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ORIGINAL RESEARCH

Storage stability of traditional Tunisian butter oil produced from spontaneous fermentation of cow's milk

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Physicochemical characteristics of traditional Tunisian butter (TTB) and thermal stability of the butter oil were studied. Thermal stability of samples was estimated by using the accelerated shelf life testing method. Effect of heating on some quality characteristics of traditional Tunisian butter oil (TTBO) was investigated and compared at different temperatures (60°C, 100°C and 130°C). Induction period of sample heated at 60°C was important compared to that at 100°C and 130°C. This result may indicate the sensitivity of TTBO to elevated temperature. Absorption at 232 and 270 nm, acidity and PV increased rapidly after reaching the oxidation induction time. The temperature had a significant effect on the formation of oxidation products in traditional butter oil (TTBO). Fatty acid composition of TTBO was also changed after heating. This may explain the observed modification on some quality characteristics of TTBO such as viscosity, hardness, cohesiveness and colour. As a consequence, heat treatment produced alterations in the oxidative status of the butter oil that could affect the shelf life.

Keywords Physicochemical composition, Traditional Tunisian butter, Butter oil, Thermal stability.

INTRODUCTION

Lactic acid fermentation, as a means of food preservation, is probably one of the oldest biotechnological processes rooted in the cultural history of mankind (Tamang *et al.* 2005). Traditional fermentation of milk remains one of the most practical and widely applied empirical methods for preserving and often enhancing the organoleptic and nutritional quality of milk. A variety of fermented milk products are prepared in Tunisia, mostly by rural women using their traditional knowledge of fermentation for the bio-preservation of milk for storage and future consumption. Two products result from the spontaneous fermentation of milk in Tunisia: the first is rich in proteins and locally called 'Leben'. The second is rich in fat and is locally called 'traditional butter' or 'Zebda beldi'. Traditional Tunisian Butter (TTB) is widely produced and consumed, and is still a popular Tunisian product. Its characteristic aroma plays an important role in its popularity. According to De Noni and Resmini (2005), the term 'traditional' may be used, together with the name 'butter', provided the cream is obtained directly from milk by either centrifugation or natural creaming. Churning is the most important step for butter-making, during which the oil-in water emulsion is broken, leading to the formation of water-in oil emulsion (Rousseau 2000). Butter oil is obtained from traditional butter after the separation of the fat from the milk serum by melting or heating. A major portion of butter oil is utilised for culinary cooking and for the frying of different foods. Deterioration (lipolysis and oxidation) of milk fat, due to several factors, causes flavour impairment, lowers nutritional quality, and creates serious problems for storage stability (Özkanli and Kaya 2005). Oxidation has a significant financial impact due to the development of positive flavours as well as rancid flavours that reduces the sensory characteristics, deteriorates essential fatty acids and heat-sensitive vitamins, and forms oxidised products which may be hazardous to health. Several studies have been carried out to determine the effects of cooking (e.g. method, temperature, time and end-point temperature) on lipid oxidation (Varela 1998; Romero *et al.* 2000; Özkanli and Kaya 2005; Ramírez and Cava 2005), High cooking temperatures, above 100°C, have been reported to reduce the development of lipid oxidation (Spanier *et al.* 1998). This effect has been attributed to the antioxidant action of Maillard reaction products (MRPs) that is induced at elevated cooking temperatures (Bailey 1998). Additionally, the frying process causes modifications in the colour of food due to the formation of low molecular weight chromophores closely related to MRPs (Hofmann 1998). The onset of rancidity in butter may be due to the oxidation of unsaturated glycerides leading to the development of peroxides and/or due to hydrolysis of glycerides resulting in increased levels of free

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fatty acids (Amr 1991; Joshi and Thakar 1994). It has been reported that, both storage time and type of treatment have highly significant effects on the peroxide value and free fatty acid content of anhydrous butter oil (Amr 1991).

The aim of this paper was to determine the physicochemical properties of TTB and to evaluate heat treatment effects on oxidation indicators to determine thresholds for each heat treatment.

MATERIALS AND METHODS

Raw butter production

Fresh raw cows' milk (Holstein breed) used in this study was obtained from a local farm in the Southern of Tunisia. Cows' milk was collected, kept at ≈4°C and transported to our laboratory within 24 h.

Raw milk was left at room temperature in a jar (25 ± 2°C) until coagulation occurred (~18 hours). Microorganisms growing in the raw milk during fermentation steps depend on the environmental conditions (result not published). On gelation, the product is called 'rayeb'. By churning the 'rayeb' is separated into an aqueous fraction (protein, lactose, mineral…) called 'Leben' and raw butter called 'Zebda beldi'. Traditionally, churning, takes place in a goat skin bag called '*Checoua*'. The churning is achieved by hanging the *Checoua* filled with 'rayeb' and vigorously shaking it back and forth until fluidisation of the contents and coalescence of the fat globules. The end of churning is discerned by the sound of the butter lumps when shaking. Warm water (40° C, 10% v/v) was added at the beginning of the churning period to enhance the coalescing of the fat globules and, thus, to increase the yield of butter (zebda beldi).

