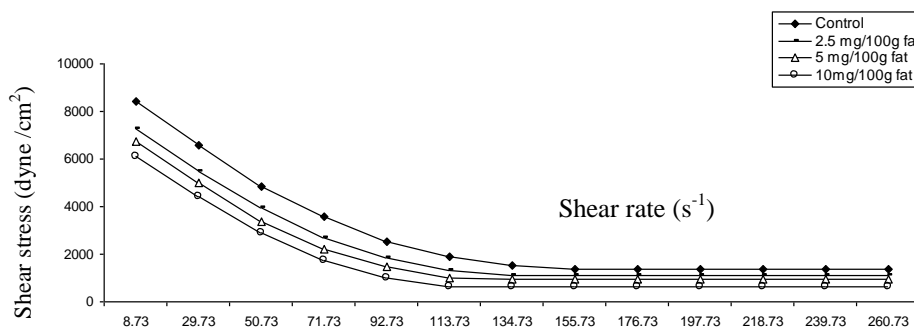


Fig.2: Flow behavior of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) stored under accelerated

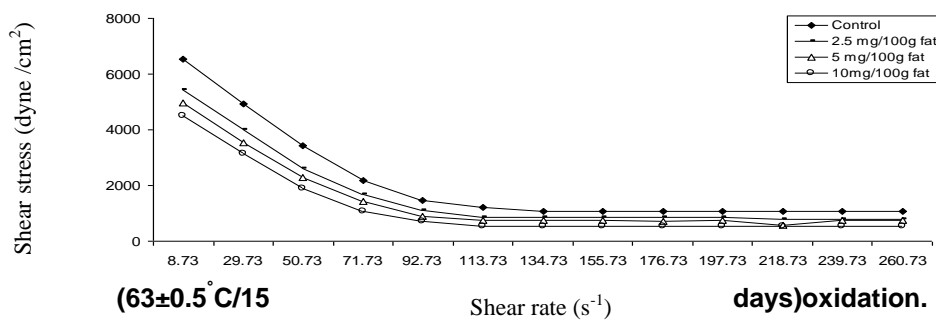


Fig.3: Flow behavior of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) stored under accelerated ($63\pm 0.5^\circ\text{C}/30$ days) oxidation.

Also, with extending the accelerated oxidation process till the 30th day (Fig.3); viscosity values decreased ($p \leq 0.05$) of all butter oil treatments, resulted in downward shifting of the flow curve. This could be due to, the degradation of the fat matter under heating oxidation (Ozkanli and Kaya, 2005). It is well known that, the compositional properties of milk fat and the concentration of antioxidants are the main factor affecting the degree of stability to oxidation and influencing the over all properties of milk fat after heating or by another mean the degree of unsaturated fatty acids (Samet-Bali *et al.*, 2009& Hurtaud *et al.*, 2010). Also, viscosity of butter oil might be associated with mono and polyunsaturated fatty acids content (Sandoval-Castilla *et al.*, 2004 and Besbes *et al.*, 2005).

Changes in the texture parameters of goats' milk butter oil treated with fixed level of PL (1%) with different concentrations of α -tocopherol (2.5-10 mg/100g fat) are presented in Table 4. The effect of α -tocopherol on the texture properties of goats' butter oil was more pronounced ($p \leq 0.05$) and depending on the concentration of the antioxidant used and extending the oxidation period (63 ± 0.5 °C/30 days). All texture parameters for all treatments as well as the control decreased ($p \leq 0.05$) during the accelerated oxidation. Texture characteristics of milk fat were significantly ($p \leq 0.05$) influenced by the presences of both SFAs and USFAs. Geller and Goodrum (2000) concerning the existence of a strong relationship between fatty acid composition, chain length and rheological properties of milk fat. The addition of α -tocopherol significantly ($p \leq 0.05$) decreased the hardness of butter oil and that was proportional to the percentage of the added α -tocopherol.

