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Traditional Tunisian butter: Physicochemical and microbial characteristics and storage stability of the oil fraction

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ABSTRACT

Physicochemical and microbiological characteristics, fatty acid composition and thermal stability of traditional Tunisian butter (TTB) were studied. Changes in microbiological and physicochemical parameters were monitored during storage at 4 and 10 °C. The physicochemical characterisation shows a fat level lower than 80% and a high value of water activity. The content of saturated fatty acid was higher (71.84%) than the unsaturated one (27.09%). The major fatty acids of butter samples were myristic, palmitic, stearic and oleic acids. During storage at 4 and 10 °C, the pH decreased and the titratable acidity increased. Counts of lactic acid bacteria exhibited relatively small changes upon storage, at 4 and 10 °C, whereas yeasts and moulds' counts increased irrespective of storage temperature. Effect of heating on some quality characteristics (absorption at 232 and 270 nm, peroxide value, free fatty acid content, viscosity, texture, colour and fatty acid composition) of traditional Tunisian butter oil (TTBO) has been investigated at 60 °C. Results show that TTBO was resistant to oxidation. All these characteristics consolidate the incorporation of TTB on food formulation.

1. Introduction

Fermentation is one of the oldest food processing technologies in the world, which has been adopted across generations (Kebede, Viljoen, Gadaga, Narvhus, & Lourens-Hattingh, 2007). According to Walshe, Grindle, Nell, and Bachmann (1991), most of the knowledge about fermentation has not been documented. Thus, it is in danger of being lost since technologies are evolving and families are forgetting about traditional food preservation practices.

Several studies have documented the quality and diversity of traditional fermented dairy product, including natural fermented milk (Abdelgadir, Ahmed, & Dirar, 1998; Benkerroum & Tamine, 2004; El-Gendy, 1983; Gran, Wetlesen, Mutukumira, & Narvhus, 2002; Tantaoui-Elaraki & El Marrakchi, 1987).

A variety of fermented milk products are prepared in Tunisia, mostly by rural women that usually use their traditional knowledge of fermentation in the bio-preservation of milk for storage and future consumption. Two products result from the spontaneous fermentation of milk in the South of Tunisia: the first is rich in proteins and is locally called "Leben". The second is rich in fat and is locally called "traditional butter" or "Zebda beldi". This traditional butter is widely produced and consumed, and is still a popular Tunisian product. Churning is the most important step for 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 governed by such factors as cream ripening, the manufacturing process, good hygienic practice (GHP) when processing and handling, the storage temperature, and type of animal feeding (Fearon, Mayne, & Charlton, 1998). Despite the application of preventive measures (refrigeration and GHP) during its production and its distribution, TTB has a limited shelf-life. During its distribution TTB must be stored at 4 °C but in the practice some deviations in which the temperature could reach 10 and 12 °C were noted.

Butter oil (called sman) is obtained by heating traditional butter and separating fat (butter oil) from milk serum. A major portion of butter oil is utilized for culinary cooking (Özkanli & Kaya, 2005). Deterioration (lipolysis and oxidation) of butter oil due to several factors causes flavour impairment, lowers nutritional quality, and creates serious problems for storage stability. The onset of rancidity in butter may be usually 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 fatty acids (Joshi & Thakar, 1994; Muir, 1996). 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 butter oil (Amr, 1991).

In this paper physicochemical and microbiological characteristics and microstructure of TTB were presented. Changes in selected microbiological and physicochemical parameters during storage at different temperature were monitored and the relationship between

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physicochemical parameters and microbiological growth was discussed. The effect of accelerated oxidative conditions (60 °C) on TTB oil was studied to evaluate the potential use of traditional Tunisian butter as an ingredient for food preparation.

2. Materials and methods

2.1. Raw butter production

Fresh raw cows' milk (Holstein breed) samples were obtained from a local farm in the South area of Tunisia (Sfax). Samples of cows' milk were collected, kept refrigerated and transported to our laboratory within 24 h. Each sample was a pooled skim milk taken from 20 to 25 animals for bovine milk.

Raw milk was left at room temperature (25 ± 2 °C) until coagulation which takes up after ~18 h. During the gelation step, the product is called "rayeb". By churning, the "rayeb" is separated into an aqueous fraction (protein, lactose, mineral…) giving "*Leben*" and a fat-rich fraction called raw butter "*Zebda beldi*" (TTB). Traditionally, churning takes place in a skin bag called "*Checoua*" which is manufactured from a goat in one piece. The churning is achieved after hanging the "*Checoua*" which is filled with "rayeb" and vigorously shaking it back and forth till the coalescence of the fat globules. The end of churning is discerned by the sound of the butter lumps when shaking. 10% (v/v) of warm water (40 °C) is added towards the beginning of the churning period to enhance the coalescing of the fat globules and, thus, to increase the yield of butter (*Zebda beldi*).

