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Effects of pH values on the properties of buffalo and cow butter-based low-fat spreads

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SUMMARY: The objective of this study was to characterize the effects of pH values (5, 5.5, 6, 6.5 and 7) on the properties of buffalo and cow butter-based low-fat spreads. Sensory evaluation of the samples decreased with an increase in pH values and during the storage periods. In addition, phase separation occurred with pH 6, 6.5 and 7. The differences in peroxide values and oil stability index among the samples compared to the control samples were slight, while peroxide values and oil stability index decreased during the storage periods. Changes in fatty acid composition among the pH treatments and during the storage periods were detected. Differences in solid fat contents among pH treatments separately and during the storage periods were negligible. A decline in the hardness and viscosity of the samples were accompanied by an increase in pH values, and the treatments had increased effects during the storage periods. Generally, an increase of pH values did not affect the melting profiles of the spreads. Additionally, changes between the melting profiles of buffalo and cow butter-based low-fat spreads were detected.

KEYWORDS: *Buffalo butter; Cow butter; Fatty acids composition; Low fat spreads; Melting behavior; Sensory evaluation; Viscosity*

RESUMEN: ***Efecto del pH en las propiedades de mantequillas para untar baja en grasa de búfalos y vacas***. El objetivo fue determinar los efectos del pH (5, 5.5, 6, 6.5 y 7) en las propiedades de mantequillas para untar bajas en grasa de búfalos y vacas. La puntuación sensorial de las muestras disminuyó con el aumento del pH y durante los períodos de almacenamiento, además, la separación de fases se produjo con pH de 6, 6,5 y 7. Se observaron diferencias en los valores de peróxido e índice de estabilidad de la grasa de las muestras en comparación con las muestras control, mientras que los valores de peróxido incrementaron, el índice de estabilidad de la grasa disminuyó durante los períodos de almacenamiento. Se observan cambios en la composición de ácidos grasos entre los tratamientos de pH y durante los períodos de almacenamiento. Las diferencias en el contenido de grasa sólida entre los tratamientos de pH por separado y durante los períodos de almacenamiento fueron no significativas. La disminución en la dureza y la viscosidad de las muestras fueron proporcionales al incremento del pH, y los tratamientos aumentan los efectos durante los períodos de almacenamiento. En general, un aumento de los valores de pH no afectó a los perfiles de fusión de los untables. Además, se observaron cambios entre los perfiles de fusión de los untables bajos en grasa a base de mantequilla búfalos y vacas.

PALABRAS CLAVE: *Comportamiento de fusión; Composición en ácidos grasos; Evaluación sensorial; Mantequilla de búfalo; Mantequilla de vaca; Untable bajo en grasa; Viscosidad*

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1. INTRODUCTION

In recent years scientists all over the world have come up with general nutritional recommendations which aim at reducing calories and tending towards healthier habits have resulted in the production of different types of low fat butter spreads with a fat content of 40%. This has increased market interest and drawn extensive attention for food technologists. The fat phase in low fat butter spread makes an important contribution to its physical properties, rheological measurements and chemical reactions as well as organoleptic properties. The overall goals are to inhibit water droplet aggregation and to make the product's process and shelf life stable, and to provide emulsions that break down easily and give good flavor release in the mouth (Mageean and Jones 1989).

The factors that have influenced low fat spreads can be generalized as follows: fat phase, stabilizers, emulsifiers, homogenization and aqueous phase. Such large reductions in fat content alter the nature of the emulsion structure and it is difficult to maintain the continuous fat nature of such products. In order to overcome this problem, stabilizers have to be added to immobilize the aqueous phase by increasing its viscosity. The most widely used aqueous phase stabilizers in low-fat spreads are milk proteins, alginates, starch derivatives and gelatin. In particular, gelatin is used in many formulations to provide the aqueous phase with a consistency and melting behavior close to those of the fat phase (Janssens and Muyldermans 1994).

Four types of such agents have been identified (Moran 1991). These are viscous (high levels of milk protein or high-molecular-weight polysaccharides), gelling (hydrocolloid agents used to gel the aqueous phase), phase-separating (with thermodynamically incompatible hydrocolloids) and synergistic (exploiting known synergistic interactions between hydrocolloids).

An appreciable portion of the population in both developing and developed countries, particularly young children adolescents, the elderly, and women of child-bearing age can suffer from nutrient deficiencies at borderline or pathological levels (Richardson 1990).

In the last three decades, due to economic and health factors, low fat spreads have been produced with reduced fat contents while attempting to retain the texture and flavor of butter. An increase in the water phase associated with the fat phase reduction in spreads significantly changes the rheological properties and sensory evaluation of W/O spread above a certain water level. This introduces specific problems in low-fat spreads such as the occurrence of loose moisture upon spreading. The properties required for W/O spreads include having a relatively firm consistency and a plastic rheology so that the product does not become much thinner during spreading (Bot and Vervoort 2006).

The main objective of the present study was to investigate the effects of the pH values on the sensory and morphological evaluations, peroxide values (PV), oil stability index (OSI), fatty acid composition (FAC), solid fat content (SFC), rheological and melting properties of buffalo butter-based low-fat spreads (B-LFS) & cow butter-based low-fat spreads (C-LFS).

2. MATERIALS AND METHODS

2.1. Materials

Buffalo butter (Table 1) was obtained from the Department of Dairy Science, Faculty of Agriculture, Suez Canal University (Ismailia, Egypt). Cow butter (Table 1), skim milk powder and sodium chloride (table salt) were purchased from a local market in Wuxi (Jiangsu, China). Halal gelatin (80-280 BLOOM) was purchased from Gelatin & Protein Co., Ltd (Hangzhou, China). DIMODAN®HP-C distilled monogelyceride was obtained from Danisco Co. (Shanghai, China). Citric acid anhydrous, sodium bicarbonate and k-sorbate were purchased from Shanghai Honghao Chemical Co., Ltd (Shanghai, China). All other reagents and solvents were of analytical or chromatographic grade to suit analytical requirements.