Butter oil treated with 10 mg α -tocopherol /100g fat exhibited the lowest hardness values (softer butter) among all treatments, while the control characterized by the highest hardness values. This result could also confirm the resistance of butter to oxidation, following its higher USFAs (Table 2) content with increasing the α -tocopherol concentration, due to protective effect of α -tocopherol against milk fat oxidation. Similar findings were reported by Hurtaud *et al.*, (2010). Initially, butter oil seemed to be harder and then became softer after heat treatment (63 °C \pm 0.5/30 days). These results could be explained by the change in the fatty acid composition. In fact, the decrease in USFAs was compensated by the slightly increase of the SFA during the oxidative stability experiment Samet-Bali *et al.*, (2009).

The springiness is the tendency to recover from large deformation after removal the deformation stress. Also, it is a characteristic for the materials' elasticity Fox *et al.*, (2000). It is evident that, the elasticity of the butter decreased with increasing the α -tocopherol concentration being the lowest value with using 10 mg α -tocopherol /100g fat, which is means that the ability of the butter to go back to its undeformed condition after removing force was the lowest or, in other words it was less elastic. The springiness values of all treatments as well as the control significantly decreased ($p \leq 0.05$) during accelerated oxidation (63 °C \pm 0.5). These results could be explained by the absence of milk proteins, which are responsible of the elasticity characteristics in the dairy products (Sandoval-Castilla *et al.*, 2004). Also, the changed occurred in the USFAs during the autoxidation stored (Table 2). On the other hand, the original butter oil showed the highest

springiness values; this means that the ability of the butter to go back to its undeformed condition after removing the deforming force was the highest or in another word it was more elastic.

Table (4): Texture profile (mean ±standard deviation) of goats' butter oil made with and without α-tocopherol (2.5-10 mg/100g fat) and with 1% phospholipids (PL)

Samples	Characteristics				
	Initial				
	Hardness (N)	Springiness (cm)	Cohesiveness (--)	Gumminess (N)	Chewiness (N/cm)
Control	24.26±0.46	0.444 ^a ±0.01	6.67 ^a ±0.46	161.81 ^a ±0.86	71.85 ^a ±0.72
T1	24.25±0.55	0.442 ^c ±0.01	6.65 ^a ±0.35	161.26 ^c ±0.94	71.28 ^b ±0.64
T2	24.24±0.48	0.443 ^b ±0.02	6.63 ^b ±0.37	160.71 ^b ±0.75	71.20 ^b ±0.77
T3	24.24±0.51	0.442 ^c ±0.04	6.63 ^b ±0.29	160.71 ^b ±0.90	71.03 ^b ±0.69
T4	24.24±0.49	0.443 ^b ±0.02	6.63 ^b ±0.41	160.71 ^b ±0.84	71.20 ^b ±0.58
Mean	24.25 ^a ±0.46	0.443 ^a ±0.03	6.64 ^a ±0.50	161.04 ^a ±0.88	71.31 ^a ±0.70
	Middle (15 days)				
Control	22.58 ^a ±0.58	0.383 ^a ±0.02	6.58 ^a ±0.42	148.58 ^a ±1.01	56.90 ^a ±0.57
T1	22.35 ^a ±0.33	0.371 ^b ±0.03	6.52 ^b ±0.44	145.72 ^b ±1.02	54.06 ^b ±0.62
T2	22.28 ^a ±0.45	0.364 ^b ±0.04	6.47 ^c ±0.32	144.15 ^b ±0.96	52.47 ^c ±0.45
T3	22.16 ^a ±0.40	0.358 ^c ±0.01	6.45 ^c ±0.46	142.93 ^c ±0.86	51.17 ^d ±0.72
T4	21.68 ^b ±0.54	0.351 ^c ±0.02	6.42 ^d ±0.38	139.19 ^d ±0.72	48.85 ^e ±0.69
Mean	22.21 ^b ±0.42	0.365 ^b ±0.03	6.49 ^b ±0.46	144.11 ^b ±0.95	52.69 ^b ±0.68
	Final (30 days)				
Control	20.77 ^a ±0.34	0.361 ^a ±0.01	6.49 ^a ±0.39	134.80 ^a ±0.99	48.66 ^a ±0.61
T1	19.84 ^b ±0.51	0.365 ^a ±0.02	6.46 ^b ±0.35	128.17 ^b ±1.03	46.78 ^b ±0.76
T2	19.42 ^b ±0.29	0.352 ^b ±0.02	6.42 ^{cd} ±0.42	124.68 ^c ±0.84	43.89 ^c ±0.65
T3	19.21 ^b ±0.43	0.349 ^b ±0.04	6.40 ^d ±0.27	122.94 ^d ±0.94	42.91 ^{cd} ±0.53
T4	19.09 ^b ±0.49	0.340 ^c ±0.01	6.38 ^e ±0.40	121.79 ^d ±1.05	41.41 ^d ±0.75
Mean	19.67 ^c ±0.38	0.353 ^b ±0.02	6.43 ^b ±0.35	126.48 ^c ±0.92	44.73 ^c ±0.65

