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APPLICATION OF ARRHENIUS KINETICS FOR SHELF-LIFE PREDICTION OF ANHYDROUS BUFFALO AND COW MILK FAT DETERMINED UNDER RANCIMAT TEST

BY

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SUMMARY

Oxidation kinetics of cow and buffalo anhydrous milk fat (AMF) manufactured by different processing methods was evaluated as a function of temperature at 100, 110, 120 and 130 °C under Rancimat test conditions. The kinetic behavior of primary (peroxide value and conjugated dienes) and secondary (conjugated trienes and hexanal concentration determined by HS-SPME-GC/MS) oxidation indices were fitted to a first-order reaction model. The temperature dependence of these oxidation indices were well described by the linear Arrhenius equation with a good correlation coefficient (R^2>0.99). Cow AMF (CAMF) treatments showed lower (P<0.05) reaction rate constants (k) than the buffalo AMF (BAMF), indicating more stability towards oxidative deterioration. Activation energy, calculated from Arrhenius equation, and Q_{10} values of CAMF were in the range of 81.11-104.71 kJ.mol^{-1} and 2.19-2.41, respectively, while their corresponding values for BAMF were 117.34-154.58 kJ.mol^{-1} and 2.53-2.88, with significant differences (P<0.05) found between the different treatments. Enthalpy (ΔH) and entropy (ΔS) values were calculated based on the activated complex theory. CAMF showed higher (P<0.05) ΔH (67.34-89.92 kJ.mol^{-1}) and ΔS (-92.52 to -109.33 J.mol^{-1}.K^{-1}) as compared to BAMF where these parameters were 40.64-58.59 kJ.mol^{-1} and -118.98 to -135.02 J.mol^{-1}.K^{-1}, respectively which was ascribed to differences (P<0.05) in fatty acids composition.

Key words: Arrhenius, Rancimat, Kinetics, Accelerated shelf-life, Oxidation, Butter oil, Ghee, Anhydrous milk fat.

INTRODUCTION

Milk fat is a natural product with unique organoleptic characteristics, which make it an important ingredient in a wide variety of food applications. Milk fat is one of the most complex fats found in nature. This complexity stems from the extreme diversity of its fatty acids (e.g., chain length and degree of unsaturation), which more than 400 of them have been identified (Jensen, 2002). The diversity of milk fat fatty acids pattern reflects, consequently, on its content of triacylglycerol (TAG). Gresti *et al.* (1993) quantified 223 individual molecular species of TAG accounting for 80 % of the total milk fat TAG content.

Ghee is a pure form of milk fat (> 99.5 %). Ghee, the Indian name of clarified butter fat, is fat-rich dairy product prepared by evaporating water from milk, cream or butter followed by milk solids not fat separation. The product has a uniformly grainy semi-solid texture with a pleasant odor and an excellent taste (Munro *et al.*, 1998; Gonfa *et al.*, 2001). Butter oil is another pure form of milk fat that is produced by melting the butter (60 °C) using either direct or indirect heating by steam injection. The melted butter has to be held at this temperature for 20-30 min to ensure complete melting and protein aggregation, followed by centrifugation for a phase separation. The final product is a bright oil of 99.5 % milk fat minimum (Illingworth and Bissell, 1994)

Edible oils and fats go under deterioration when exposed to an oxidative environment. Oxidation of lipids, RH, proceeds

through a radical initiated chain mechanism involving: initiation ($RH \rightarrow R^{\bullet}$), propagation ($R^{\bullet}+O_2 \rightarrow RO_2^{\bullet}$, $RO_2^{\bullet}+RH \rightarrow RO_2H+R^{\bullet}$), and termination ($R^{\bullet}+R^{\bullet} \rightarrow RR$, $R^{\bullet}+RO_2^{\bullet} \rightarrow RO_2R$, $RO_2^{\bullet}+RO_2^{\bullet} \rightarrow RO_2R+O_2$) (Shahidi and Wanasundara, 2002). The reaction creates compounds such as aldehydes, ketones, hydrocarbones and polymers that cause off-flavors, loss of nutritional value and color changes conducive to consumer rejection (Min and Boff, 2002). In this context, milk fat autoxidation, also known as oxidative rancidity, is a process which occurs fairly slowly at room temperature. Hence, accelerated oxidation is used to estimate the oxidative stability in a short period of time which can't be accomplished under normal storage conditions. Several factors can be used to increase the rate of the reaction, and consequently development of rancidity, such as temperature, light, metal catalysis, and rise in oxygen partial pressure (Frankel, 2005a). However, since the rate of the reaction increases exponentially with the temperature, this parameter is usually chosen to accelerate the oxidation process (Farhoosh *et al.*, 2008).

The Rancimat method has become very common for determining Oxidative Stability Index (OSI) of oils and fats owing to its ease of use and reproducibility (Anwar *et al.*, 2003; Kowalski *et al.*, 2004; Farhoosh, 2007; Gonzaga *et al.*, 2007). It depends on detecting oxidation products generated upon thermal oxidation in the presence of an air stream. Decomposition of hydroperoxides and the accompanying formation of free fatty acids (such as formic acid) cause the drastic change in the conductivity of the water that collects volatiles released from a degrading lipid (Ganguli *et al.*, 2003). The time interval needed for a lipid to reach this stage is termed the induction period (IP) which is an indicative of the OSI.

Under the Rancimat test conditions, several kinetic parameters can be determined for predicting the oxidative stability of AMF during heat processing, storage, and distribution conditions. Calculations of these kinetic data can be performed quite rapidly and Arrhenius model is the most accepted one for studying the effect of temperature on rate of oxidation as a chemical reaction. This model, developed theoretically on a molecular basis for chemical reactions, has been shown to hold empirically for a wide range of complex chemical and physical phenomena occurring in foods (Labuza and Riboh 1982). By measuring the rate that the quality index changes at 3 different temperatures at least, the reaction rate at a desired temperature can be extrapolated by the Arrhenius linear plot. Nevertheless, this subject has received no attention for BAMF or CAMF, and data on their oxidative degradation kinetics do not exist. Hence, the objective of the present work was to study the temperature dependence of the oxidation rate as well as Arrhenius kinetic parameters of BAMF and CAMF produced by different processing methods.