2.2. Preparation of buffalo and cow butter oil

Butter oil preparation was performed according to Fatouh *et al*. (2003) with some modifications. Both buffalo and cow butter were melted separately at 50 °C instead of 60 °C, and the top oil layer was decanted and filtered through glass wool. The oil was then re-filtered under vacuum to obtain clear buffalo and cow butter oil.

2.3. Preparation of B-LFS and C-LFS with pH values

The procedure for the pH treatments (B-LFS and C-LFS) was carried out according to Madsen (2000) with some modifications. The treatments consisted of the following (percentage, w/w): Buffalo and cow butter oil 40%, DIMODAN®HP-C distilled monogelyceride 0.5%, halal gelatin 2%, skim milk powder

TABLE 1. Buffalo and cow butter specifications

Characteristics	Buffalo butter	Cow butter
Fat (%)	83.48	82.68
Solid not fat (%)	2.91	1.75
Moisture (%)	13.61	15.57
Peroxide value	0.145	0.135

1%, sodium chloride 1%, k-sorbate 0.1% and distilled water (to 100%). The sample preparation steps were as follows:

1. Water phase. The ingredients: Halal gelatin, skim milk powder, NaCl and k-sorbate were blended together with distilled water at 70 °C for 10 min using a JJ-1B Electric Blender (Changzhou Runhua Electric Appliance Co., Ltd, China).
2. The temperature of the water phase was then reduced to 40 °C and the pH was adjusted to 5, 5.5, 6, 6.5 and 7 [with citric acid 20% (w/w) and sodium bicarbonate 20% (w/w)] while blending.
3. Fat phase. A portion of the melted buffalo and cow butter oil (~5×the weight of the emulsifier) was removed and heated to 70 °C with blending until the emulsifier dissolved, which was then added back to the melted butter oil at 40 °C.
4. The water phase was then slowly added to the fat phase while mixing using a homogenizer (IKA®T18 Basic ULTRA-TURRAX®, Germany) for 5 min at speed No. 2.
5. The mixture was then pasteurized at 75 °C for 10 min in a water bath while blending.
6. The mixture was homogenized once using a laboratory Homogenizer (Model: GYB, Donghua High Pressure Homogenizer Factory, Shanghai, China) at a pressure of 17 MPa at 60 °C.
7. The treated samples were kept in sterilized plastic cups (30 g) at room temperature for 15 hours (h) and then moved to the refrigerator (4 °C).

2.4. Sensory evaluations

Sensory evaluations of the samples (B-LFS ad C-LFS) were carried out according to Patange *et al.* (2013) using a panel of 12 judges selected from Egypt, Sudan and Yemen. Both B-LFS and C-LFS samples were approximately 30 g and were presented to the panelists at refrigeration temperature (4 °C). The color and appearance, spreadability, body and texture, flavor and overall acceptability, of the products were rated on a 9-point scale ranging from 1 (disliked extremely) to 9 (liked extremely). Spreadability was assessed by the panelists using a slice of bread onto which the sample was spread at 4 °C.

2.5. Morphology evaluation

Morphology evaluations of the pH treatments were recorded with a digital camera (Sony Camera T500, Japan).

2.6. Peroxide value

The PV was modified from International Dairy Federation (IDF) Standard 74:1974 (Alexa *et al.* 2010). The samples of pH treatments (B-LFS and C-LFS) (40 g each) were placed into 50 mL conical centrifuge tubes and placed in a 50 °C water bath for 20 min, followed by centrifugation (RJ-TDL-50A, Low-speed desktop centrifuge, China) for 20 min at 5000 rpm. The top fat layers were decanted into a beaker and then dried over excess anhydrous sodium sulfate to remove residual water. The fat was separated from the anhydrous sodium sulfate by vacuum filtration through a Whatman No. 4 filter paper to obtain a clear fat. A 0.1 mL of melted fat was dissolved with 10 mL of a chloroform/methanol (70:30) mixture, followed by the addition of ammonium thiocyanate (0.05 mL) and ferrous chloride (0.05 mL), respectively. Using glass stoppers, the tubes were inverted and placed in a dark cupboard for 10 min. At the same time, a blank test with only reagents and no sample was carried out. The absorbance of the samples was read at 505 nm on a Spectrophotometer (Alpha-1500, China). After calibration, the blank value was subtracted from the sample values (1) and the PVs were calculated. All of the experiments were carried out in triplicate and the mean results are reported.

$$OD = Abs_{sample} - Abs_{standard} \tag{1}$$

where, OD is the optical density.

2.7. Oil stability index

The oxidation induction time (OIT) of the extracted fat (see PV) was determined by the AOCS method Cd 12b-92 (Firestone 2004) with the Rancimat 743 apparatus (Metrohm AG, Herison, Switzerland). Samples of pH treatments (B-LFS and C-LFS) were prepared in triplicate by weighing 3 g of extracted fat into the reaction vessels. Distilled water (50 mL) was added to the measuring vessels, which were maintained at room temperature. Electrodes were attached for measuring changes in conductivity. The samples were heated at 120 °C under a purified air flow rate of 20 $L \cdot h^{-1}$. The induction time is defined as the time necessary to reach the inflection point of the conductivity curve.

2.8. Fatty acids composition

The preparation of the methyl esters of the fatty acids was determined according to GB/T 17376 (2008). Briefly, 60 mg of extracted fat were weighed (see PV) into a 10 mL screw-capped test tube. Then, 5 mL of n-hexane to dissolve the sample, and 250 µL of 2 M potassium hydroxide in MeOH were added to the test tube. The mixtures were vigorously shaken for 2 min, and then 1 g $NaHSO_4$ was added into the tube and the mixtures were vigorously shaken for 2 min. After vortexing, 2 mL from the separated upper layer was added into the screw-capped test tube, and then centrifuged at high speed (TGL-16B, Shanghai Anting scientific factory, China) for 10 min at 10,000 rpm. One μL of purified hexane extract was injected into a GC-14B gas chromatograph (GC) equipped with a fused-silica capillary column (CP-Sil88, 100 m×0.25 mm×0.2 mm) and a flame ionization detector (Shimadzu, Tokyo, Japan). Both, injector and detector temperatures were set at 250 °C. The column oven temperature was as follows: 45 °C for 4 min, raised at 13 $°C·min^{-1}$ to 175 °C, held for 27 min, raised at 4 $°C·min^{-1}$to 215 °C, held for 20 min. Nitrogen was the carrier gas. The identification of the peaks was achieved by comparing the retention times with authentic standards analyzed under the same conditions. Results were expressed as w/w (%) total fatty acid.