For this work triplicate jars were used to perform fermentation steps (about 15 L of milk in each jar) and a goat skin bag (traditional *checoua*) was used to perform churning process. Duplicate analyses were performed on each replicate.

Raw butter analysis

Total nitrogen (TN) was achieved using the Kjeldahl method (AFNOR 1993) with a Büchi 325 apparatus (Büchi, Flawil, Switzerland). Dry matter, ash, lactose and fat contents were determined according to standard methods (AFNOR 1993). NaCl (chloride content) was measured using the International Dairy Federation method (IDF 1969). Titrable acidity was determined by titration of sample with N/9 sodium hydroxide to pink endpoint using phenolphthalein as indicator and pH was determined using a pH metre (744 pH Meter Metrohm) (AFNOR 1993).

Traditional Tunisian butter (TTB) samples were placed in glass jars open to the atmosphere in temperature controlled ovens at 60°C, 100°C and 130°C (Memmert-GmbH+Co.KG, Germany) to perform stability tests. Instantaneous separation occurs (density phenomena) in heated TTB sample leading to two phases: an aqueous phase [containing protein, minerals, lactose…) and an oil phase (traditional Tunisian butter oil (TTBO)]. In addition, TTBO was thoroughly separated by centrifugation and filtration. Samples of TTBO was collected and used to perform heat stability. Peroxide value (PV), free fatty acid (FFA), coefficient extinctions, colour, viscosity, texture and fatty acid composition were measured at different periods of heating to evaluate the deterioration state of traditional Tunisian butter oil (TTBO).

AOCS (1997) official methods were used for the determination of the peroxide and acidity values (method number Cd 8-53 and Cd 3d-63, respectively).

ε_{232} and ε_{270} extinction coefficients were calculated from absorption at the corresponding waves length, with an UV spectrophotometre (UV mini 1240, UV-Vis SPECTROPHOTOMETER, Schimadzu, JAPAN), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

The CieLab coordinates (L*, a*, b*) were directly read with a spectrophotometre MS/Y-2500 (Hunterlab, In., Reston, VA, USA), calibrated with a white tile. Under the tristimulus colour coordinate system, the L* value is a measure of lightness and varies from −100 (black) to 100 (white), the a* value varies from −100 (green) to +100 (red), and the b* value varies from −100 (blue) to +100 (yellow).

Viscosity of the butter oil samples was followed at 37°C with a Stress Tech Rheologica Rheometre (Rheologica Instruments AB, Lund, Sweden) conducted with a steel cone-plate (C40/4) under a constant shear rate of 100/s.

Textural analyses were performed using uniaxial compression test (Texture analyser: LLOYD instruments, England). For each temperature (60°C, 100°C and 130°C) three samples of TTBO were taken at different heating time and stored at 4°C. All textural analyses were performed at solidified TTBO and at 4°C. A Textural Procedure Analysis was performed under the following conditions: cylindrical probe (diametre = 1 cm^2), compression speed of 40 mm/min and a compression rate of 2 cm. textural test were performed at triplicate.

Fatty acid composition was performed using Gas Chromatography (GC). TTBO samples were warmed to 37°C immediately prior to analysis, then vortexed vigorously to achieve sample uniformity. Fatty acid methyl esters (FAMES) were prepared according to Maxwell and Warner (1983). Fifty microlitre of the butter oil were converted to methyl esters using 2 mL hexane and 200 μL of

2 N KOH in methanol. Aqueous fraction were recuperated and mixed with 200 µL of sodium acetate solution. After centrifugation, organic fraction was washed with 0.5 mL of distilled water. Fatty acid methyl esters (FAMES) were presented in the organic phase for GC analyses.

Gas Chromatography analyses were performed on a Shimadzu, GC 17 A chromatograph, equipped with a flame hydrogen ionisation detector and a capillary column (FFAD, 50 m × 0.32 mm × 0.5 µm, PERICHROM Sarl, France). The oven temperature was programmed as follow: the initial temperature (100°C) was raised to 150°C at a rate of 30°C/min and held at this temperature for 5 min, then increasing at 10°C/min to 190°C and held at this temperature for 14 min, and then increasing at 5°C/min to 255°C and held at this temperature for 10 min. The injector and detector temperatures were 255°C and 270°C, respectively. Nitrogen was the carrier gas. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analysed under the same conditions. Peak areas of triplicate injections were measured with a HP computing integrator. Results were expressed as w/w (%) total fatty acid (Sağdiç *et al.* 2004).

Statistical analysis

Values of different tests were expressed as the mean ± standard deviation (x ± SD). SPSS packet program for Windows was used for the statistical analysis. Significant differences between mean ($P < 0.05$) were determined by using a one-way ANOVA (Duncan's test).