MATERIALS AND METHODS

1. Materials

Buffalo milk used was obtained from the herd of College of Agriculture, Ain Shams University, Cairo, Egypt, while cow milk was obtained from the herd of College of Agriculture, Cairo University, Giza, Egypt. The milk was separated at 37±0.5°C (Alfa-Laval, Lund, Sweden). The resultant cream was pasteurized at 85°C /18 s, rapidly cooled to 5°C and aged at this temperature overnight. The cream obtained (~ 40 % fat) was divided into two portions. One portion was used for preparing ghee, and the other was churned into unsalted butter which was used for making butter oil as well as ghee (Fatouh *et al.*, 2003).

2. Methods

2.1. Anhydrous milk fat preparation

2.1.1. Ghee

Direct cream ghee (CG) and butter ghee (BG) were manufactured by the traditional "boiling-off" method where moderate heating of the cream or butter with a regular stirring being applied. After the water was evaporated (110 °C-125 °C), the slurry of milk solids in

the liquid fat was cooled to 50 °C and filtered through cheesecloth (Ganguli and Jain, 1973; Sserunjogi *et al.*, 1998). Cream ghee manufactured using buffalo and cow milk was designated as BCG and CCG, respectively, while butter ghee manufactured using buffalo and cow milk was designated as BBG and CBG, respectively.

2.1.2. Butter oil

Butter oil (BO) was prepared by the method of Amer *et al.* (1985). Butter was melted at 60 °C and the top oil layer was decanted and centrifuged at 3392 ×*g* for 5 min (Sigma 3-16P, Sartorius, Gottingen, Germany). The oil was filtered under vacuum to obtain a clear product. The resultant BO was approximately 99.5% pure milk fat. BO manufactured using buffalo milk was designated as (BBO), while that made using cow milk was designated as (CBO).

2.2. Rancimat test

A Metrohm Rancimat model 743 (Metrohm A G, Herisau, Switzerland) was used for measuring the Oxidative stability index (OSI) following the AOCS method Cd 12b-92 (AOCS, 1997). The glassware used was rigorously cleaned between runs to avoid any contamination that would catalyze peroxidation. The tubes were cleaned by boiling them with sodium hydroxide solution (2%) for 1 h, followed by cooling and soaking in concentrated hydrochloric acid. The acid was washed-off and the tubes were rinsed with distilled water. The clean glassware was thoroughly dried in an oven. Measuring vessels, electrodes, and connecting tubes were cleaned several times with alcohol and distilled water, and were blown out with nitrogen before the experiment (Farhoosh *et al*, 2008).

The OSI tests were carried out in triplicates with 3 g of AMF at temperatures of 100, 110, 120, and 130 °C and an airflow rate of 20 $L.h^{-1}$. The OSI values were automatically recorded by the apparatus software and taken as the break point of the plotted curves (the intersection point of the two extrapolated parts of the curve).

2.3. Analytical determinations

2.3.1. Peroxide value

Peroxide value (PV) was determined as $meq.O_2.kg^{-1}$ fat by the iodometric titration method according to AOAC (2012) method no. 965.33.

2.3.2. Conjugated dienes and trienes

The Conjugated dienes (CD) and trienes (CT) content was measured as described in the AOCS method Ti 1a-64 using a molar absorption coefficient of 25,200 $M^{-1}.cm^{-1}$. (AOAC, 2012). AMF sample was dissolved in isooctane and the absorbance of the solution was measured at 232 for CD and 270 for CT using a Cary 50 UV-Vis spectrophotometer (Varian, Sint-Katelijne Waver, Belgium).

2.3.3. Fatty acids composition

Fatty acids (FAs) composition of AMF was determined after conversion of the FAs into their corresponding methylesters (FAMEs) following the AOCS method Ca 5a-40 (AOCS, 1997) method. The FAMEs were dissolved in isooctane, and 0.1 µL was injected into a CP-Sil 88™ capillary column (60 m x 0.25 mm I.D x 0.2 µm film thickness) previously installed in an Agilent 6890N series gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a flame-ionization detector (FID) and cool-on-column injector. Heleium was the carrier gas (1 $mL.min^{-1}$) and make up gas was supplied at a flow rate of 20 $mL.min^{-1}$. The oven temperature was programmed as follow: initially held at 50°C for 4 min, and then increased at a rate of 12°$C.min^{-1}$ until a temperature of 225 °C was reached and held at this temperature for 25 min. The injector and detector temperatures were 53°C and 300°C, respectively. The FAMEs peaks were identified by comparing the retention times with those of a standard mixture of the FAMEs (GLC 68D, Nu-Check-Prep Inc., Elysian, MN, USA). For quantification, 50 mg of the FAMEs standard mixture were dissolved in 50 mL isooctane. The stock solution was then diluted to 500, 200, 100, 50, 20, 10, 5, and 1 ppm (total concentration) solutions with spiked 100 ppm nonadecanoic acid as internal standard.

2.3.4. Hexanal

Hexanal concentration was measured by head space-solid phase micro extraction-gas chromatography-mass spectrometry (HS-SPME–GC/MS) technique following the method of Panseri *et al.*, 2011).