2.9. Solid fat content

The SFC was performed according to the AOCS Official Method Cd 16b-93 (Firestone 2004). The SFC of the samples was determined on a PC120 pulsed nuclear magnetic resonance (pNMR) spectrometer (Bluker, Karlsrube, Germany). A 2.5 mL melted fat (see PV) added by the micropipette into glass tubes of pNMR. The samples were tempered by heating in a water bath at 100 °C for 15 min^{-1}, then at 60 °C for 15 min^{-1} followed by 60 min at 0 °C, and finally 30 min at each chosen measuring temperature. The determination of SFC was performed in the temperature range of 0–40 °C at 5 °C intervals. All of experiments were carried out in triplicate and the mean results are reported.

2.10. Rheological measurements

2.10.1. Hardness

The pH treatments (B-LFS and C-LFS) in plastic cups (diameter×height =4×2.5 cm) were kept in the refrigerator at 4 °C before the determination of the texture evaluation. The hardness was defined as the necessary force to reach the maximum penetration using a probe. The samples were removed from the refrigerator, and quickly placed on the platform of a TA-XT 2i texture analyzer (Stable Micro System, Ltd, UK). A puncture test was performed immediately using a probe (P/5–0.50 cm-diameter cylindrical probe) at pretest speed 1 $mm·s^{-1}$, test speed 1 $mm·s^{-1}$, posttest speed 1 $mm·s^{-1}$ and a data acquisition rate of 200 $points·s^{-1}$. The test was stopped when a penetration of 12 mm had been reached. All measurements were repeated at least 3 times in each test series.

2.10.2. Apparent viscosity

Both B-LFS and C-LFS with pH values were removed from the refrigerator (4 °C), and kept at room temperature for 1 h, then the apparent viscosity of the samples was measured at 25 °C with the 5 cm parallel-plate geometry of the Physica MCR 301 Rheometer (Anton Paar, Austria). The shear rates were from 0 to $200·s^{-1}$, whereas the apparent viscosity was determined at a shear rate of $100·s^{-1}$.

2.11. Melting behavior

Differential scanning calorimetry (DSC Q2000 V24.9 Build 121, TA Instruments, New Castle, DE, USA) was used to determine the melting behavior of the samples. The system was purged with nitrogen gas at 20 $mL·min^{-1}$ during the analysis, and liquid nitrogen was used as a refrigerant to cool the system. Calibration was performed with indium, eicosane, and dodecane standards. An empty aluminum pan was used as a reference. The samples (5–8 mg) were hermetically sealed in an aluminum pan, heated to 80 °C and held for 5 min to completely destroy the previous crystal structure. The samples were then cooled to −40 °C and maintained for 5 min. Following this step, the melting profiles were obtained by heating the samples to 80 °C at a rate of 10 $°C·min^{-1}$. DSC melting curves were recorded from −40 °C to 80 °C. Data analysis was carried out with the software provided with the DSC.

2.12. Statistical analysis

B-LFS and C-LFS with different pH values were analyzed separately, and values from the different tests were expressed as the mean ± standard deviation. One–way analysis of variance using SPSS 16 for windows (SPSS Inc., Chicago, USA) was performed on all experimental data sets. The Duncan analysis was applied to evaluate the significance of differences between means at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Effects of pH values on the sensory and morphological evaluations of B-LFS and C-LFS

Results from the sensory evaluation tests (color and appearance, body and texture, spreadability, flavor and overall acceptability) for the pH treatments (B-LFS and C-LFS) are presented in Table 2 (a and b). The yellow color of the pH treatments (C-LFS) reflected the coloring agent (β-carotene) in the fat phase of C-LFS. In general, the differences in sensory evaluation tests between B-LFS and C-LFS with pH 5 were negligible, while with pH 6, 6.5 and 7, the differences were clear when compared to the control samples. In addition, the scores of all the treatments with pH 6, 6.5 and 7 were decreased in the following order: pH 6>6.5>7. On the other hand, all sensory evaluation values were decreased during the storage periods (3 to 90 days).

The sensory evaluation of color and appearance was in correlation with the morphology evaluation of pH treatments, especially with increasing pH values (Fig. 1). In addition, no separated phase was observed for pH 5 of B-LFS and C-LFS compared to the control samples. In contrast, the treatments with pH 6, 6.5 and 7 had a separated phase (Fig. 1) compared to the control samples, and the phase separation was increased in the following order: pH 7>pH 6.5>pH 6. Furthermore, the phase separation occurred due to the fact that the attraction potential (attractive van der Waals forces) was greater than the repulsion potential, and vice versa with both pH 5 and 5.5. Also, the pH was far from the isoelectric point of the protein molecules when compared to the control samples (Cheng *et al.* 2008). No darkness was observed in the color or appearance of the samples during the storage periods, while both darkness and mould growth were observed at 80 days with the samples of pH 7. This observation is quite different when compared to Kristensen *et al.* (2000), who observed a darker and more yellow color during storage.

The decline in body and texture scores of pH treatments (B-LFS and C-LFS) during the storage periods is presumably due to the proteolytic action for microorganisms in the non-fat portion of the table spread (Patange *et al.* 2013).

With regards to spreadability, we found changes in the sensory evaluation of spreadability in our treatments during storage attributed to the changes in the overall consistency of the product due to protein degradation and/or decreased water holding by the non-fat fraction resulting in an increased softening of the spread particularly towards the end of the storage period (Patange *et al.* 2013). The flavor scores of all samples had decreased effects during the storage periods, which can be explained by a loss in freshness (Patange *et al.* 2013). Furthermore, no rancid flavor in the samples was observed, due to the storing of samples at 4 °C, the addition of k-sorbate and the pasteurization, which led to the inhibition of lipase.

The fresh samples were highly acceptable in overall acceptability. In addition, the scores of samples decreased during the storage periods due to the decline in flavor of the spread as well as to softening of the product (Patange *et al.* 2013).