Means in the column with the same small (^{a,b}), and in row with capital (^{A,B}) superscript letter indicate no significant (P≤0.05) Differences between α-tocopherol treatments and storage time, respectively.

Control: butter oil without α-tocopherol added; T1: 2.5mg α-tocopherol /100g fat; T2:5mg α-tocopherol /100g fat;

T3: 7.5 mg α-tocopherol /100g fat; T4:10mg α-tocopherol /100g fat.

These findings were confirmed from the data obtained of fatty acids composition (Table 2). It could be due to, the highest SFAs (higher oxidation rate) due to less deformable butter becomes. These findings were confirmed with Baliolfa *et al.*, (2010).

Cohesiveness values of butter oil treatments are also illustrated in Table (4). These results clear that the strength of the internal bonds making up the body of the butter (cohesiveness) increased with increasing the ratio of SFA and long chain fatty acids being the highest in the control (6.58N/cm) while the highest treated α-tocopherol butter oil (10 mg/100g fat) had the lowest value (6.42N/cm) after 15 days of stored under accelerated oxidation conditions. This means that the control butter was able to keep its body and shape for longer periods. In addition, spreadability of the butter could be

evaluated by the cohesiveness values (Sagdic *et al.*, 2004 and Baliolfa *et al.*, 2010).

The gumminess value decreased with adding α -tocopherol and it is significantly differ ($p \leq 0.05$) according to the concentration of antioxidant added. Gumminess value which is the energy required to disintegrate the butter sample to state ready for swallowing was related to values of hardness and cohesiveness. Butter oil treated with 10 mg α -tocopherol /100g fat characterized by the lowest value. Conversely, the control had the highest gumminess value either when initial or at the end of oxidation storage, which is readily explained from the fatty acids profile (Table, 3). Different values of gumminess among treatments are due to the differences in physical and chemical properties of butter oil during autoxidation at $63 \pm 0.5^\circ\text{C}/30$ days (Samet-Bali *et al.*, 2009).

Chewiness values are related to primary parameters of hardness. Increasing the concentration used of α -tocopherol decrease the chewiness value. Butter oil treated with 10 mg α -tocopherol /100g fat had the lowest chewiness value, which is due to the highest USFA and lowest SFA content as previously discussed (Table, 3). However, original butter fat had the highest chewiness value 56.90 N/cm after 15 days of accelerated oxidation. Possible explanation could be due to that, decreasing the USFAs content in butter oil make the butter need more work to masticate or less chewy. In opposite way, with increasing SFAs, the required work to masticate the butter increased. This is in agreement with Baliolfa *et al.*, (2010).