2.3.4.1. HS-SPME conditions

5 grams of oxidized AMF were weighed in a 20 mL headspace vial (Sigma-Aldrich, St. Louis, MO, USA). The vial was sealed with a PTFE septum cap and the sample was equilibrated at 60°C for 10 min. A 50/30 µm divinylbenzene/carboxen/ poly-dimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) was exposed to the sample headspace for 30 min, while the AMF sample was stirred continuously, by CombiPAL system injector autosampler (CTC Analytics, Zwingen, Switzerland).

2.3.4.2. GC/MS analysis

Chromatographic analysis was performed using an Agilent 7890A GC (Agilent, Palo Alto, CA, USA) equipped with a 5975C quadrupole mass spectrometer (MS) operating in electronic impact ionization mode (70 eV). A SPB-5 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Supelco, Bellefonte, PA, USA) was used. The column temperature was programmed as follow: initial temperature at 30 °C for 1 min, then 15 °C.min^{-1} to 200 °C and kept at this temperature for 2 min, followed by a ramp of 20 °C.min^{-1} to 250 °C isothermally for 10 min. Helium was used as carrier gas at a flow rate of 1.2 ml. min^{-1} and a constant pressure of 20 psi. Injector temperature was 250 °C and time for fiber desorption was fixed at 10 min in splitless mode. Capillary direct interface temperature of the MS was 250 °C and data collection was carried out at a rate of 2.42 scans.s^{-1} over a range of 30–150 *m/z*. Hexanal was identified using its mass spectrum as well an authentic standard, and quantification was carried out by preparing an external calibration curve using hexanal at a range of 0.06 to 1 mM.

2.4. Kinetic data analysis

Kinetic analysis was carried out following the methods of Labuza (1984) and Taoukis and Labuza (1996). The measured oxidation parameters were fitted to first order reaction model:

$$\frac{-d[C]}{dt} = k[C] \quad (1)$$

Where C is the concentration of an oxidation product (e.g., PV) at time t (h), and k is the reaction rate constant. By integrating Eq. 1:

$$\ln C = \ln C_o + kt \quad (2)$$

Where C_o is the initial concentration of the oxidation product. Plots of ln C versus t were linear with slopes of k which was then fit to Arrhenius equation to determine the effect of temperature on AMF oxidation rate was:

$$K = Ae^{(Ea/RT)} \quad (3)$$

Where A is the pre-exponential or frequency factor (h^{-1}), Ea is the activation energy (kJ.mol^{-1}), R is the molar gas constant (8.31 J.K^{-1}.mol^{-1}) and T is the absolute temperature (K). A and E_a were determined from slopes and intercepts, respectively, of the plots generated by regressing ln$k$ vs. 1/T:

$$\ln k = \ln A - E_a/RT \quad (4)$$

Since shelf-life can be predicted by extrapolating k from high temperatures to low temperatures, the exponential relation between the time required to reach the upper limit of acceptability (shelf- life, t_s), when PV reaches 5 meq. O_2.Kg^{-1} fat, and temperature is determined according to:

$$t_s = ae^{-bT} \quad (5)$$

Where a is the shelf-life plot intercept and b is the slope. Integration of Eq. 5 gives:

$$\ln t_s = \ln a + b \ln T \quad (6)$$

Q_{10}, the change of t_s when the temperature is raised by 10 °C, was calculated as follow:

$$Q_{10} = \frac{ts\ at\ T}{ts\ at(T+10)} = e^{10b} = e^{\left[\frac{Ea}{R}\frac{10}{T(T+10)}\right]} \quad (7)$$

Enthalpies (ΔH) and entropies (ΔS) of activation were determined by regression ln (k/T) vs. 1/T using the activated complex theory:

$$\ln (k/T) = \ln (k_B/h) + (\Delta S/R) - (\Delta H/RT) \quad (8)$$

Where k_B is Boltzman constant (10380658 × 10^{-23}J.K^{-1}), and h is Planck constant (6.62606957×10^{-34} J.s). From slopes and intercepts of these lines, both ΔH and ΔS were calculated.

2.5. Statistical analysis

Three individual batches of either cow or buffalo AMF including BO and ghee were manufactured. All Rancimat experiments and measurements were carried out in triplicate, and the obtained data were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS software version 9.22 (SAS, 2010). The differences were considered significant at $P < 0.05$. Regression analyses were performed using GraphPad Prism 5 (GraphPad Prism Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

1. FAs composition and PV

Table 1 presents FAs profile and initial PV of CAMF and BAMF manufactured by different processing methods. PV of CAMF and BAMF was less than 0.2 meq O_2/Kg fat indicating that they were unoxidized and of very high quality. Regarding the FAs composition, myrstic (C14:0), palmetic (C16:0), stearic (C18:0), and oleic (C18:1) acids were the major FAs detected with variable differences ($P<0.05$) found in C16:0, C18:0, and C18:1. In general, saturated fatty acids (SFAs) were more abundant ($P<0.05$) in BAMF as compared to CAMF, while the opposite was observed for unsaturated fatty acids (USFAs) where CAMF treatments was higher ($P<0.05$) by almost 5 % than the buffalo ones. Consistent with the results obtained, Hammad and Baiomy (2010) who found that, C14:0, C16:0, C18:0 and of C18:1 represented 12.40, 25.69, 8.83, and 30.82 %, respectively, of the total FAs profile of cow milk fat, and 11.46, 29.27, 13.28, and 27.60 % for buffalo milk fat following the same order. Our results are also in agreement with previous studies of Hussein *et al.* (2001), Varrichio *et al.* (2007), Blasi *et al.* (2008), Ménard *et al.* (2010), and Islam *et al.*, (2014).

Table (1):Fatty acids composition and peroxide value (PV) of anhydrous milk fat (AMF) prepared by different processing methods[1].