It could be noted that the pH treatments (B-LFS and C-LFS) of all the parameters were accepted by the panelists. Furthermore, the highest scores in the sensory evaluations of color and appearance, body and texture, spreadability, flavor and overall acceptability related to B-LFS as follows: 8.77 (pH 5), 8.61 (pH 5), 8.67 (pH 5.5), 8.66 (pH 5) and 8.62 (pH 5) respectively at 3 days, while the lowest scores at 90 days were 6.13 (pH 7 with B-LFS), 5.90 (pH 7 with C-LFS), 6.18 (pH 7 with B-LFS), 6.95 (pH 7 with C-LFS) and 6 (pH 7 with C-LFS), respectively.

3.2. Effects of pH values on the PV of B-LFS and C-LFS

The effects of pH values on the oxidative stability of the pH treatments as measured by the PV test are presented in Table 3. The rate of increasing PVs in each B-LFS and C-LFS with pH values was higher from 3–30 days, but after 30 to 90 days of storage, the rate became lower. The differences among all the pH treatments compared to the control samples were slight. Moreover, the PVs of the pH treatments (B-LFS) were greater than C-LFS, due to the fact that the fat phase in the cow butter for the C-LFS samples contained a color agent (β-carotene), and β-carotene has been reported to be an antioxidant (Mallia 2008). In addition, Britton (1995) reported that β-carotene has been shown to protect lipids from free radical autoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction. Furthermore, the PVs of all pH treatments increased noticeably ($P<0.05$) during the storage periods. On the other hand, the pH treatments were in accepted in an industrial setting, because the highest PV was 0.486 (pH 7 with B-LFS at 30 days); however, the samples are considered rancid and unacceptable when the PVs are over 5, while the ideal PV should be below 1–1.5 (Stathopoulos *et al.* 2009).

It is remarkable that, the oxidation was promoted in our treatments due to the incorporation of air and the commencement of oxidation during the preparation of the butter oil (Alexa *et al.* 2010). Furthermore, the heat treatments caused the oxidation of samples (Mallia 2008). Interestingly, the viscosity of each B-LFS and C-LFS with pH values increased during the storage periods; however, the viscosity was not able to delay the process of oxidation during the storage periods (Basaran *et al.* 1999).

3.3. Effects of pH values on the OSI of B-LFS and C-LFS

The effects of pH values on the OSI values of the samples are given in Table 4. As indicated, no significant differences ($P<0.05$) were observed in the OIT between each B-LFS and C-LFS samples and the control samples, while the OIT significantly decreased ($P<0.05$) during the storage periods. However, our results were in agreement with those observed for the OSI of NaCl and $CaCl_2$ treatments, which are still under study in our lab. Likewise, Krause *et al.* (2008) noticed that the OSI values for stick cow butter decreased during the storage periods under refrigeration conditions. The correlation between the OSI values and the PVs (Table 3) were reversible. In addition, all OSI values in the pH treatments (B-LFS) were lower than C-LFS (see PV). Furthermore, β-carotene led to a prolonging of the OIT for C-LFS samples as compared to B-LFS.