All texture parameter significantly ($p \leq 0.05$) decreased during the accelerated oxidation storage ($63 \pm 0.5^\circ\text{C}/30$ days). It could be attributed to the polymerization reaction and the formation of high molecular weight compounds including carbon-carbon bonds and carbon – oxygen –carbon bridges between fatty acids (Ebru *et al.*, 2011). Also, the effect of α -tocopherol on these texture properties depends on the concentration used; butter oil with the highest concentration of α -tocopherol (10mg /100g) was less springy, harder, and not able to keep its body and shape for longer periods at higher temperature. Also, less gummy and chewy comparing with the other treatments. (Tabe, 3). Finally, one can say that, addition of α -tocopherol as an antioxidant (2.5-10 mg α -tocopherol /100g fat) with fixed level of milk phospholipids (1%) had significant ($p \leq 0.05$) effects of all butter oil characteristics and increase the oxidative stability of butter oil against heat treatment.

The efficiencies of various combinations of 1%PL and different levels of α -tocopherol (2.5-10 mg/100g fat) on the physical properties (slip melting point (SMP), specific gravity (sp gr) and refractive index (RI) of goats' butter oil stored under accelerated oxidation ($63 \pm 0.5^\circ\text{C}/30$ days) are shown in Figs. 4, 5 and 6, respectively. All physical properties significantly differed ($p \leq 0.05$) between treatments, where the concentration of antioxidant used and oxidation storage time were the principle factors influencing the physical properties of butter oil treatments. During the accelerated oxidation storage, the highest SMP was observed for the control BO, indicating its faster oxidation rate than all antioxidant treated treatments (Fig.4). It might be due to the grater content of high melting triglycerides and lowest USFAs in the

original butter oil than all treated butter oil treatments (Table,2). Same findings reported by Lakshminarayana & Rama-Murthy, (1985); Ramesh& Bindal, (1987) and Bindal & Wadhwa, (1993). In addition, SMP decreased ($p \leq 0.05$) with increasing the concentration of added α -tocopherol being the lowest with the highest α - tocopherol concentration (10 mg α -tocopherol /100g fat).

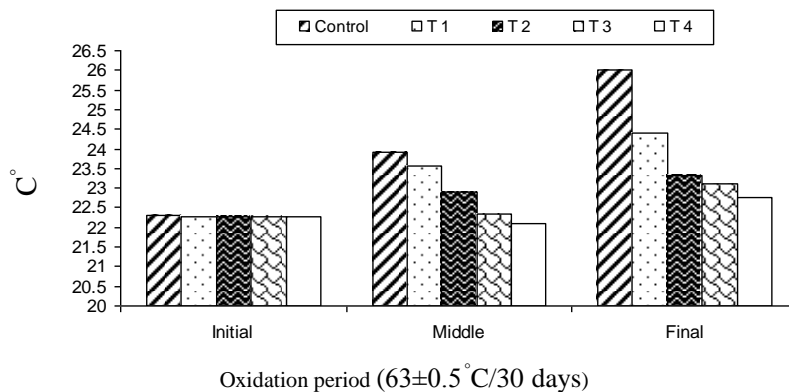


Fig.(4): Slip melting point (C) of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) stored under accelerated ($63\pm 0.5^\circ\text{C}/30$ days) oxidation

Possible explanation could be due to that, α - tocopherol protected milk fat from oxidation, which makes butter oil containing high amount of USFAs and as a result lowest melting point (Samet-Bali *et al.*, 2009 and Hurtaud *et al.*, 2010). Changes in SMP of the butter oil treatments are mainly due to the changes that occurred in proportions of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ acids, which have melting points of 62.9, 69.6 and 16.3 C, respectively. The melting point of these fatty acids, their distribution on the glycerol backbone is the main factor affecting the changes occurred in the melting point. Same findings reported by Formo, (1979); Laakso et al, (1992) and Deffense, (1993). Also, the melting point of fat also is influenced by fatty acid profile. The higher the number of double bonds in the fatty acids chain, the lower the melting point and softer butter at any temperature (Walstra, 1995 and Ashes et al., 1997). Also, Fig.1 shows that, SMP of all butter oil treatments increased ($p \leq 0.05$) with extending the exposed heat treatment, it could be due to the decreasing in the USFAs (Table, 2), being the lowest with the highest antioxidant concentration. Possible explanation could be due to that, α - tocopherol protected milk fat from oxidation (Hurtaud *et al.*, 2010) and plays a crucial role in preventing or delaying fat oxidation (Havemose *et al.*, 2006).