Fatty acid	AMF treatments[2]						±SE
	CCG	CBG	CBO	BCG	BBG	BBO	
C4:0	1.46^{a}	1.38^{a}	1.30^{ab}	1.37^{a}	1.32^{ab}	1.25^{b}	0.09
C6:0	2.39^{a}	2.32^{ab}	2.21^{b}	2.43^{a}	2.40^{a}	2.34^{ab}	0.10
C8:0	1.19^{a}	1.17^{a}	1.17^{a}	1.10^{ab}	1.06^{ab}	1.02^{b}	0.04
C10:0	3.56^{a}	3.49^{a}	3.41^{a}	2.50^{b}	2.52^{b}	2.45^{b}	0.11
C12:0	3.84^{a}	3.86^{a}	3.87^{a}	2.83^{b}	2.91^{b}	2.98^{b}	0.12
C14:0	12.77^{a}	12.65^{a}	12.62^{a}	11.82^{ab}	11.70^{ab}	11.62^{b}	0.34
C16:0	28.15^{b}	28.11^{b}	28.04^{b}	31.96^{a}	31.93^{a}	31.82^{a}	0.39
C18:0	12.98^{b}	12.82^{b}	12.76^{b}	17.10^{a}	17.06^{a}	16.98^{a}	0.35
C18:1	30.21^{a}	30.58^{a}	30.69^{a}	26.19^{b}	26.31^{b}	26.53^{b}	0.46
C18:2	2.45^{abc}	2.60^{ab}	2.85^{a}	1.99^{d}	2.04^{cd}	2.16^{bcd}	0.14
C18:3	1.00^{a}	1.02^{a}	1.04^{a}	0.71^{b}	0.75^{b}	0.81^{b}	0.05
SFAs	66.34^{c}	65.80^{c}	65.42^{c}	71.11^{a}	70.90^{ab}	70.50^{ab}	1.43
USFAs	33.66^{a}	34.20^{a}	34.58^{a}	28.89^{b}	29.10^{b}	29.50^{b}	0.57
PV[3]	0.12^{ab}	0.13^{ab}	0.08^{b}	0.16^{a}	0.16^{a}	0.09^{b}	0.05

[1] Means within the same row followed by different superscripts are significantly different ($P<0.05$).
[2] CCG, cow cream ghee; CBG, cow butter ghee; CBO, cow butter oil; BCG, buffalo cream ghee; BBG, buffalo butter ghee; BBO, buffalo butter oil.
[3] meq.O_2.Kg^{-1} fat.

2. Oxidative Stability Index (OSI)

Stability of CAMF and BAMF was evaluated by the Rancimat test and expressed as OSI at 100, 110, 120, and 130 °C (Table 2). Data presented indicate that the longer the IP, the more stable is the sample. Considering the milk type and at all tested temperatures, CAMF treatments illustrated a longer IP

(P<0.05) than the buffalo ones. The IP of CCG was the longest (P<0.05) among all samples evaluated followed by CBG, CBO, BCG, BBG, and finally BBO was the shortest (P<0.05). The Rancimat test temperatures used displayed an inverse relation with the IP with significant variations (P<0.05) found between treatments. For example, the IP of CCG was 20.48 h at 100 °C that was reduced by 35.8% when the test was carried out at 130°C. Similarly the IP of BBO was 8.79 and reduced by 66.1% following the same order of temperatures. Pawar *et al.* (2014) found that, IP of CBG determined under the Rancimat test was 8.5 h at 120 °C, while longer IP (10.38 h) was reported by Patel *et al.* (2013) for CCG at the same temperature. For BBO, IP determined under the Rancimat test was 8.2 h at 110 °C (Fatouh *et al.*, 2003).

The method of manufacture used has an impact on the stability of AMF. Preparation of BO does not include using high temperatures (> 100 °C), contrary to ghee where boiling-off, either for cream or butter, is the main principle of processing. The prolonged shelf-life of ghee, consequently the longer IP, as compared to BO is attributed to several factors including the low moisture (≤ 0.3 %), the high content of phospholipids (~ 400 mg.kg^{-1}) acting as antioxidants, and a possible role of sulfur free amino acids like methio-nine, cysteine that are liberated from the phospholipids-protein adduct into the fat phase during the boiling-off process (Achaya, 1997). Moreover, CAMF was less susceptible to oxidative deterioration than BAMF treatments owing to its high content of β-carotene, a natural antioxidant, which does not exist in buffalo milk fat. Kumar *et al.* (2010) reported a 6.98 µg/g of β-carotene in CCG. In accordance also with our findings previous work of Mariod *et al.* (2010) and Olfa *et al.* (2010).

Table (2): Oxidative stability index (OSI) of anhydrous milk fat (AMF) manufactured by different processing methods[1].

Temperature (°C)	OSI (h)						±SE
	AMF treatments[2]						
	CCG	CBG	CBO	BCG	BBG	BBO	
100	20.48^{a}	17.63^{b}	15.95^{c}	13.65^{d}	11.32^{e}	8.79^{f}	0.283
110	18.43^{a}	15.51^{b}	13.28^{c}	11.37^{d}	9.82^{e}	7.98^{f}	0.445
120	16.51^{a}	13.27^{b}	10.88^{c}	8.70^{d}	6.23^{e}	4.86^{f}	0.376
130	13.14^{a}	10.26^{b}	7.75^{c}	6.53^{d}	4.85^{e}	2.98^{f}	0.306

[1] Means within the same row followed by different superscripts are significantly different (P<0.05).
[2] CCG, cow cream ghee; CBG, cow butter ghee; CBO, cow butter oil; BCG, buffalo cream ghee; BBG, buffalo butter ghee; BBO, buffalo butter oil.