RESULTS AND DISCUSSION

For the production of traditional butter, the physicochemical characteristics of fresh raw cow's milk samples obtained from local farm are presented in Table 1. The fresh raw milk sample gave suitable technological properties.

Table 1 Physicochemical characterisation of milk and traditional Tunisian butter

Component	*Milk*	*Raw butter*
Dry matter (%)	11.51 ± 0.02	70.60 ± 0.15
Fat (%)	3.45 ± 0.05	65.70 ± 2.16
Caseins (%)	2.67 ± 0.05	–
Lactose (%)	4.13 ± 0.04	1.01 ± 0.09
Ash (%)	0.82 ± 0.08	1.80 ± 0.01
Chlorides (%)	0.15 ± 0.02	0.29 ± 0.05
pH	6.7 ± 0.0	4.7 ± 0.0
Density	1.03 ± 0.00	0.92 ± 0.00
Titratable acidity (°Dornic)	16.00 ± 0.01	22.50 ± 0.01

All values given are means of three determinations.

Physical and chemical composition

Table 1 shows the physicochemical properties of the traditional Tunisian butter (TTB). The fat content of the TTB (65.70 ± 2.16%) was lower compared to the content reported with most common butters. This result could be explained by the low dry matter content in TTB (70.60 ± 0.15%). In deed, the traditional churning process (with 'chekwa') doesn't allow a good separation performance. In addition, mean Chloride content was less than those reported by Hayaloğlu (1999) for other butters. Mean pH value (4.7) and titratable acidity value (22.5°D) were similar to those reported by Filkensen (1987) and Sağdiç *et al.* (2004) respectively.

Heat stability

TTBO oxidation was followed by testing a TTBO sample at regular intervals using PV, ε_{232} and ε_{270} extinction coefficients, acidity, viscosity, texture, colour values determinations and fatty acids composition.

Figure 1 shows the change in TTBO PV values at 60°C, 100°C and 130°C. Hydroperoxide is the primary product of lipid oxidation; therefore, the determination of the peroxide value can be used as an oxidative index for the early stage of lipid oxidation (Ramadan and Mörel 2004). The initial stage of slow oxidation (the induction period) can be measured as the time required to reach an end point of oxidation corresponding either to a detectable level of rancidity (a defined peroxide value) or to a sudden change in oxidation rate (Fearon *et al.* 1998). The peroxide values obtained for TTBO stored at 60°C (Figure 1a) proceeded at a lower rate initially. This period of time is called the induction period (IP) or induction time (IT) (Nissiotis and Tasioula-Margari 2002). After that, the oxidation rate increases considerably. Absence of induction time and an increase of the slope were observed during heating at 100°C and 130°C (Figure 1b). Temperature had a significant effect on the formation of oxidation products in TTBO, with higher PV levels obtained at higher temperatures (Figure 1b) and low PV levels obtained at lower temperature (Figure 1a). Indeed, the induction period is 14 days for TTBO stored at 60°C with the peroxide values reaching approximately 1.495 mequiv.O_2/ Kg fat. However, for higher temperature (100°C and 130°C), absence of induction period was observed. The peroxide values of TTBO at 100°C and 130°C increased gradually during the whole storage period. After 8 hours (0.3 day) of heating at 100°C the measured value was 4.45 mequiv.O_2/Kg fat. In contrast, 4.95 mequiv.O_2/kg fat was identified after 2 hours of heating at 130°C. Consequently, TTBO shows the highest stability at lower heating temperature. Ndjouenkeu and Ngassoum (2002) reported that

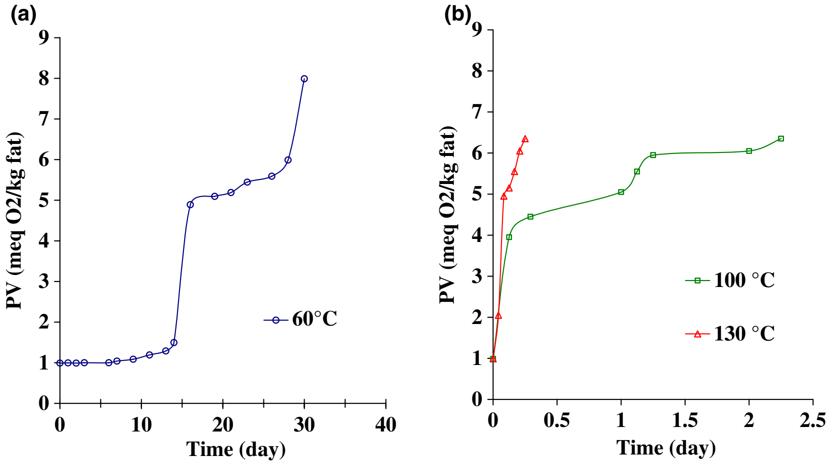

Figure 1 Peroxide value of TTBO during storage: (a) at 60°C; (b) at 100°C and 130°C.