TABLE 2A. Effect of different pH values on the sensory evaluation of B-LFS

	Storage (days)	Sensory evaluation scores[a] B-LFS pH 5.00	pH 5.50 (control)	pH 6.00	pH 6.50	pH 7.00
Color & appearance	3	8.77 ± 0.09^{aA}	8.47 ± 0.11^{aB}	7.98 ± 0.09^{aC}	7.44 ± 0.10^{aD}	$6.43\pm0.07^{a}E$
	15	8.64 ± 0.09^{abA}	8.42 ± 0.08^{aA}	7.94 ± 0.11^{aB}	7.41 ± 0.25^{abC}	6.41 ± 0.12^{abD}
	30	8.58 ± 0.12^{abcA}	8.36 ± 0.09^{abB}	7.87 ± 0.11^{abC}	7.37 ± 0.05^{abD}	$6.38\pm0.10^{ab}E$
	45	8.55 ± 0.07^{bcA}	8.33 ± 0.14^{abB}	7.82 ± 0.11^{abC}	7.17 ± 0.11^{bcD}	$6.27\pm0.08^{abc}E$
	60	8.41 ± 0.14^{cdA}	8.33 ± 0.09^{abA}	7.81 ± 0.12^{abB}	7.10 ± 0.10^{cdC}	6.25 ± 0.15^{bcD}
	75	$8.32\pm0.13^{d}e^{A}$	8.21 ± 0.09^{bcA}	7.79 ± 0.13^{abB}	$6.90\pm0.18^{d}e^{C}$	6.17 ± 0.04^{cD}
	90	$8.19\pm0.13e^{A}$	8.13 ± 0.07^{cA}	7.70 ± 0.13^{bB}	$6.81\pm0.11e^{C}$	6.13 ± 0.05^{cD}
Body & texture	3	8.61 ± 0.09^{aA}	8.49 ± 0.07^{aA}	8.17 ± 0.20^{aB}	7.17 ± 0.13^{aC}	6.41 ± 0.06^{aD}
	15	8.60 ± 0.10^{aA}	8.41 ± 0.11^{abAB}	8.14 ± 0.02^{aB}	7.10 ± 0.06^{abC}	6.37 ± 0.31^{abD}
	30	8.56 ± 0.10^{abA}	8.42 ± 0.10^{abA}	8.05 ± 0.20^{abB}	6.95 ± 0.13^{abcC}	6.31 ± 0.10^{abcD}
	45	8.43 ± 0.06^{bcA}	8.33 ± 0.08^{abcA}	7.94 ± 0.33^{abB}	6.87 ± 0.11^{bcC}	6.15 ± 0.13^{abcdD}
	60	8.36 ± 0.13^{cA}	8.24 ± 0.12^{bcdAB}	7.90 ± 0.15^{abB}	6.81 ± 0.32^{cC}	6.11 ± 0.23^{bcdD}
	75	8.36 ± 0.09^{cA}	8.16 ± 0.11^{cdB}	7.84 ± 0.10^{abC}	6.80 ± 0.12^{cD}	$6.04\pm0.11^{cd}E$
	90	8.17 ± 0.09^{dA}	8.08 ± 0.12^{dA}	7.76 ± 0.31^{bB}	6.76 ± 0.11^{cC}	6.00 ± 0.10^{dD}
Spreadability	3	8.45 ± 0.09^{aB}	8.67 ± 0.08^{aA}	8.10 ± 0.11^{aC}	7.12 ± 0.05^{aD}	$6.58\pm0.14^{a}E$
	15	8.41 ± 0.22^{abA}	8.56 ± 0.10^{abA}	8.00 ± 0.11^{abB}	7.06 ± 0.09^{abC}	6.52 ± 0.13^{aD}
	30	8.35 ± 0.08^{abcA}	8.55 ± 0.09^{abA}	7.94 ± 0.10^{abcB}	6.92 ± 0.09^{bcC}	6.51 ± 0.21^{aD}
	45	8.32 ± 0.12^{abcA}	8.41 ± 0.29^{abcA}	7.89 ± 0.12^{abcB}	6.87 ± 0.09^{cdC}	6.47 ± 0.12^{aD}
	60	8.33 ± 0.13^{abcA}	8.38 ± 0.08^{bcdA}	7.82 ± 0.30^{bcB}	$6.73\pm0.11^{d}e^{C}$	6.40 ± 0.14^{abD}
	75	8.18 ± 0.20^{bcA}	8.25 ± 0.14^{cdA}	7.78 ± 0.11^{bcB}	$6.68\pm0.08e^{C}$	6.35 ± 0.11^{abD}
	90	8.11 ± 0.07^{cA}	8.11 ± 0.22^{dA}	7.69 ± 0.06^{cB}	$6.61\pm0.12e^{C}$	6.18 ± 0.12^{bD}
Flavor	3	8.66 ± 0.08^{aA}	8.53 ± 0.11^{aA}	8.00 ± 0.19^{aB}	7.47 ± 0.12^{aC}	7.54 ± 0.07^{aC}
	15	8.62 ± 0.12^{abA}	8.51 ± 0.10^{abA}	7.94 ± 0.10^{abB}	7.41 ± 0.10^{abC}	7.50 ± 0.29^{abC}
	30	8.56 ± 0.09^{abcA}	8.44 ± 0.10^{abA}	7.88 ± 0.13^{abB}	7.35 ± 0.08^{abC}	7.44 ± 0.09^{abC}
	45	8.51 ± 0.07^{abcA}	8.36 ± 0.11^{abA}	7.87 ± 0.12^{abB}	7.28 ± 0.14^{bcC}	7.37 ± 0.10^{abcC}
	60	8.47 ± 0.15^{bcA}	8.33 ± 0.10^{bcA}	7.79 ± 0.13^{abB}	7.25 ± 0.09^{bcdC}	7.31 ± 0.27^{abcC}
	75	8.39 ± 0.10^{cdA}	8.15 ± 0.13^{cdB}	7.70 ± 0.10^{abC}	7.13 ± 0.09^{cdD}	7.25 ± 0.07^{bcD}
	90	8.21 ± 0.11^{dA}	8.13 ± 0.11^{dA}	7.69 ± 0.32^{bB}	7.07 ± 0.11^{dC}	7.14 ± 0.07^{cC}
Over-all acceptability	3	8.62 ± 0.08^{aA}	8.55 ± 0.11^{aA}	7.85 ± 0.12^{aB}	7.23 ± 0.09^{aC}	6.44 ± 0.14^{bD}
	15	8.53 ± 0.10^{abA}	8.48 ± 0.08^{abA}	7.81 ± 0.10^{aB}	7.23 ± 0.13^{aC}	6.37 ± 0.14^{aD}
	30	8.51 ± 0.10^{abA}	8.45 ± 0.07^{abA}	7.75 ± 0.10^{abB}	7.18 ± 0.09^{aC}	6.26 ± 0.09^{abD}
	45	8.47 ± 0.11^{abcA}	8.36 ± 0.32^{abcA}	7.68 ± 0.11^{abB}	7.11 ± 0.11^{abC}	6.17 ± 0.11^{bcD}
	60	8.38 ± 0.15^{bcdA}	8.31 ± 0.08^{abcA}	7.66 ± 0.30^{abB}	6.97 ± 0.10^{bcC}	6.16 ± 0.14^{bcD}
	75	8.27 ± 0.21^{cdA}	8.22 ± 0.13^{bcA}	7.52 ± 0.11^{bB}	6.83 ± 0.08^{cdC}	6.11 ± 0.08^{bcD}
	90	8.18 ± 0.11^{dA}	8.14 ± 0.09^{cA}	7.51 ± 0.09^{bB}	6.78 ± 0.10^{dC}	6.04 ± 0.07^{cD}

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. [a]mean±S.D; n=12.

3.4. Effects of pH values on the FAC of B-LFS and C-LFS

The effects of the pH values on the FAC of each B-LFS and C-LFS are shown in Table 5 (a and b). Obviously, the differences among pH treatments (B-LFS) were significant compared to the control samples, while saturated fatty acids (SFA) (at 3 and 90 days), C14 (at 90 days), C15 (at 3 days), C15:1 (at 90 days), C16:1 (at 90 days), C18:2C (at 3 days), and the total FA (at 3 and 90 days) were not significant. Likewise, the differences among pH treatments (C-LFS) were significant compared to the control samples, while the SFA (at 90 days), C14 (at 90 days),