3. Primary oxidation products

The applicability of Arrhenius model (Eq. 3), which is conventionally used to describe the effect of temperature on k, was evaluated in terms of PV (k_{pv}) and CD (k_{232}) at reference temperatures of 100, 110, 120, and 130°C (Table 3). Since k is a function of temperature, as accelerated oxidation temperature increased, a corresponding increase was observed in k. Significant differences (P<0.05) were found between k_{pv} of all treatments in the temperature range 100-120 °C where CCG was always the lowest and BBO the highest. Similar differences (P<0.05) were also found between some of the treatments at 130 °C like that between CCG, BBG, and BBO. Considering CD, measuring this parameter is a sensitive method to follow the early stages of lipid oxidation under conditions in which hydroperoxides undergo little or no decomposition (Frankel, 2005b). For k_{232}, the evolution at the temperatures used presented variations (P<0.05) between the different treatments as in the case of k_{pv}. The dependence of k_{pv} and k_{232} on the oxidation temperature is presented in Figs 1 and 2. Either CAMF or BAMF that prepared by different methods showed a linear dependency with a good correlation coefficient (R^2 > 0.99).

Table (3): Reaction rate constant (k, h^{-1}) of primary oxidation of anhydrous milk fat (AMF) manufactured by different processing methods[1].

Temperature (°C)	AMF treatments[2]						±SE
	CCG	CBG	CBO	BCG	BBG	BBO	
PV[3]							
100	0.630^{f}	0.675^{e}	0.719^{d}	0.783^{c}	0.821^{b}	0.889^{a}	0.011
110	0.830^{f}	0.905^{e}	0.989^{d}	1.071^{c}	1.142^{b}	1.297^{a}	0.013
120	1.105^{f}	1.230^{e}	1.371^{d}	1.505^{c}	1.633^{b}	1.914^{a}	0.020
130	1.413^{e}	1.614^{de}	1.821^{cd}	2.027^{bc}	2.236^{b}	2.685^{a}	0.093
K_{232}[4]							
100	0.219^{e}	0.241^{de}	0.263^{d}	0.298^{c}	0.336^{b}	0.375^{a}	0.008
110	0.260^{e}	0.317^{d}	0.369^{c}	0.412^{c}	0.465^{b}	0.558^{a}	0.016
120	0.355^{f}	0.409^{e}	0.463^{d}	0.517^{c}	0.585^{b}	0.692^{a}	0.018
130	0.508^{e}	0.571^{e}	0.659^{d}	0.764^{c}	0.848^{b}	0.931^{a}	0.022

[1] Means within the same row followed by different superscripts are significantly different ($P<0.05$).
[2] CCG, cow cream ghee; CBG, cow butter ghee; CBO, cow butter oil; BCG, buffalo cream ghee; BBG, buffalo butter ghee; BBO, buffalo butter oil.
[3] Peroxide value.
[4] Conjugated dienes.

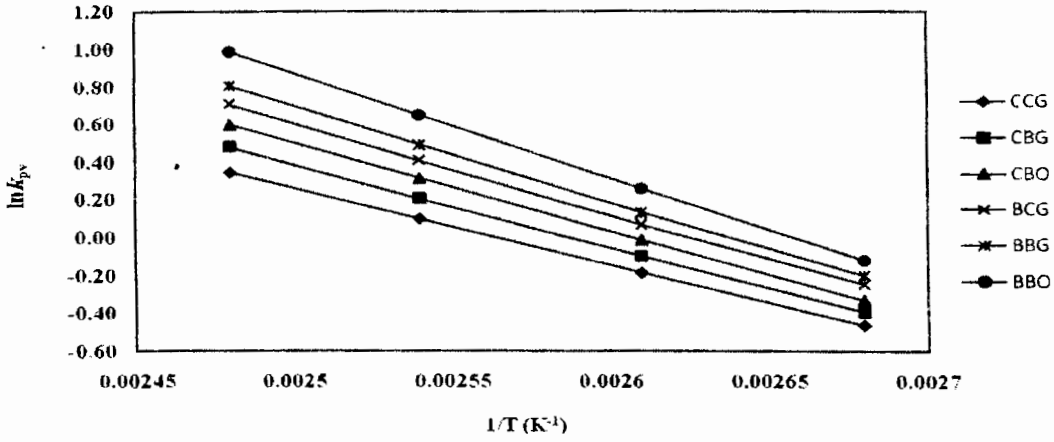

Fig (1): Arrhenius plot: Effect of temperature on oxidation rate constant for peroxide value (k_{pv}) of anhydrous milk fat manufactured by different processing methods.

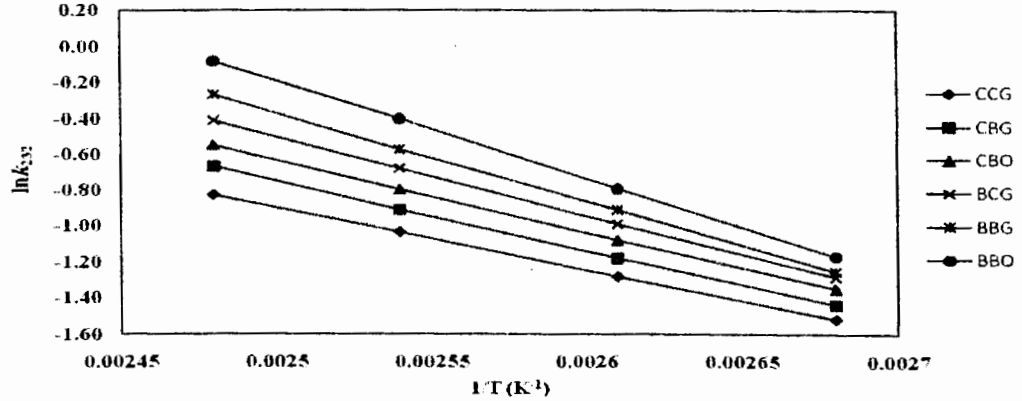

Fig (2): Arrhenius plot: Effect of temperature on oxidation rate constant for conjugated dienes (k_{232}) of anhydrous milk fat manufactured by different processing methods.