PV values must normally increase during heating because of the oxidative activation. This result could be explained by the fact that reactions and mechanisms taking place in the degradation of TTBO was dependant on the heating treatment. Gertz *et al.* (2000) studied the oxidative stability of fats and oils heated at frying temperature (170°C) and reported that for temperatures <120°C, main oxidation reactions are: either hydrolysis or oxidation induced by moisture and atmospheric oxygen. Whereas, for temperature above 120°C, the most obvious and important reactions consisted in polymerisation. Whereas, Özkanli and Kaya (2005) studied the stability of pasteurised and nonpasteurised butter oil, and reported that stability of pasteurised butter oil can be attributed to pasteurisation. Thus, through pasteurisation and heat treatment of milk, microorganisms and enzyme activities, which could initiate lipid oxidation, are eliminated. Whereas heat treatment was not applied to butter oil produced from nonpasteurised milk in the melting process. This result leads the authors to suppose that butter oil is more susceptible to enzyme oxidation.

It can be seen that the traditional method for determining PV serves as an indicator of butter oil quality does not distinguish between various unsaturated fatty acids that undergo oxidation; It also does not supply information about the secondary oxidation products formed by hydro peroxide decomposition. However, it can generally be stated that the PV is an indicator of the primary level of oxidation.

The formation of hydro peroxides is accompanied by the generation of conjugated compound, measured by absorption at a wavelength of 232-234 nm (Guillén and Ruiz 2004). The hydro peroxide and the conjugated compound reflect the degree of primary products formation during lipid oxidation (Guillén and Ruiz 2004). Figure 2 illustrates evolution of absorption at 232 nm during the storage period at different temperatures: 60°C (Figure 2a), 100°C and 130°C (Figure 2b). Absortivity at 232 nm of TTBO at different storage temperatures evolves in the same way. The primary products of lipid oxidation of TTBO heated at 130°C shows a higher absorption at 232 nm compared to samples heated at 60°C and 100°C. This

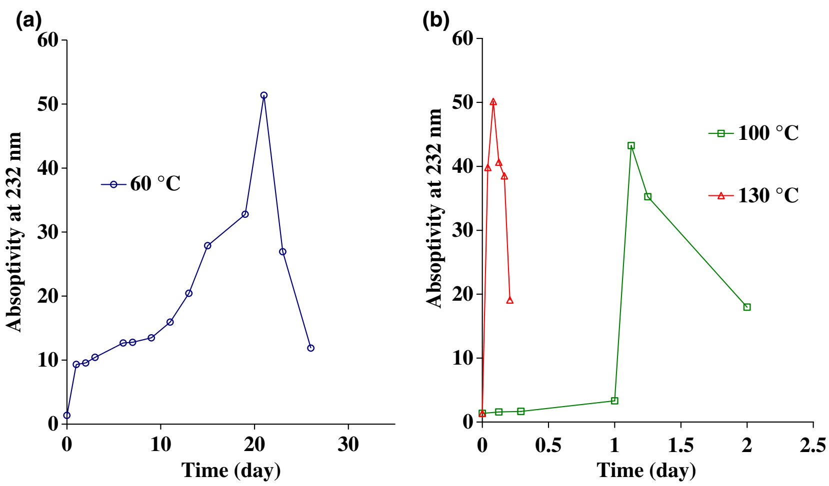

Figure 2 Absorptivity at 232 nm of TTBO during storage: (a) at 60°C; (b) at 100°C and 130°C.

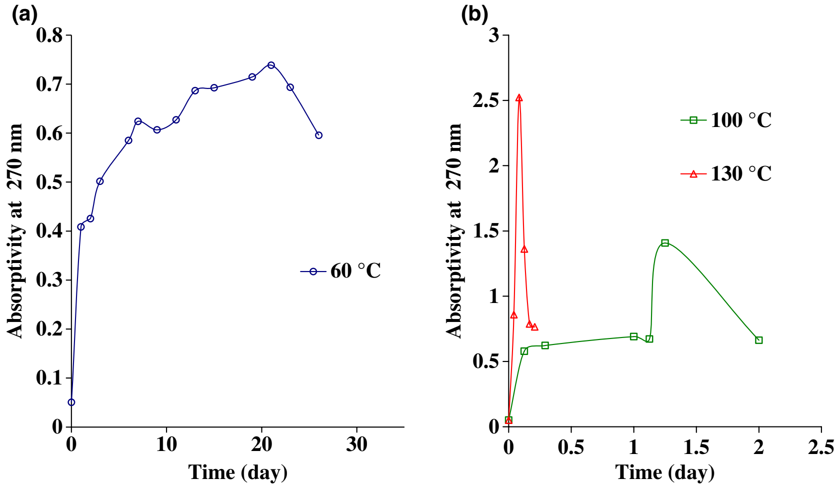

Figure 3 Absorptivity at 270 nm of TTBO during storage: (a) at 60°C, (b) at 100°C and 130°C.

result could explain the higher sensitivity of TTBO to temperature during storage, leading to a higher rate of the oxidation primary products content. The coincidence in the changes in absorption at 232 nm with hydro peroxide formation was observed. As can be seen, absorption at 232 nm increased immediately for samples heated at 130°C (Figure 2b). However, induction period was more important for samples stored at 60°C (Figure 2a) and 100°C (Figure 2b).