TTB samples were produced according to the traditional method. 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. Samples were stored at 4 °C and 10 °C to perform storage stability tests during ~6 weeks. Samples were used to produce butter oil and perform heat stability tests.

2.2. Microscopic study

2.2.1. Optical microscopy

Emulsion evolution versus churning time was estimated by optical microscopy observations. Emulsion samples were spread between a slide and cover glass and observed under a contrast phase microscope, Olympus B X 50 (Olympus Optical Co., Tokyo, Japan) equipped with a video system (Attia, Kherouatou, Fakhfakh, Khorchani, & Trigui, 2000).

2.2.2. Scanning electron microscopy (SEM)

Samples of TTB were prepared according to the method described by Attia, Bennasar, and De La Fuente (1991), and were observed under a scanning electron microscope Philips XL30 (Philips, France) after drying to CO_2 critical point using a Baltec CPD 030 apparatus and coating with gold using a Baltec MED 20 apparatus (Balzers Union, Balzers, Germany).

2.3. Analytical methods

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation ($X \pm SD$).

2.3.1. Physicochemical analysis

Total nitrogen (TN) was achieved using the Kjeldahl method (AFNOR, 1993) with a Büchi 325 apparatus (Büchi, Flawil, Switzerland). Total solids, ash, lactose and fat contents were determined according to standard methods (AFNOR, 1993). NaCl (chlorides) was obtained using the method of the International Dairy Federation (1969). Water activity was measured via the Novasina apparatus (Aw Sprint TH-500). Titratable acidity, expressed in Dornic degrees, was determined by titration of sample with N/9 sodium hydroxide to a pink endpoint using phenolphthalein as indicator (AFNOR, 1993). The pH was determined using a pH meter (744 pH Meter Metrohm) (AFNOR, 1970).

2.3.2. Microbiology counts

Sample preparation and decimal dilutions were made according to IDF (1992). Total bacteria were enumerated in plate count agar (PCA, Difco) after incubation at 30 °C for 48 h. MRS medium (Difco) was used for counting lactic acid bacteria (LAB) at 30 °C for 48 h (Garrotte, Abraham, & De Antoni, 2001). Yeasts and moulds were enumerated in Sabouraud dextrose agar after incubation at 30 °C for 3 days (Tantaoui-Elaraki, Berrada, El Marrakchi, & Berramou, 1983).

2.3.3. Oxidative stability

Samples of TTB were kept in glass jars, opened to the atmosphere with a controlled temperature (60 °C) in a drying oven (Memmert-GmbH + Co. KG, Germany) to perform stability tests. The temperature of 60 °C is used as a rapid method to simulate the storage in real conditions. During heating process, the water phase of butter is separated from the oil phase. The oil fraction was recuperated and called traditional Tunisian butter oil (TTBO). Peroxide value (PV), free fatty acid (FFA), coefficient extinctions, fatty acid composition, colour, viscosity and texture were measured at different times during heating to evaluate the deterioration of butter.

PV and FFA expressed as oleic acid determinations were carried out according to the AOCS Official Methods numbers 965.33 and 940.28, respectively (AOAC, 1995).

ε_{232} and ε_{270} extinction coefficients were calculated from absorption at the corresponding wavelength, with a UV spectrophotometer (UV mini 1240, UV–Vis spectrophotometer, Japan), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Fatty acid composition: butter 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). 50 μl of the butter were converted to methyl esters using 2 ml hexane and 200 μl of 2 N KOH in methanol. Aqueous fractions were recuperated and mixed with 200 μl of sodium acetate solution. After centrifugation, the 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.

GC analyses were performed on a Shimadzu, GC 17 A chromatograph, equipped with a flame hydrogen ionization detector and a capillary column (FFAD, 50 m × 0.32 mm × 0.5 μm, PERICHROM Sarl, France). The oven temperature was programmed as follows: 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 increased at 10 °C/min to 190 °C and held at this temperature for 14 min, and then increased at 5 °C/min to 255 °C and held at this temperature for 10 min. The injector and detector temperatures were 255 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 an HP computing integrator. Results were expressed as w/w (%) total fatty acid (Sağdiç, Dönmez, & Demirci, 2004).