The increase in k values, either k_{pv} or k_{232}, can be attributed to the increase of oxygen solubility in AMF, and thus the susceptibility to oxidation regardless the milk type or the method of manufacture. In accordance with our results Özkanlı and Kaya (2005) who found a corresponding increase in k as the temperature of the accelerated oxidation test used increased. At 60, 70, and 80 °C, k values for BO produced by hexane extraction from sheep's non-pasteurized and pasteurized milk were 0.12, 0.21, and 0.57, respectively. In terms of USFAs/SFAs ratio, similar to our results the trend found in vegetable oils by Farhoosh *et al.* (2008) who calculated k values for canola (8.5 % SFAs, 91.5 % USFA), corn (11.76 % SFAs, 88.04 % USFA), and olive (19.13 % SFAs, 80.87 % USFA) oils using the Rancimat test. k values increased from 25.04, 44.87, and 49.42 at 100 to 215.82, 362.04, and 409.55 at 130 °C following the same order of the oils.

4. Secondary oxidation products

The primary lipid oxidation products, mainly hydroperoxides, are unstable and susceptible to decomposition. A complex mixture of volatile (e.g., hexanal), nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation (Shahidi and Zhong, 2005). By studying k values of AMF oxidation measured by CT (k_{270}) and hexanal concentration ($k_{hexanal}$) as a function of temperature, an increasing k_{270} and $k_{hexanal}$ can be observed as the Rancimat temperature increased (Table 4). For example, k_{270} for CCG and BCG were higher by 125.37 and 131.8 % at 130°C than at 100°C, respectively. Data presented also illustrate that, CAMF is more stable (P<0.05) than BAMF for oxidative deterioration as evidenced by the lower k_{270} and $k_{hexanal}$. Moreover, plotting ln k_{270} and $k_{hexanal}$ vs $1/T$ (Figs 3 and 4) generated straight and almost parallel lines which indicate that, the temperature dependence of these oxidation indices is well described by the linear Arrhenius model (R^2 > 0.99) in the temperature range tested (100-130°C). Gómez-Alonso *et al.* (2004) performed an accelerated shelf-life test on olive oil in the temperature range of 25-75 °C. Their results indicated that, the formation of hexanal followed a first order kinetic where $k_{hexanal}$ increased exponentially with the temperature.

5. Arrhenius kinetic parameters

Activiation energy (Ea) is a measure of the temperature sensitivity of the oxidation reaction, e.g., how much faster it will go if the temperature is raised (Taoukis *et al.*, 1997). Arrhenius model suggests that, if a molecule has a total energy $E \geq E$a, then it has a potential for reacting which is controlled by the value of A that is also called sometimes the collision or frequency factor because it represents the frequency of collisions between the reactant molecules. As the temperature increases, the fraction molecules with $E \geq E$a increases, thus the k increases (Taoukis and Labuza, 1996). The Ea values of AMF produced by different processing methods were in the range of 81.11-154.58 kJ.mol^{-1} (Table 5). Regardless the method of production, CAMF showed lower (P<0.05) Ea than the buffalo ones which can be attributed to differences found in the FAs composition (Table 1). All BAMF treatments contained significantly (P<0.05) higher amount of SFAs as compared to the cow ones. Khan *et al.* (2010) reported that, high values of Ea are associated with fats and oils having greater proportions of SFAs. The Ea values of CAMF and BAMF were in agreement with previous literature data, reporting typical Ea values for lipid oxidation ranging from 24 to 240 kJ.mol^{-1} based on FAs composition of the lipid material (Tan *et al.*, 2001; Frankel 2005b). The trend found in this study is in agreement with Thurgood *et al.* (2007) who evaluated the oxidation kinetics of AMF/soybean blends. Ea value of AMF (73.04 % SFAs) was 93.56 kJ.mol^{-1} that was reduced to 73.2 kJ.mol^{-1} when soybean oil (15.66 % SFAs) was used for creating a 50-50 % AMF/soybean blend containing 42.95 % SFAs. Similarly, Farhoosh *et al.* (2008) reported higher (P<0.05) Ea value of 92.42 kJ.mol^{-1} for olive oil that contains 19.13 % SFAs, mostly palmitic acid, comparing to 86.86 kJ.mol^{-1} for sunflower oil that contains 9.66 % SFAs. Similar observations for vegetable oils were also reported by Ostrowska-Ligeza *et al.* (2010).

Table (4): Reaction rate constant (k, h^{-1}) of secondary oxidation products of anhydrous milk fat (AMF) manufactured by different processing methods[1].

Temperature (°C)	AMF treatments[2]						±SE
	CCG	CBG	CBO	BCG	BBG	BBO	
K_{270}[3]							
100	0.134^{f}	0.162^{e}	0.189^{d}	0.217^{c}	0.254^{b}	0.298^{a}	0.008
110	0.179^{e}	0.204^{de}	0.236^{d}	0.271^{c}	0.315^{b}	0.378^{a}	0.011
120	0.237^{e}	0.288^{d}	0.335^{d}	0.391^{c}	0.456^{b}	0.539^{a}	0.016
130	0.302^{e}	0.361^{e}	0.427^{d}	0.503^{c}	0.589^{b}	0.684^{a}	0.021
Hexanal							
100	0.105^{f}	0.132^{e}	0.164^{d}	0.189^{c}	0.211^{b}	0.243^{a}	0.007
110	0.126^{e}	0.155^{d}	0.183^{c}	0.207^{c}	0.239^{b}	0.281^{a}	0.008
120	0.149^{e}	0.174^{e}	0.208^{d}	0.256^{c}	0.300^{b}	0.342^{a}	0.01
130	0.173^{e}	0.206^{e}	0.250^{d}	0.309^{c}	0.378^{b}	0.455^{a}	0.014

[1] Means within the same row followed by different superscripts are significantly different (P <0.05).
[2] CCG, cow cream ghee; CBG, cow butter ghee; CBO, cow butter oil; BCG, buffalo cream ghee; BBG, buffalo butter ghee; BBO, buffalo butter oil.
[3] Conjugated trienes.