The primary products of oxidation are not stable and their degradation could lead to formation of oxidation secondary product with absorption occurring at 270 nm (Vieira and Regitano d'Arce 2001). These secondary products have a role in the break-up of the acyl group chains as was suggested by Guillén and Ruiz (2004). Figure 3 shows that specific extinction at 270 nm increased during heating storage at different temperature. It can be seen clearly that secondary compounds of oxidation were detected before reaching the oxidation induction time for the TTBO.

Figure 4 illustrate evolution of TTBO acidity vs storage time at 60°C, 100°C and 130°C. Acid value of TTBO remains relatively lower and constant (stable) during 14 days of heating at 60°C (Figure 4a). This means that the TTBO hydrolysis is not sufficient to compensate or to even increase the acid functions blocked by polymerisation (Ndjouenkeu and Ngassoum 2002). Acidity of samples heated at 60°C exhibit a low increasing. However during heating treatment at 100°C and 130°C, acidity of TTBO increases immediately. In fact, acidity reaches 2.8 after 30 days of heating at 60°C, 2.79 after only 2 days heating at 100°C and 2.9 after 5 hours heating at 130°C. The acid values increased as the storage time and temperature increased. This could be attributed to the initiation of (Tunisian traditional butter oil) TTBO degradation with a high heating treatment. Indeed, butter oil undergoes lipolysis releasing free fatty acids and increasing acidity.

Table 2 presents free fatty acid profiles of TTBO at different time of heating at 60°C, 100°C and 130°C. Nontreated TTBO were characterised by the presence of four major fatty acids [palmitic ($C_{16:0}$), stearic ($C_{18:0}$), myristic ($C_{14:0}$), and oleic ($C_{18:1}$) acids]. Palmitic acid was the major fatty

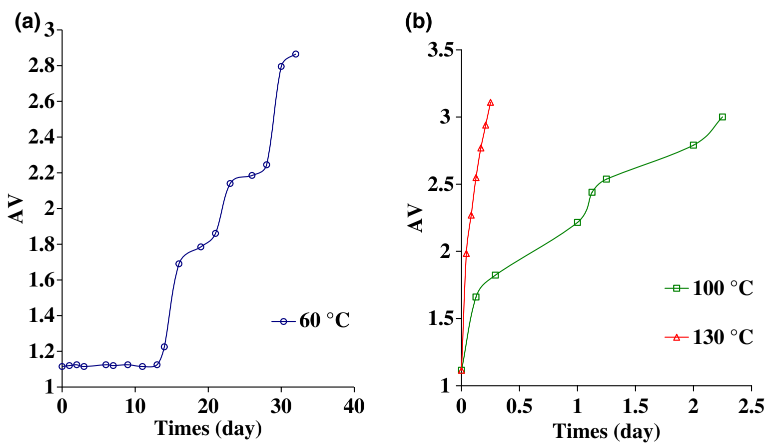

Figure 4 Acidity of TTBO during storage: at 60°C, (b) at 100°C and 130°C.

Table 2 Fatty acid composition (%) of traditional Tunisian butter oil produced from spontaneous fermentation of cows' milk and stored at different temperature (60°C, 100°C and 130°C)