TABLE 2B. Effect of different pH values on the sensory evaluation of C-LFS

	Storage (days)	Sensory evaluation scores^{a}				
		C-LFS				
		pH 5.00	pH 5.50 (control)	pH 6.00	pH 6.50	pH 7.00
Color & appearance	3	8.66±0.07^{aA}	8.48±0.05^{abB}	8.00±0.11^{aC}	7.33±0.10^{aD}	6.58±0.06^{aE}
	15	8.54±0.17^{abA}	8.52±0.10^{aA}	7.97±0.09^{abB}	7.33±0.07^{aC}	6.54±0.07^{aD}
	30	8.55±0.07^{abA}	8.44±0.09^{abA}	7.93±0.11^{abB}	7.26±0.14^{aC}	6.47±0.27^{abD}
	45	8.43±0.11^{bcA}	8.40±0.07^{abcA}	7.96±0.17^{abB}	7.17±0.14^{aC}	6.41±0.14^{abD}
	60	8.39±0.19^{bcA}	8.33±0.15^{bcA}	7.86±0.28^{abB}	7.10±0.20^{abC}	6.36±0.16^{abD}
	75	8.22±0.11^{cdA}	8.24±0.05^{cdA}	7.83±0.07^{abB}	7.12±0.06^{abC}	6.45±0.05^{abD}
	90	8.13±0.14^{dA}	8.14±0.14^{dA}	7.72±0.07^{bB}	6.89±0.17^{bC}	6.21±0.17^{bD}
Body & texture	3	8.47±0.05^{aA}	8.37±0.09^{aA}	8.10±0.05^{aB}	7.14±0.09^{aC}	6.23±0.07^{aD}
	15	8.51±0.05^{aA}	8.33±0.08^{aA}	8.05±0.14^{abB}	7.05±0.03^{abcC}	6.17±0.14^{aD}
	30	8.46±0.11^{aA}	8.35±0.06^{aA}	7.96±0.11^{abcB}	7.07±0.19^{abC}	6.11±0.13^{abD}
	45	8.41±0.17^{aA}	8.23±0.08^{abA}	7.91±0.31^{abcB}	6.92±0.06^{bcdC}	6.14±0.08^{abD}
	60	8.43±0.10^{aA}	8.18±0.04^{bB}	7.89±0.14^{abcC}	6.88±0.11^{cdD}	6.06±0.12^{abcE}
	75	8.36±0.07^{aA}	8.16±0.13^{bB}	7.81±0.09^{bcC}	6.83±0.06^{dD}	5.96±0.15^{bcE}
	90	8.17±0.13^{bA}	8.13±0.08^{bA}	7.70±0.07^{cB}	6.79±0.08^{dC}	5.90±0.05^{cD}
Spreadability	3	8.38±0.11^{aA}	8.41±0.11^{aA}	7.92±0.14^{aB}	6.96±0.06^{aC}	6.64±0.07^{aD}
	15	8.33±0.13^{abA}	8.28±0.06^{abA}	7.88±0.07^{abB}	6.91±0.17^{abC}	6.55±0.05^{abD}
	30	8.36±0.12^{aA}	8.27±0.08^{abA}	7.85±0.11^{abB}	6.85±0.12^{abC}	6.48±0.13^{abD}
	45	8.31±0.07^{abA}	8.15±0.18^{bA}	7.76±0.17^{abB}	6.81±0.31^{abC}	6.42±0.18^{bD}
	60	8.25±0.18^{abA}	8.12±0.09^{bA}	7.71±0.11^{bB}	6.83±0.14^{abC}	6.35±0.08^{bcD}
	75	8.19±0.13^{abA}	8.13±0.09^{bA}	7.73±0.08^{bB}	6.74±0.06^{abC}	6.37±0.15^{bcD}
	90	8.14±0.11^{bA}	8.11±0.09^{bA}	7.69±0.12^{bB}	6.68±0.11^{bC}	6.20±0.15^{cD}
Flavor	3	8.39±0.11^{aA}	8.30±0.04^{aA}	7.80±0.05^{aB}	7.38±0.12^{aC}	7.32±0.09^{abC}
	15	8.38±0.04^{aA}	8.31±0.05^{aA}	7.85±0.16^{aB}	7.28±0.32^{abC}	7.35±0.09^{abC}
	30	8.33±0.04^{abA}	8.28±0.06^{abA}	7.73±0.11^{abB}	7.22±0.07^{abC}	7.36±0.09^{aC}
	45	8.31±0.08^{abA}	8.22±0.07^{abcA}	7.69±0.06^{abB}	7.16±0.16^{abC}	7.21±0.11^{bcC}
	60	8.27±0.06^{abA}	8.14±0.04^{bcA}	7.66±0.11^{abB}	7.13±0.19^{abC}	7.11±0.06^{cdC}
	75	8.21±0.09^{bcA}	8.11±0.15^{cA}	7.55±0.25^{bB}	7.13±0.15^{abC}	7.03±0.07^{deC}
	90	8.11±0.05^{cA}	8.10±0.09^{cA}	7.50±0.09^{bB}	7.04±0.13^{bC}	6.95±0.08e^{C}
Over-all acceptability	3	8.43±0.09^{aA}	8.38±0.09^{aA}	7.70±0.15^{abB}	7.10±0.08^{aC}	6.36±0.07^{aD}
	15	8.39±0.05^{abA}	8.23±0.07^{abB}	7.75±0.05^{aC}	7.11±0.06^{aD}	6.25±0.09^{abE}
	30	8.27±0.14^{abcA}	8.22±0.10^{abA}	7.67±0.11^{abcB}	6.95±0.14^{abcC}	6.22±0.12^{abcD}
	45	8.23±0.16^{abcA}	8.18±0.18^{abA}	7.61±0.06^{abcB}	7.03±0.15^{abC}	6.17±0.26^{abcD}
	60	8.21±0.13^{abcA}	8.16±0.10^{abA}	7.62±0.11^{abcB}	6.88±0.07^{bcC}	6.19±0.07^{abcD}
	75	8.19±0.13^{bcA}	8.12±0.19^{bA}	7.55±0.08^{bcB}	6.83±0.14^{bcC}	6.11±0.06^{bcD}
	90	8.10±0.15^{cA}	8.10±0.11^{bA}	7.52±0.06^{cB}	6.79±0.17^{cC}	6.00±0.18^{cD}

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. ^{a}mean±S.D; n=12.

C15 (at 3 days), C17 (at 3 days), C18:2T (at 3 days) and total FA (at 3 and 90 days) were not significant. Furthermore, there were changes in the proportions of fatty acids within pH treatments (B-LFS and C-LFS) during the storage periods, presumably due to the degradation of fat under pasteurization and oxidation (Samet-Bali *et al*. 2009).