The CieLab coordinates (L^*, a^*, b^*) were directly read with a spectrophotometer 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 Rheometer (Rheologica Instruments AB, Lund, Sweden) conducted with a steel cone-plate (C40/4) under a constant shear rate of 100 s^{-1}.

Textural analyses were performed using uni-axial compression test (Texture analyser: LLOYD instruments, England). Three samples of TTBO were taken at different heating time and were 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 (diameter = 1 cm^2), compression speed of 40 mm min^{-1} and a compression rate of 2 cm. Textural test was performed at triplicate.

2.3.4. Statistical analysis

All analytical determinations were performed at least in triplicate. Values of different tests were expressed as the mean ± standard deviation ($X \pm SD$). 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).

3. Results and discussion

Physicochemical characteristics of fresh raw cow's milk sample used for butter production are presented in Table 1.

3.1. Emulsion characterisation

Fig. 1 illustrates the emulsion state evolution versus churning time. Fig. 1(a) represents the microscopy observation of a sample churned during 15 min. This photo shows the existence of an aqueous continuous phase, in which are scattered spherical shape droplets of various diameters. Fig. 1(b) (churning during 30 min) shows that the number of oil droplets decreased whereas the diameter increased. This variation in the size of droplets can be explained by the process of coalescence. Indeed, during coalescence, two droplets could form a single larger droplet (Rousseau, 2000). In spite of this evolution, the sample obtained after 30 min of churning time was still a cream (oil-in-water emulsion). With the increase of the churning time, coalescence can lead to phase inversion where an oil-in-water emulsion becomes a water-in-oil emulsion (Boode, Walstra, & DeGroot-Mostert, 1993). Fig. 1(c) shows that at least 40 min churning time is necessary to obtain a water-in-oil emulsion (TTB) as that obtained for industrial butter (Fig. 1(d)). In order to better study the microstructure of TTB, scanning electron micrograph of TTB is shown in Fig. 2. SEM observation shows a water-in-oil emulsions in which a continuous fat matrix surrounds the dispersed water droplets and then confirmed that 40 min churning time was necessary to obtain TTB. According to these results, samples of TTB used during this work were churned during at least 40 min.

Table 1
Average composition of raw cow's milk and traditional Tunisian butter.[a]

Parameters	Milk	Raw butter
Dry matter (%)	11.51 ± 0.02	70.60 ± 0.15
Total nitrogen (%)	3.34 ± 0.06	1.10 ± 0.10
Caseins (%)	2.67 ± 0.05	–
Fat (%)	3.45 ± 0.05	65.70 ± 2.16
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
Water activity	0.98 ± 0.00	0.79 ± 0.01
pH	6.70 ± 0.02	4.70 ± 0.05
Density	1.03 ± 0.00	0.92 ± 0.00
Titratable acidity (°D)	16.0 ± 0.01	22.5 ± 0.01

[a] Mean ± standard deviation.

3.2. Physicochemical and microbiological properties

Table 1 shows physicochemical properties of TTB. Fat content (65.70 ± 2.16%) was lower compared to fat rate reported in the most common butters. In addition, mean chlorides' content was lower than those reported in earlier studies on butters (Hayaloğlu, 1999). Water activity (a_w) of the TTB was relatively high (0.79) which explains its short self-life. Mean pH value (4.7) and titratable acidity value (22.5 °D) were similar to that reported by Filkensen (1987) and Sağdiç et al. (2004), respectively.

Microbial counts (log cfu/g) of TTB were 4.70 ± 0.05 and 4.81 ± 0.01 for lactic acid bacteria (LAB) and yeasts and moulds, respectively. These values were higher than that reported by Sağdiç et al. (2004) in traditional Turkish butter. Undesirable bacteria were absent in TTB. Benkerroum et al. (2002) reported that LAB inhibit the growth of undesirable bacteria by producing several antibacterial substances.

3.3. Physicochemical and microbial stability versus storage time

Fig. 3 shows the change in pH and titratable acidity evolution respectively of TTB stored at 4 and 10 °C. During the first 12 days, pH of TTB stored at 4 °C was ∼4.7, then it decreased to ∼4.45. However, pH value of the sample storage at 10 °C decreased immediately to 4.4. Titratable acidity of samples stored at 4 °C and 10 °C increased slightly during the first 12 days, then increased from 24.3 °D to 36 °D and from 27 °D to 39.6 °D after 20 stored days at 4 °C and 10 °C, respectively. After this period, titratable acidity on TTB stored at 4 °C was maintained constant during 13 days, and then increased considerably. However titratable acidity of TTB stored at 10 °C increased significantly ($p < 0.05$). These results show that storage temperature conditions have a significant effect on physicochemical stability of TTB. Physicochemical change observed during storage is probably due to the number and/or metabolic activity of acid-producing microorganisms as reported by Al-Kadamany, Khattar, Haddad, and Toufeili (2003).