Fatty acid	TTBO before heating	*After 360 hours of heating at 60°C*	*After 30 hours of heating at 100°C*	*After 3 hours of heating at 130°C*	*After 720 hours of heating at 60°C*	*After 60 hours of heating at 100°C*	*After 6 hours of heating at 130°C*
$C_{4:0}$	2.01 ± 0.20^{a}	1.98 ± 0.20^{a}	2.30 ± 0.13^{a}	2.11 ± 0.15^{a}	1.98 ± 0.13^{a}	2.02 ± 0.21^{a}	2.31 ± 0.18^{a}
$C_{6:0}$	1.20 ± 0.11^{a}	1.22 ± 0.12^{a}	1.41 ± 0.17^{b}	1.36 ± 0.14^{b}	1.21 ± 0.11^{a}	1.27 ± 0.12^{a}	1.46 ± 0.15^{b}
$C_{8:0}$	2.80 ± 0.22^{a}	2.93 ± 0.21^{a}	3.26 ± 0.14^{a}	3.30 ± 0.19^{a}	2.90 ± 0.12^{a}	3.03 ± 0.23^{a}	3.41 ± 0.11^{a}
$C_{10:0}$	0.31 ± 0.13^{a}	0.31 ± 0.24^{a}	0.33 ± 0.22^{a}	0.34 ± 0.16^{a}	0.28 ± 0.14^{a}	0.32 ± 0.22^{a}	0.34 ± 0.21^{a}
$C_{12:0}$	3.30 ± 0.23^{a}	3.53 ± 0.21^{a}	3.79 ± 0.22^{a}	3.96 ± 0.23^{a}	3.44 ± 0.25^{a}	3.60 ± 0.23^{a}	4.00 ± 0.24^{b}
$C_{14:0}$	11.38 ± 0.40^{a}	12.53 ± 0.33^{b}	12.83 ± 0.32^{b,c}	12.98 ± 0.36^{b}	11.74 ± 0.28^{b}	12.70 ± 0.31^{b}	13.05 ± 0.30^{c}
$C_{14:1}$	1.14 ± 0.10^{a}	1.21 ± 0.11^{a}	1.21 ± 0.10^{a}	1.21 ± 0.13^{a}	1.08 ± 0.14^{a}	1.18 ± 0.11^{a}	1.19 ± 0.12^{a}
$C_{16:0}$	32.04 ± 0.61^{a}	36.31 ± 0.65^{b}	34.37 ± 0.60^{b}	34.7 ± 0.61^{b}	34.61 ± 0.59^{b}	34.62 ± 0.57^{b}	34.86 ± 0.61^{b}
$C_{16:1}$	1.92 ± 0.09^{a}	2.15 ± 0.10^{a}	2.08 ± 0.12^{a}	2.00 ± 0.11^{a}	2.28 ± 0.14^{a}	2.10 ± 0.10^{a}	2.01 ± 0.11^{a}
$C_{18:0}$	18.80 ± 0.54^{a}	9.06 ± 0.56^{b}	9.92 ± 0.49^{b}	9.31 ± 0.43^{b}	9.82 ± 0.40^{b}	9.86 ± 0.39^{b}	9.35 ± 0.51^{b}
$C_{18:1}$	21.62 ± 0.42^{a}	24.73 ± 0.43^{b}	24.58 ± 0.46^{b}	24.33 ± 0.40^{b}	26.88 ± 0.41^{b}	25.17 ± 0.42^{b}	24.14 ± 0.40^{b}
$C_{18:2}$	2.41 ± 0.10^{a}	2.86 ± 0.11^{a}	2.82 ± 0.14^{a}	3.31 ± 0.20^{a}	2.51 ± 0.11^{a}	3.06 ± 0.14^{a}	2.76 ± 0.10^{a}
Total	98.93	98.82	98.90	98.92	98.73	98.93	98.88
Saturated (S)	71.84	67.87	68.21	68.06	65.98	67.42	68.78
Unsaturated (U)	27.09	30.95	30.69	30.85	32.75	31.51	30.10
U/S	0.38	0.46	0.45	0.45	0.49	0.47	0.44

Data are the mean of three measurements. Different letters in the same line indicate significant differences ($P < 0.05$).

acid found (32.04%) in TTBO. These results are in agreement with previous studies on different butter oil produced from cow's milk. Indeed, Glew *et al.* (1999) reported that saturated fatty acid level in 'Fulani butter oil' made from cow's milk was 53.3% and the major fatty acid was palmitic acid (30.2%). Whereas, percentage of saturated fatty acid in traditional Turkish butter was 67.06% and palmitic acid was the major one (33.72%) (Sağdic *et al.* 2004). In addition, Fatouh *et al.* (2007) reported that saturated fatty acid rate in butter oil made from buffalo milk was 70.72% and palmitic acid was also the major fatty acid (31.89%).

Butter oil made from milk of other animal species was studies (Sağdiç *et al.* 2004; Özkanlı and Kaya 2005). Özkanli and Kaya (2005) found a lower saturated fatty acid level (59.13%) and the major fatty acid was oleic acid (31.08%) in butter produced from sheep's milk. Sağdiç *et al.* (2004) reported that percentage of saturated fatty acid was 73.88% and 69.10% in butter oil made from Goats' and Ewes' milk, respectively. These authors reported too that palmitic acid was the major one for the two butter oils.

Milk fatty acid composition depends on several factors as animal species and feed, climate and the environmental conditions. TTBO showed higher saturated fatty acid content (especially stearic acid) than butter oil produced from sheep's milk (Özkanlı and Kaya 2005).

Changes were observed in fatty acid composition of traditional Tunisian butter oil (TTBO) during heat treatment. Indeed, a decrease in the relative percentages (%) of stearic acid (~50%) and an increase in the percentage of palmitic ($C_{16:0}$), myristic ($C_{14:0}$) and oleic ($C_{18:1}$) acids were detected. These changes in the fatty acid composition could be attributed to the degradation of fat matter under heating and oxidation. Özkanli and Kaya (2005) reported that fatty acid composition of oil butter made from sheeps' milk varies slightly under heating treatment. Özkanli and Kaya (2005) attribute this stability to temperature effect which inhibits lipolitic activity.