Specific gravity (sp gr) of goats' butter oil treatments decreased ($p \leq 0.05$) with increasing SMP (Fig.5), which may be ascribed to the decreased in the USFAs content (Table, 2). sp gr of low melting treatment (10 mg α -

tocopherol /100g fat) was higher than that of the control butter oil. It could be due to the lower USFAs and greater high melting triglycerides content of the control (Table,2) during the exposed heat treatment ($63\pm 0.5^{\circ}\text{C}/30$ days), which is reflecting the lowest stability against oxidation. Same findings reported by Lakshminarayana & Rama-Murthy, (1985); Ramesh& Bindal, (1987) and Bindal & Wadhwa, (1993).

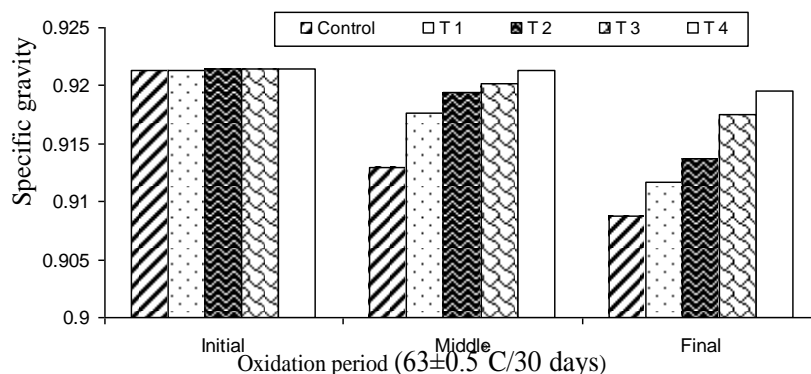


Fig.(5): Specific gravity of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) stored under accelerated ($63\pm 0.5^{\circ}\text{C}/30$ days) oxidation

Also, Fig.5 shows that, sp gr of all butter oil treatments decreased ($p \leq 0.05$) with extending the exposed heat treatment, it could be due to the decreasing in the USFAs and increasing in SMP values, being the highest with the highest antioxidant concentration. Possible explanation could be due to that, α -tocopherol protected milk fat from oxidation (Hurtaud *et al.*, 2010) and plays a crucial role in preventing fat oxidation (Havemose *et al.*, 2006).

The greater the degree of unsaturation, the higher refractive index (RI) content (Sankhla and Yadava, 1981; Farah *et al.*, 1989 and Fatouh, 2005). Among all butter oil treatments; RI of butter oil treated with 10 mg α -tocopherol /100g fat was higher than the corresponding values of the original goat butter oil which has lower content of USFAs during the accelerated oxidation storage (Fig.6). In addition, the rate of RI changes significantly ($p \leq 0.05$) increased with increasing the α -tocopherol concentration due to protective effect of α -tocopherol against milk fat oxidation.

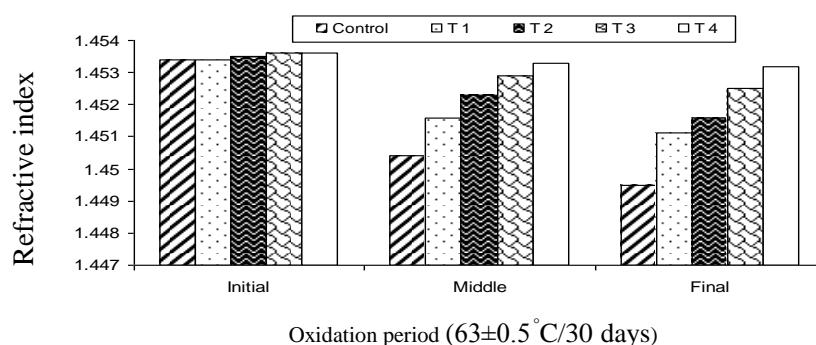


Fig.(6). Refractive index of goats' butter oil treated with mixtures of 1% PL and different concentrations of α -tocopherol (2.5-10 mg/100g fat) stored under accelerated ($63\pm 0.5^\circ\text{C}/30$ days) oxidation