With regard to the differences among pH treatments (B-LFS and C-LFS together), we found the percentages of SFA and trans FA (TFA) to be lower and monounsaturated fatty acids (MUFA) to be higher for all the pH treatments in B-LFS than in C-LFS. In addition, the percentages of polyunsaturated fatty acids (PUFA) were close to each other in

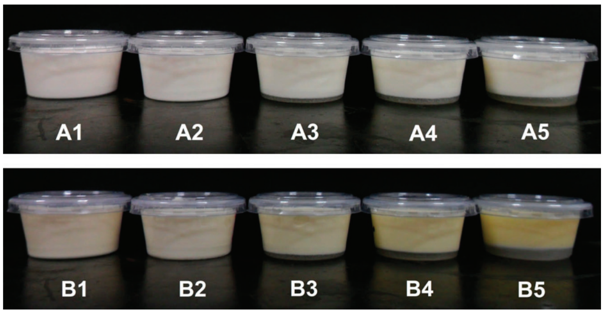


FIGURE 1. Effects of different pH values on the morphological evaluation of B-LFS and **C-LFS**.
A1) B-LFS with pH 5; A2) B-LFS with pH 5.5 (control); A3) B-LFS with pH 6; A4) B-LFS with pH 6.5; A5) B-LFS with pH 7.
B1) C-LFS with pH 5; B2) C-LFS with pH 5.5 (control); B3) C-LFS with pH 6; B4) C-LFS with pH 6.5; B5) C-LFS with pH 7.

the B-LFS and C-LFS samples although our results were in contrast with those observed by Varricchio *et al*. (2007), because they found that buffalo milk fat contained higher amounts of SFA and lower amounts of unsaturated fatty acids than cow milk fat. However, results of the previous authors were from other breeds which are different from the breed of Egyptian buffalo animals. Furthermore, Samet-Bali *et al*. (2009) reported that the FAC depends on several factors such as animal species, nutrition, climate and environmental conditions. However, our results were in agreement with those observed by Haggag *et al*. (1987), who reported that unsaturated fatty acids for Egyptian buffalo milks were higher than Egyptian cow milks.

The proportions of C4, C15, C16, C17, C14:1, C15:1, C16:1, C17 and C18:2T with pH treatments (B-LFS) were higher than the C-LFS samples, while C6, C8, C10, C11, C12, C13, C14 and C18:1T with C-LFS were higher than the B-LFS samples. Moreover, Patel *et al*. (2002) found that an averages of C4, C16, C17 and C18 in buffalo milk fat was higher than cow milk fat, while C6, C8, C10, C10:1, C12, C14, C14:1 and C18:1 in cow milk fat was higher than buffalo milk fat.

It is clear that the changes in FAC during the storage periods of the samples were slight; although our results were in agreement with those found by Mallia (2008), who mentioned that the differences in FAC before and after 8 weeks of storage were negligible in each unsaturated fatty acids/conjugated linoleic acid enriched and conventional butter. On the other hand, the differences observed during the storage periods of the samples, are presumably attributed to degradation of non-enzymatic, pasteurization and microbiological aspects.

3.5. Effects of pH values on the SFC values of B-LFS and C-LFS

The effects of the pH values on the pH treatments are shown in Table 6 (a and b). The SFC was defined at a number of temperatures, typically from 0 to 40 °C, covering the range of practical uses. The pH treatments (B-LFS and C-LFS) exhibited a gradual decreasing in the SFC with an increase in the temperature from 0 °C to completely melting. In addition, the differences in SFC among the pH treatments and during the storage periods were negligible.

The SFC of the pH treatments (C-LFS) was higher than B-LFS from 0 to 15 °C, while both C-LFS and B-LFS were completely melting at 30 and 35 °C, respectively. Our results resembled those observed for the SFC of $CaCl_2$ and the NaCl

TABLE 3. Effect of different pH values on peroxide values (meq $O_2 \cdot kg^{-1}$ of fat) of B-LFS and C-LFS

Storage (days)	pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
			B-LFS		
3	0.259±0.026^{cA}	0.239±0.024^{cA}	0.246±0.014^{cA}	0.229±0.014d^{A}	0.236±0.013d^{A}
30	0.356±0.024^{bB}	0.383±0.021^{bAB}	0.392±0.017^{bAB}	0.388±0.024^{cAB}	0.414±0.017^{cA}
60	0.390±0.013^{abC}	0.414±0.013^{abB}	0.408±0.015^{bBC}	0.422±0.013^{bB}	0.455±0.009b^{A}
90	0.416±0.017^{aC}	0.443±0.024^{aBC}	0.455±0.026^{aAB}	0.454±0.012^{aAB}	0.486±0.012^{aA}
			C-LFS		
3	0.195±0.026^{cA}	0.170±0.011^{cA}	0.186±0.024^{cA}	0.198±0.020^{cA}	0.204±0.013d^{A}
30	0.330±0.015^{bA}	0.340±0.012^{bA}	0.319±0.014^{bA}	0.327±0.023^{bA}	0.333±0.026^{cA}
60	0.364±0.011^{abAB}	0.392±0.017^{aA}	0.345±0.014^{bB}	0.355±0.026^{bB}	0.367±0.019b^{AB}
90	0.394±0.027^{aA}	0.417±0.019^{aA}	0.409±0.021^{aA}	0.414±0.027^{aA}	0.427±0.009^{aA}

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. [a]mean±S.D; n=3.