CieLab coordinates (L*, a*, b*) of the TTBO during oxidation were given in Figure 5. The initial colour (t = 0 h) of TTBO was reported to be yellow, and this is due to its richness in yellow pigments (carotenoids). Figures 5a,b show that heating gives immediately a considerable increase in L* parameter at a rate of ~ 50% from the initial value. Furthermore, b* (Figures 5c,d) values increase at the beginning of the heating at a rate of ~60% from the initial rate, and then decrease during treatment. However, what was observed for a* (Figures 5e,f) parameter was different: a decrease in values was observed at the beginning of heating, then values remained practically constant and finally increased considerably at the end of the treatment. This colour change (darkness and green-brown colour) was essentially marked by the high loss of yellow colour and then of yellow pigments, essentially β-carotenes, beyond the oxidation induction period. On the other hand, the colour formation in traditional Tunisian butter oil (TTBO), during heating processes, could be attributed to both nonenzymatic browning and phospholipids degradation during heating (Husain

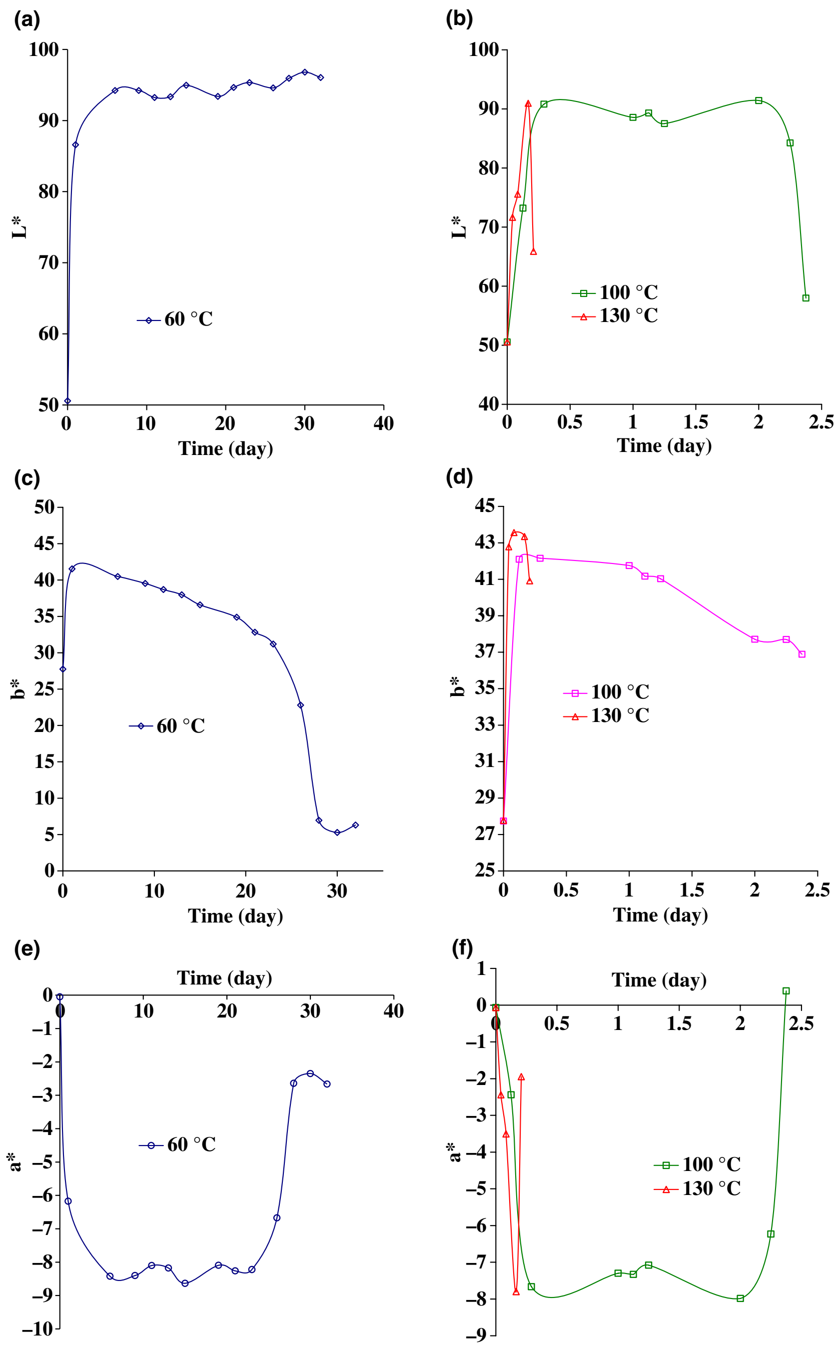

Figure 5 CieLab coordinates (L*, a*, b*) of TTBO during storage: (a) L* at 60°C, (b) L* at 100°C and 130°C, (c) b* at 60°C, (d) b* at 100°C and 130°C, (e) a* at 60°C, (f) a* at 100°C and 130°C.

et al. 1986). Nonenzymatic browning is favoured by heat treatment and includes a wide number of reactions such as Maillard reaction, caramilisation and chemical oxidation of phenols (Manzocco *et al.* 2001). TTBO could contain compound which would result in formation of Maillard reaction products (MRPs). In fact, TTBO could contain a trace of lactose and milk protein depends on the separation operation between aqueous and oil phases. Thus, colour formation during heat treatment is partly due to the formation of coloured MRPs. These MRPs correspond to the compound of low-molecular-weight and melanoidins with high-molecular-weight (Ames 1992).