LAB and yeast and moulds' growths during storage time were shown in Fig. 4. At 4 °C, LAB counts exhibited initially a lag phase (∼12 days), then manifested an exponential phase growth and decreased slowly afterwards. At 10 °C, the lag phase was absent in the evolution of LAB in TTB. LAB growth increased immediately for TTB stored at 10 °C. Evolution of LAB bacteria counts, in traditional butter stored at 4 °C and 10 °C, is not only due to the inhibitory effects of relatively increased acidity on growth of butter culture as it is reported by Rohm, Lechner, and Lehner (1990), but it is also due to the fact that LAB are exigent microorganisms towards nutritional substances (Lee, Lee, Kim, Moon, & Park, 2001).

At 4 °C, yeasts and moulds exhibited initially a lag phase (∼12 days), with counts increasing to a maximum at the twenty fifth day of storage and sharply decreasing afterwards. At 10 °C, these strains increased sharply to a maximum of 9.76 log cfu/ml after 11 days of storage and then decreased markedly. As soon as the number of lactic bacteria increased yeasts and moulds increased. A symbiosis between yeasts and lactic acid bacteria has been suggested: whereby the bacteria provide the acidic condition favourable for the growth of yeasts. The latter provide vitamins and other growth factors to the bacteria (Gobbetti, Corsetti, & Rossi, 1994). The viable population of TTB microorganisms increased initially after the butter manufacture, and then decreased during prolonged refrigerated storage of the product. This result suggested that microbial count can be affected by the storage temperature.

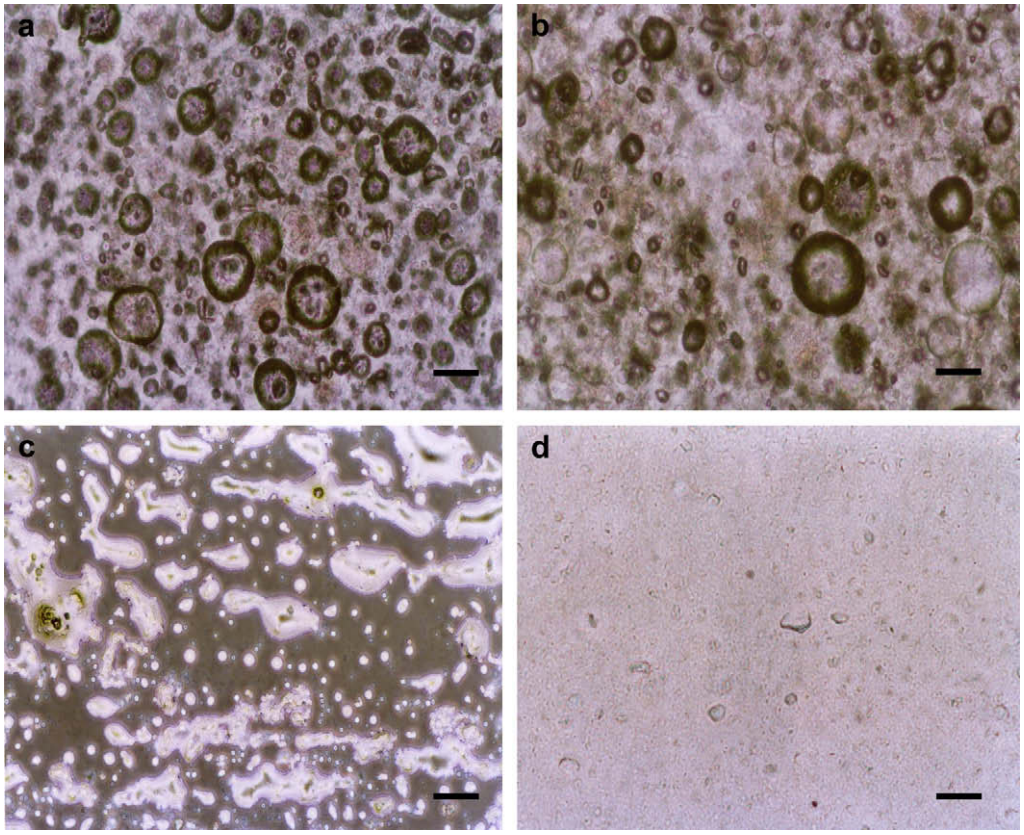

Fig. 1. Emulsion state versus churning time. (a) Sample churned during 15 min, (b) sample churned during 30 min, (c) sample churned during 40 min (traditional butter) and (d) industrial butter. Magnification is 50 and scale bars = 7 μm.