Similar findings were reported by Hurtaud *et al.*, (2010). Also, Fig.6 shows that, RI of all butter oil treatments decreased ($p \leq 0.05$) with extending the exposed heat treatment. It could be due to the decreasing in the USFAs (Hurtaud *et al.*, 2010).

Finally, it could be concluded that there is a possibility for the development the use of goats' butter produced from naturally fermented (as a traditional processing method) goats' milk by the addition of milk phospholipids and α -tocopherol. The oxidative stability of butter fat significantly ($p \leq 0.05$) increased with the increase of the α -tocopherol concentration from 2.5-10 mg / 100g fat. The effect of α -tocopherol on the fatty acid profile, physico-chemical characteristics as well as textural profile and rheological properties of goats' milk butter oil was more pronounced ($p \leq 0.05$) and depending on the concentration of the antioxidant used and extending the oxidation period ($63\pm 0.5^\circ\text{C}/30$ days).

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**الخواص الفيزيوكيميائية للسمن المصنع من لبن الماعز بالطريقة التقليدية أثناء التخزين
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تم دراسة تأثير إضافة تركيزات مختلفه من ألفا توكوفيرول (٢,٥ - ١٠ ملجم / ١٠٠ جم دهن) و نسبة ثابتته من الفسوليبيدات (١ %) على بعض الخصائص الفيزيوكيميائية والريولوجية لزيت الزبدة (٩٩% دهن) المصنوعة من لبن الماعز عن طريق التخمر الذاتي و ذلك تحت شروط اسراع الأكسده 0.5 ± 63 م/٣٠ يوم. أظهرت النتائج وجود فروق معنويه في جميع الخصائص المدروسة نتيجة اختلاف تركيز ألفا توكوفيرول المستخدم وخلال مده اسراع الأكسده سجلت نتائج الكنترول أقل الدرجات ثباتا ضد الأكسده واطهر تباينا كبيرا في جميع الخصائص المدروسة مقارنة بالمعاملات المختلفه. أظهرت المعامله المحتويه علي اعلي نسبه من التوكوفيرول احتوائها علي نسبه مرتفعه من الحمض الدهنيه غير المشبعه، بالإضافة الي وجود فروق معنويه في الصفات الكيميائية لثوابت الدهن (رقم اليودي، رقم التصبن، و رقم حامض) والأحماض الدهنية الحره مما يعكس اعلي درجه حمايه للدهن ضد الأكسده بالمقارنه مع التركيزات الأخرى و الكنترول. اظهرت النتائج انخافضا معنويا في قيم نقطه الانصهار مع زيادة تركيز ألفا توكوفيرول المضاف، بينما أظهرت قيم كلا من الوزن النوعي و معامل الانكسار زياده معنويه مع زياده التركيز المضاف خلال فتره اسراع الأكسده. إضافة تركيز ١٠ ملجم ألفا توكوفيرول / ١٠٠ جم دهن أدى إلى إنخفاض معنويا في قيم كل من اللزوجة الظاهرية والخواص التركيبية أثناء فتره اسراع الأكسده. بصفه عامه ادت اضافته تركيزات مختلفه من ألفا توكوفيرول الي رفع درجه حمايه الدهن أثناء التخزين تحت شروط اسراع الأكسده من التأثير الضار لكلا من عاملى الوقت والحماض. بينما أظهرت المعامله ١٠ ملجم / ١٠٠ جم دهن من ألفا توكوفيرول + ١ % فسفوليبيد إلى اعلي ثبات الدهن ضد الأكسده و تحسين جميع في الخصائص المدروسة بالمقارنه بالتركيزات الأخرى و الكنترول.

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