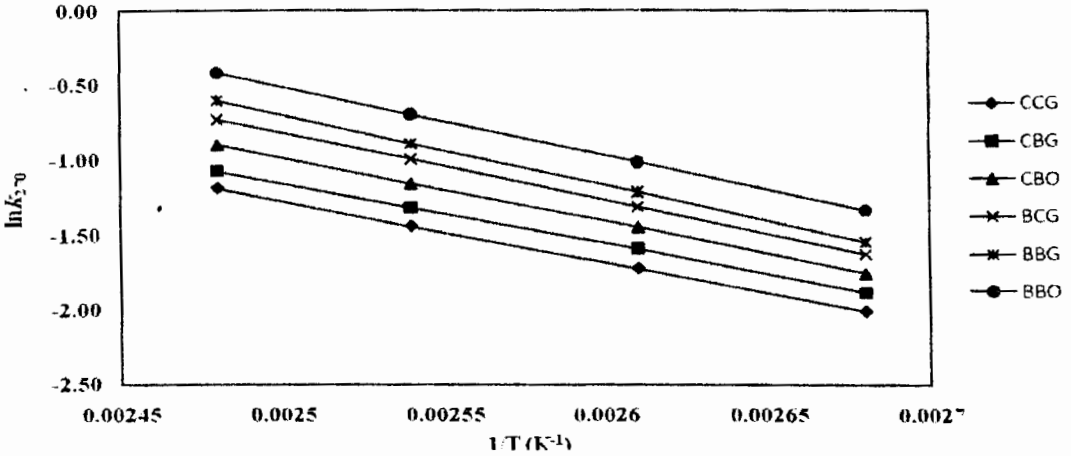

Fig (3): Arrhenius plot: Effect of temperature on oxidation rate constant for conjugated trienes (k_{270}) of anhydrous milk fat manufactured by different processing methods.

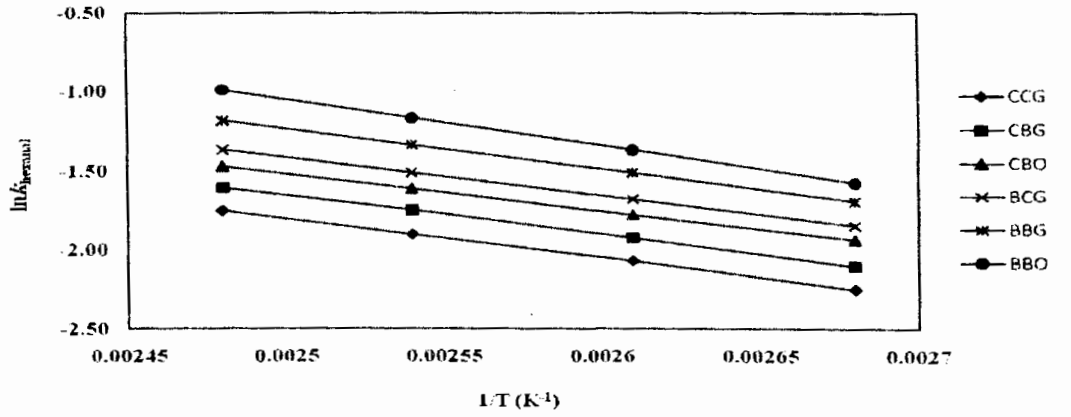

Fig (4): Arrhenius plot: Effect of temperature on oxidation rate constant for hexanal concentration ($k_{hexanal}$) of anhydrous milk fat manufactured by different processing methods.

Table (5): Arrhenius parameters, Temperature acceleration factor (Q_{10}), enthalpy (ΔH) and entropy (ΔS) values of anhydrous milk fat (AMF) manufactured by different processing methods[1].

Parameter	AMF treatments[2]						±SE
	CCG	CBG	CBO	BCG	BBG	BBO	
Ea[3]	81.11^{f}	93.56^{e}	104.71^{d}	117.34^{c}	133.02^{b}	154.58^{a}	3.69
$A^4 \times 10^{11}$	8.92^{f}	11.06^{e}	13.35^{d}	16.69^{c}	18.51^{b}	22.14^{a}	0.54
Q_{10}	2.16^{f}	2.29^{e}	2.41^{d}	2.53^{c}	2.71^{b}	2.88^{a}	0.05
ΔH^5	89.92^{a}	78.55^{b}	67.34^{c}	58.59^{d}	49.00^{e}	40.64^{f}	2.81
ΔS^6	-92.52^{a}	-100.45^{b}	-109.33^{c}	-118.98^{d}	-127.64^{e}	-135.02^{f}	3.07

[1] Means within the same row followed by different superscripts are significantly different (P<0.05).
[2] CCG, cow cream ghee; CBG, cow butter ghee; CBO, cow butter oil; BCG, buffalo cream ghee; BBG, buffalo butter ghee; BBO, buffalo butter oil.
[3] Activation energy (kJ.mol^{-1}).
[4] Pre-exponential factor or frequency factor (h^{-1}).
[5] kJ.mol^{-1}
[6] J.mol^{-1}.K^{-1}

The pre-exponential or frequency factor (A) represents the frequency of collisions between reactant molecules (Labuza and Schmidt, 1985). The A values of CAMF and BAMF treatments under the Rancimat test were evaluated in the temperature range 100-130 °C (Table 5). The data indicated a direct proportional relation between A and k which agrees with Arrhenius equation (Eq.3). Significant differences (P<0.05) were found between all different treatments with CCG and BBO are the lowest (8.92×10^{11} h^{-1}) and highest (22.14×10^{11} h^{-1}) A values calculated, respectively. In accordance with the trend found in this work, Adhvaryu *et al.* (2000) who studied Arrhenius oxidation kinetic parameters of several vegetable oils using the differential scanning calorimeter technique. Their results indicated a corresponding increase in k as A values increased. Cottonseed oil exhibited the highest k and A values (0.37 min^{-1}and 9.25×10^{6} h^{-1}) followed by corn (0.43 min^{-1}and 2.43×10^{8} h^{-1}), and canola oils (0.51 min^{-1}and 7.67×10^{9} h^{-1}). Thurgood *et al.* (2007) reported k and A values, calculated at 200°C, of AMF prepared by melt-centrifugation to be 0.57 min^{-1} and 1.2×10^{10} min^{-1}, respectively.