3.4. Oxidative stability tests of TTBO (heating at 60 °C)

Quality indexes such as specific extinctions at 232 nm and 270 nm, PV, acidity, colour, viscosity, texture and fatty acids composition were followed up in order to study the resistance of TTBO to oxidation phenomena using an accelerated procedure (oven test at 60 °C).

Table 2 presents free fatty acid profiles of TTBO at different time of heating at 60 °C. Non-treated TTBO was 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 acid found (32.04%) in TTBO. These results are in agreement with previous studies on different butter oil produced from cow's milk. Glew, Okolo, Chuang, Huang, and VanderJagt (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%). Percentage of saturated fatty acid in traditional Turkish butter was

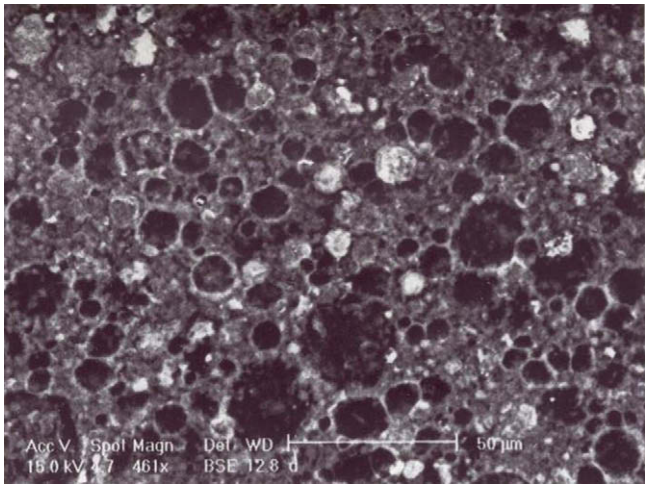

Fig. 2. SEM micrograph of traditional Tunisian butter produced from cow's milk.

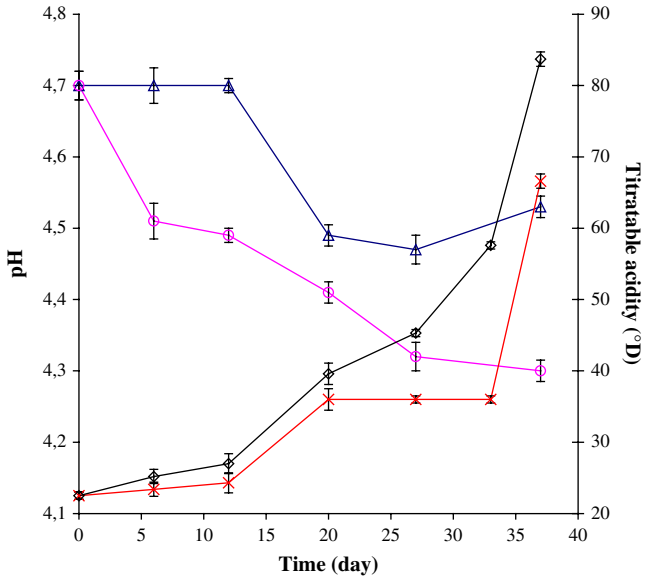

Fig. 3. Changes in pH and titratable acidity during storage of traditional Tunisian butter at 4 and 10 °C. (△): pH at 4 °C, (○): pH at 10 °C, (×): acidity at 4 °C, (◇): acidity at 10 °C.

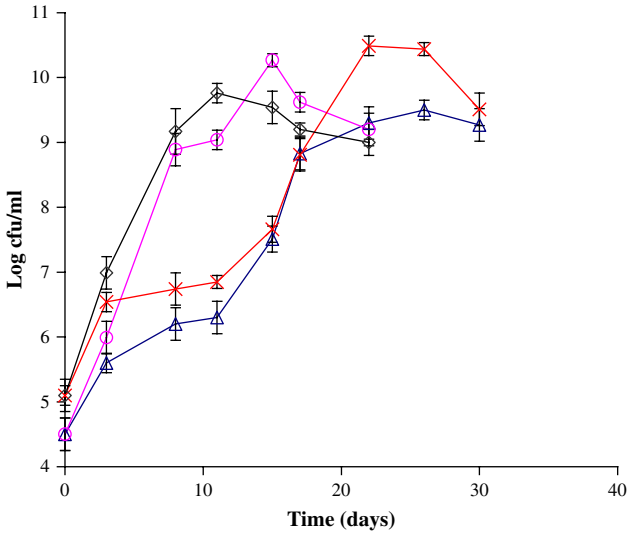

Fig. 4. Changes in viable counts of lactic acid bacteria, yeasts and moulds during storage of traditional Tunisian butter at 4 and 10 °C. (△): LAB at 4 °C, (○): LAB at 10 °C, (×): yeasts and moulds at 4 °C, (◇): yeasts and moulds at 10 °C.