At 60°C and 100°C, L* and b* parameters remain constant at the beginning of the experiment. This result, not being the case for a temperature of 130°C, could be explained by the effect of the oxidation reaction, induced essentially by atmospheric oxygen at lower heat temperature (60°C and 100°C). Whereas for temperature above 120°C, oxidative colour change could be essentially attributed to the polymerisation reactions induced by high treatment temperature.

Table 3 shows viscosity value, cohesiveness, springiness and hardness of TTBO after different time of heating at 60°C, 100°C and 130°C. The low initial viscosity of TTBO (5.85 mPa.s) could

Table 3 Viscosity, cohesiveness, springiness, adhesiveness and firmness coefficients of Tunisian traditional butter oil produced from spontaneous fermentation of cows' milk and stored at different temperature (60°C, 100°C and 130°C)

Textural parameters	*TTBO before heating*	*After 360 hours of heating at 60°C*	*After 30 hours of heating at 100°C*	*After 3 hours of heating at 130°C*	*After 720 hours of heating at 60°C*	*After 60 hours of heating at 100°C*	*After 6 hours of heating at 130°C*
Viscosity (mPa/s)	5.85 ± 0.90^{a}	2.07 ± 0.60^{b}	1.19 ± 0.40^{b}	9.22 ± 1.2^{c}	1.2 ± 0.31^{b}	1.12 ± 0.10^{b}	$6.33 \pm 0.85^{a,c}$
Cohesiveness	0.13 ± 0.10^{a}	0.21 ± 0.12^{b}	0.21 ± 0.22^{b}	0.29 ± 0.21^{b}	0.25 ± 0.24^{b}	0.28 ± 0.13^{b}	0.25 ± 0.12^{b}
Springiness (mm)	4.99 ± 0.22^{a}	4.93 ± 0.33^{a}	4.90 ± 0.32^{a}	4.98 ± 0.33^{a}	4.96 ± 0.31^{a}	4.88 ± 0.32^{a}	4.97 ± 0.35^{a}
Hardness (N)	101.87 ± 5.12^{a}	63.28 ± 2.15^{b}	47.29 ± 4.10^{b}	175.47 ± 6.22^{c}	56.39 ± 4.11^{b}	46.99 ± 4.50^{b}	42.96 ± 3.81^{b}

Data are the mean of three measurements. Different letters in the same line indicate significant differences ($P < 0.05$).

be explained by the relatively level count of mono-unsaturated and polyunsaturated fatty acids as previously shown (Table 2) and by the presence of high medium and short-chain fatty acids content. These results reinforce the finding of Geller and Goodrum (2000), concerning the existence of a strong relationship between fatty acid chain length and viscosity. During heat stability tests, viscosity and hardness of TTBO decreases, then remains constant till the end of the oxidation process, except for the sample stored at 130°C for about 3 hours. In addition, during storage cohesiveness increases then remains constant till the end of oxidation process. Initially, butter seemed to be harder and then became softer after heat treatment at 60°C and 100°C. These results could be explained by the change in the fatty acid composition. Indeed, the decrease of saturated fatty rate was compensated by the increase of the unsaturated one during the heat stability experiments (Table 2).

An increase in viscosity and hardness value of traditional butter was observed after 3 hours heating at 130°C. This significant increase could be attributed to the polymerisation reaction and the formation of high-molecular-weight compounds including carbon–carbon bonds and carbon–oxygen–carbon bridges between fatty acids (Stevenson *et al.* 1984).

Spreadability of the butter could be evaluated by both cohesiveness and hardness values. However springiness is a characteristic for the materials' elasticity. Table 3 shows that springiness' value during heat stability test, remains constant for all storage temperatures. These results could be explained by the absence of milk proteins, which are responsible of the elasticity characteristic, in dairy products (Sandoval-Castilla *et al.* 2004).

CONCLUSION

To conclude, the present study has allowed physico-chemical characterisation and heat stability evaluation of Tunisian traditional butter (TTB). Fatty acids compositions were also determined. Predominant fatty acids in Tunisian traditional butter oil (TTBO) are: myristic, palmitic and oleic acids. TTBO Heat stability tests show that induction period was 14 days for samples stored at 60°C and absence of this induction period for samples stored at 100°C and 130°C. Change in colour parameters and texture characteristics were observed depending on storage temperature. Indeed, TTBO stored at 130°C exhibit a change in colour parameters and in viscosity and hardness values.

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