Temperature dependence often is expressed as Q_{10}, the ratio of k at temperatures differing by 10°C, or the change of shelf-life (t_s) when the temperature of the lipid is raised by 10°C (Fu and Labuza, 1993). The magnitude of the temperature effect on oxidation rate of CAMF and BAMF treatments manufactured by different processing methods was evidenced by the Q_{10} (Table 5). In general, the higher Q_{10} values (P<0.05) observed in BAMF treatments comparing to the CAMF ones were associated with their higher Ea (Table 5) which is readily explained based on differences in the USFAs/SFAs ratio (Table 1) as previously shown. Kochhar and Henry (2009) studied the oxidative stability of selected culinary oils. Q_{10} values of almond (7 % SFAs), avocado (14 % SFAs) and macadamia (18.5 % SFAs) oils increased as the oil SFAs content increased. Q_{10} values were 1.96, 2.05 and 2.15 following the same order of the oils. The same trend was also observed in the work of Farhoosh *et al.* (2008) for vegetable oils with different USFAs/SFAs ratios.

Enthalpy (ΔH) and entropy (ΔS) values were calculated based on the activated complex theory and the corresponding regression parameters are summarized in Table 5. A high correlation of determination (R^2 > 0.99) indicated good fit for characterization of the temperature dependence of AMF oxidation. The ΔH and ΔS values ranged from 40.64 to 89.92 kJ.mol^{-1} and -92.52 to -135.02 J.mol^{-1}.K^{-1}, respectively with significant differences found (P<0.05) between CAMF and BAMF treatments. CAMF showed greater values for both ΔH and ΔS as compared to the BAMF ones. These results are readily explained based on their FAs composition differences (Table 1). Buffalo milk fat contains

more SFAs (70.5-71.11%) and less USFAs (28.89-29.1%) as compared to cow milk fat where these values were 65.42-66.34 and 33.66-34.2 % following the same order. Tan *et al.* (2001) reported higher ($P<0.05$) ΔH and ΔS values for oils with high USFAs content (e.g., safflower, 89.6 % USFAs) than for oils with greater amounts of SFAs (e.g., coconut, 96.0 % SFAs). The negative values for ΔS indicate that, the activated complexes are more ordered than the molecules of the reactants, and its greater negative values indicate fewer numbers of species are in the activated complex state, and hence lesser probability of the activated complex for lipid oxidation and therefore slower reaction rate (Tan *et al.*, 2002).

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تم دراسة حركيات تأكسد دهن اللبن البقرى والجاموسى اللامائى والمصنع بطرق مختلفة و ذلك عند درجات حرارة ١٠٠، ١١٠، ١٢٠ و ١٣٠°م و ذلك تحت ظروف إختبار الـ Rancimate أوضحت النتائج أن سلوك حركيات منتجات الأكسدة الأولية (رقم البيروكسيد و الروابط الزوجية المتبادلة) و كذلك الثانوية (الروابط الثلاثية المتبادلة و تركيز مركب الهكسانال و الذى تم تقديره بتقنية HS-PMES-GC/MS) قد كان منطبقا على نموذج التفاعل من الدرجة الأولى. أظهرت النتائج أيضا أن ثابت معدل التفاعل لدهن اللبن البقرى كان أقل معنويا (P<٠.٠٥) من نظيره الجاموسى مما يشير إلى ثبات أعلى للتزنخ التأكسدى. باستخدام معادلة Arrhenius تم حساب طاقة التنشيط لدهن اللبن البقرى وقد تراوحت قيمتها ما بين ٨١,١١-١٠٤,٧١ كيلوجول/مول بينما كانت قيمتها أعلى معنويا (P<٠.٠٥) فى حالة دهن اللبن الجاموسى وبلغت ١١٧,٣٤-١٥٤,٥٨ كيلو جول/مول. كانت قيم الـ Q١٠ فى حالة دهن اللبن البقرى ٢,١٩-٢,٤١ وهى أقل معنويا (P<٠.٠٥) عن مثيلتها فى دهن اللبن

الجاموسى و التى بلغت ٢,٥٣-٢,٨٨. تم حساب قيم كل من الإنثالبي و الإنتروبى باستخدام نظرية المعقد النشط وقد أظهرت النتائج وجود فروق معنوية (P<٠.٠٥) ما بين دهن اللبن البقرى والجاموسى حيث بلغت قيم الإنثالبي والإنتروبى لدهن اللبن البقرى ٨٩,٩٢-٦٧,٣٤ كيلوچول/مول و -٩٢,٥٢- (-)١٠٩,٣٣ چول/مول.كالڤين على التوالى، بينما كانت ٤٠,٦٤-٥٨,٥٩ كيلوچول/مول و ١١٨,٩٨-- (-)١٣٥,٠٢ چول/مول.كالڤين فى دهن اللبن الجاموسى على التوالى و قد عزيت هذه النتائج إلى الإختلاف فى تركيب الأحماض الدهنية ما بين دهن اللبن البقرى والجاموسى.