67.06% and palmitic acid was the major one (33.72%) (Sağdiç et al., 2004). In addition, Fatouh, Mahran, El Gandour, and Singh (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 studied (Özkanli & Kaya, 2005; Sağdiç et al., 2004). Özkanli et al. (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 also that palmitic acid was the major one for the two butter oils.

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

Table 2
Fatty acid composition (%) of Tunisian traditional butter oil produced from spontaneous fermentation of cows' milk heated at 60 °C. Data are the mean of three measurements. Different letters in the same line indicate significant differences ($p < 0.05$).

Fatty acids	Initial (before heating at 60 °C)	Middle (after 360 h of heating at 60 °C)	Final (after 720 h of heating at 60 °C)
Saturated (S)	71.84 ± 0.30	67.87 ± 0.58	65.98 ± 0.67
$C_{4:0}$	2.01 ± 0.01^a	1.98 ± 0.20^a	1.98 ± 0.13^a
$C_{6:0}$	1.20 ± 0.01^a	1.22 ± 0.12^a	1.21 ± 0.11^a
$C_{8:0}$	2.80 ± 0.01^a	2.93 ± 0.21^a	2.90 ± 0.12^a
$C_{10:0}$	0.31 ± 0.00^a	0.31 ± 0.24^a	0.28 ± 0.14^a
$C_{12:0}$	3.30 ± 0.02^a	3.53 ± 0.21^a	3.44 ± 0.25^a
$C_{14:0}$	11.38 ± 0.06^a	12.53 ± 0.33^b	11.94 ± 0.28^b
$C_{16:0}$	32.04 ± 0.12^a	36.31 ± 0.65^b	34.61 ± 0.59^b
$C_{18:0}$	18.80 ± 0.31^a	9.06 ± 0.56^b	9.82 ± 0.40^b
Unsaturated (U)	27.09 ± 0.28	30.95 ± 0.46	32.75 ± 0.68
Monounsaturated	24.68 ± 0.43	28.09 ± 0.35	30.24 ± 0.50
$C_{14:1}$	1.14 ± 0.01^a	1.21 ± 0.11^a	1.08 ± 0.14^a
$C_{16:1}$	1.92 ± 0.01^a	2.15 ± 0.10^a	2.28 ± 0.14^b
$C_{18:1}$	21.62 ± 0.41^a	24.73 ± 0.43^b	26.88 ± 0.41^b
Polyunsaturated	2.41 ± 0.01	2.86 ± 0.11	2.51 ± 0.11
C18:2	2.41 ± 0.01^a	2.86 ± 0.11^a	2.51 ± 0.11^a
Total	98.93 ± 0.79	98.82 ± 0.51	98.73 ± 0.63
Others	1.07 ± 0.01	1.18 ± 0.12	1.27 ± 0.11
U/S	0.38	0.46	0.49

Changes were observed in fatty acid composition of TTBO during heat treatment. Indeed, a significant ($p < 0.05$) decrease in the relative percentages (%) of stearic acid (∼50%) and a significant increase ($p < 0.05$) in the % 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 et al. (2005) reported that fatty acid composition of oil butter made from sheep's milk varies slightly under heating treatment. They attribute this stability to temperature effect which inhibits lipolitic activity.

Fig. 5(a) shows the change in PV values of TTBO heated at 60 °C. Hydro peroxide 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 & Mörsel, 2004). The initial stage of slow oxidation (the induction period) can be measured as the time required to reach an endpoint of oxidation corresponding either to a level of detectable rancidity (a defined peroxide value) or to a sudden change in oxidation rate (Fearon et al., 1998). The peroxide values obtained for TTBO proceeded at a lower rate initially. This period of time is called the induction period (IP) or induction time (IT) (Nissiotis & Tasioula-Margari, 2002). TTBO induction time is ∼14 days under heating at 60 °C with peroxide values reaching approximately 1.495 mequiv O_2/kg fat. FFA of TTBO (Fig. 5(a)) remains relatively lower and constant (stable) during 14 days of heating at 60 °C. These results suggested

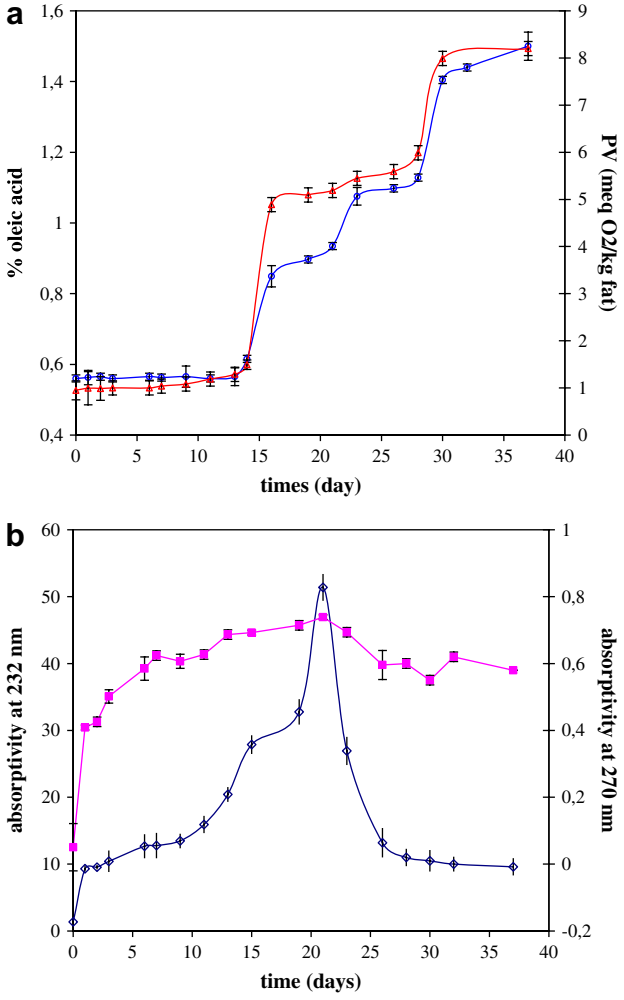

Fig. 5. Oxidative stability tests under heat treatment at 60 °C of traditional Tunisian butter oil. (a) Peroxide value (△), free fatty acids (FFA) content (○). (b): Absorptivity at 232 nm (◇), absorptivity at 270 nm (■).

that TTBO was resistant to oxidation which may be explained firstly because TTBO presented a low polyunsaturated fatty acid content (as previously shown (Table 2)), which is more sensitive to oxidation, and secondly by the presence of natural antioxidant.

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 compounds, measured by absorption at a wavelength of 232–234 nm (Guillén & Ruiz, 2004). The hydro peroxide and the conjugated compounds reflect the degree of primary products formation during lipid oxidation (Guillén & Ruiz, 2004). Fig. 5(b) illustrates the evolution of the absorption at 232 nm during heating at 60 °C. Formation of primary compounds of oxidation occurred initially at a lower rate. Specific extinction at 232 nm did not considerably change during ~14 days in an oven. Formation of primary compounds of oxidation reached its maximal value after 20 days of heating. After this period, specific extinction at 232 nm decreased continually to reach a value of ~10. This decrease could be explained by the instability of this primary compounds. Primary products of oxidation are not stable under heating and then their degradation could promote the formation of secondary products of oxidation that absorb at about 270 nm (Vieira & Regitano d'Arce, 2001). These secondary products have as a consequence a role in the break-up of the acyl group chains as was suggested by Guillén & Ruiz (2004). Fig. 5(b) shows that specific extinction at 270 nm increased slowly during heating storage. In fact, the extinction coefficient at 270 nm passed only from ~0.05 to ~0.70. This slight increase could also confirm that TTBO has a good resistance against heating and oxidation.

CieLab coordinates (L^*, a^*, b^*) of the TTBO during accelerated self-life procedure were given in Fig. 6. The initial colour seen in time $t = 0$ h of TTBO was reported to be yellow, and this is due to its richness in yellow pigments (carotenoids). Fig. 6 shows that heating gave immediately a considerable increase in L^* parameter at a rate of ~50%. Furthermore, b^* values increase at the beginning of the heating at a rate of ~60%, and then decrease during treatment. However, what was observed for a^* parameter was different: a decrease in values was observed at the beginning of heating, and

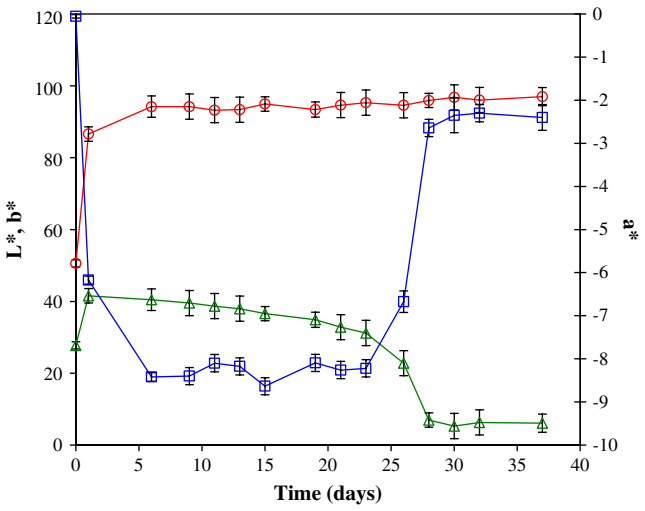

Fig. 6. CieLab coordinates (L^*, a^*, b^*) of traditional Tunisian butter during heating at 60 °C. (○): L^*, (△): b^*, (□): a*.

Table 3
Viscosity, cohesiveness, springiness, adhesiveness and firmness coefficients of Tunisian traditional butter oil produced from spontaneous fermentation of cows' milk heated at 60 °C. Data are the mean of three measurements. Different letters in the same line indicate significant differences ($p < 0.05$).

Textural parameters	Initial (before heating at 60 °C)	Middle (after 360 h of heating at 60 °C)	Final (after 720 h of heating at 60 °C)
Viscosity (mPa s^{-1})	5.85 ± 0.90^a	2.07 ± 0.60^b	1.2 ± 0.31^b
Cohesiveness	0.13 ± 0.10^a	0.21 ± 0.12^b	0.25 ± 0.24^b
Springiness (mm)	4.99 ± 0.22^a	4.93 ± 0.33^a	4.96 ± 0.31^a
Hardness (N)	101.87 ± 5.12^a	63.28 ± 2.15^b	56.39 ± 4.11^b

then values remained practically constant and finally increased considerably at the end of the treatment. This colour change (dark 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 change in TTBO, during heating processes, could be attributed to the phospholipids degradation during heating (Husain, Terao, & Matsushita, 1986). Several pigments are associated with these phospholipids.

It is worth noting that L^* and b^* parameters remained constant for an important period of time. This result could be explained by the effect of the oxidation, induced essentially by atmospheric oxygen at 60 °C.

Table 3 shows initial, middle and final viscosity value, cohesiveness, springiness and hardness versus time. The low initial viscosity of TTBO (~5.85mPa s) could be explained by the relatively low level count of monounsaturated and polyunsaturated fatty acids as previously shown (Table 2) and by the presence of high medium and short-chain fatty acids content. Droplet size and high water content of TTB could explain also this low viscosity value. 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 hardness and cohesiveness of butter oil decreased then remained practically constant till the end of the oxidation process. This result could also confirm the resistance of butter to oxidation, following its lower unsaturated fatty acids content as previously shown. It is known that viscosity decreases when molecular weight decreases (Besbes et al., 2005). Initially, butter seemed to be harder and then became softer after heat treatment at 60 °C. These results could be explained by the change in the fatty acid composition. In fact, the decrease of saturated fatty rate was compensated by the increase of the unsaturated one during the heat stability experiments (Table 2).

Spreadability of the butter could be evaluated by both cohesiveness and hardness values. However springiness is a characteristic of the materials' elasticity. Table 3 shows that springiness' value of the TTBO, during heat stability test, remained constant during oxidation process at 60 °C. These results could be explained by the absence of milk proteins, which are responsible of the elasticity characteristic in the dairy products (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004).

4. Conclusion

Samples of traditional Tunisian butter were produced according to the traditional method used in Tunisia. Physicochemical composition shows a high dry matter and water activity values. The predominant fatty acids are: myristic, palmitic, stearic and oleic acids.

Microbiological and physicochemical parameters were monitored during storage at 4 and 10 °C. Based on these results, we can conclude that the shelf-life of TTB stored at 4 °C could be expected to range of 12 days. In the contrast TTB stored at 10 °C could not be consumed after 1 or 2 days.

Accelerated shelf-life procedure (heat stability at 60 °C) shows that traditional butter oil was resistant to thermal treatment during a long period (~14 days). According to these specificities of TTBO, the value of this product as an ingredient in food may be justified.

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