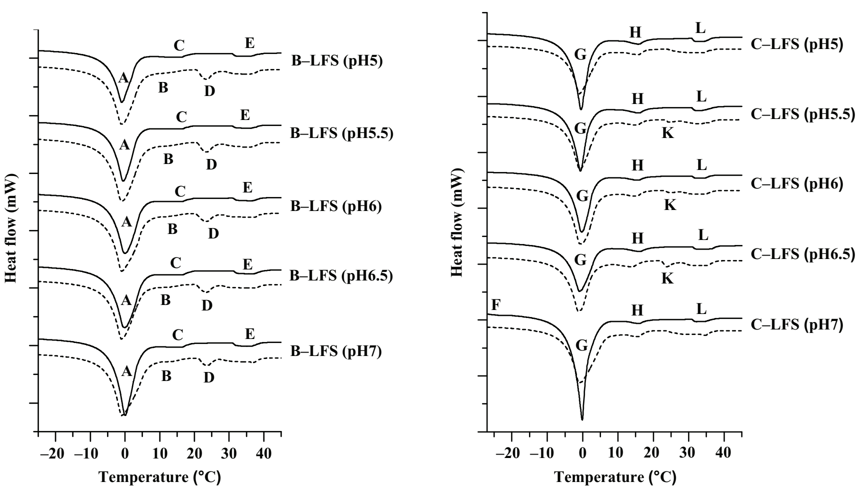
TABLE 4. Effect of different pH values on OSI values (h) of B-LFS and C-LFS

Storage periods (days)	pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
			B-LFS		
3	4.33±0.09^{aA}	4.27±0.17^{aA}	4.39±0.12^{aA}	4.20±0.16^{aA}	4.24±0.12^{aA}
30	4.21±0.12^{aAB}	4.14±0.08^{abAB}	4.30±0.16^{aA}	4.10±0.10^{aAB}	4.02±0.17^{abB}
60	3.94±0.06^{bB}	3.96±0.16^{bcAB}	4.14±0.10^{abA}	3.99±0.09^{abAB}	3.95±0.09^{bB}
90	3.89±0.08^{bA}	3.86±0.18^{cA}	3.94±0.15^{bA}	3.87±0.09^{bA}	3.90±0.07^{bA}
			C-LFS		
3	5.33±0.09^{aA}	5.24±0.17^{aA}	5.36±0.15^{aA}	5.47±0.06^{aA}	5.33±0.16^{aA}
30	5.14±0.18^{abA}	5.12±0.13^{aA}	5.23±0.07^{abA}	5.22±0.16^{bA}	5.15±0.09^{abA}
60	4.94±0.13^{bcA}	4.85±0.10^{bA}	5.04±0.11^{bA}	4.95±0.10^{cA}	4.94±0.16^{bA}
90	4.76±0.07^{cA}	4.63±0.05^{bA}	4.75±0.12^{cA}	4.78±0.15^{cA}	4.66±0.14^{cA}

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. [a]mean±S.D; n=3.

treatments (data not shown). Furthermore, there are correlations between the SFC of the pH treatments (from 30 to 35 °C) and the melting behavior, with regard to the high melting zones (Fig. 2).

It is worth noting that the SFC of our treatments was not increased during the storage periods. In contrast, Laia *et al.* (2000) found that the SFC values of table margarine showed an increasing



pH values	Storage (days)	Melting zones (°C) B-LFS A	B	C	D	E	C-LFS F	G	H	K	L
5	3	–0.97	–	16.51	–	31.86 to 36.39	–	–0.48	16.03	–	31.65 to 34.54
	0	–0.97	11.86	–	23.56	31.86 to 36.14	–	–0.86	15.65	–	28.37 to 34.29
5.5	3	–0.60	–	16.64	–	31.99 to 36.77	–	–0.60	16.03	–	31.90 to 34.54
	90	–0.97	12.49	–	23.43	31.48 to 36.26	–	–0.73	15.14	24.34	31.27 to 36.18
6	3	–0.03	–	16.89	–	32.11 to 37.14	–	–0.2	15.65	–	31.77 to 34.80
	90	–0.97	12.61	–	23.43	30.35 to 37.52	–	–0.35	15.14	24.97	30.76 to 34.80
6.5	3	–0.22	–	16.76	–	32.49 to 37.14	–	–0.86	16.28	–	32.02 to 36.56
	90	–1.10	12.86	–	23.56	30.10 to 37.77	–	–1.11	15.01	23.84	30.76 to 35.43
7	3	–0.03	–	16.64	–	32.24 to 36.77	–23.41	–0.10	15.90	–	31.77 to 35.05
	90	–0.97	13.49	–	23.68	30.10 to 37.27	–	–0.98	15.65	–	28.12 to 34.92

FIGURE 2. Effect of different pH values on the melting behavior of B-LFS and C-LFS at 3 days (solid lines) and after 90 days (dashed lines). The letters indicate the main endothermic peaks.

Table 5a. Effect of different pH values on FAC of B-LFS

	Storage (days)	FAC (%)[a]				
		B-LFS				
		pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
SFA	3	68.20±1.95^{aA}	67.79±2.11^{aA}	65.73±2.41^{aA}	66.19±1.24^{aA}	67.26±1.41^{aA}
	90	67.16±2.09^{aA}	64.94±1.71^{aA}	66.91±2.83^{aA}	65.67±1.82^{aA}	67.51±1.09^{aA}
C4	3	3.48±0.11^{aB}	2.89±0.08^{aC}	2.76±0.12^{bC}	2.15±0.13^{aD}	3.85±0.16^{bA}
	90	3.67±0.14^{aB}	2.32±0.17^{bC}	4.29±0.15^{aA}	2.40±0.12^{aC}	4.48±0.13^{aA}
C6	3	1.56±0.12^{aA}	0.51±0.15^{bC}	0.87±0.14^{bB}	0.63±0.05^{bBC}	1.46±0.20^{aA}
	90	1.34±0.17^{aB}	1.00±0.20^{aC}	1.87±0.17^{aA}	0.88±0.02^{aC}	1.63±0.16^{aA}
C8	3	1.05±0.02^{aA}	0.60±0.04^{aB}	0.59±0.26^{aB}	0.60±0.02^{aB}	1.00±0.03^{aA}
	90	0.53±0.04^{bAB}	0.45±0.06^{bB}	0.81±0.31^{aA}	0.53±0.05^{aAB}	0.72±0.20^{aAB}
C10	3	1.75±0.05^{aAB}	1.79±0.20^{aAB}	1.44±0.19^{aB}	1.70±0.21^{aB}	2.08±0.27^{aA}
	90	1.24±0.05^{bBC}	1.26±0.07^{bBC}	1.55±0.23^{aAB}	0.97±0.20^{bC}	1.84±0.22^{aA}
C11	3	0.08±0.02^{aBC}	0.14±0.05^{aAB}	0.11±0.04^{aABC}	0.05±0.01^{aC}	0.15±0.04^{aA}
	90	0.07±0.01^{aB}	0.26±0.07^{aA}	0.10±0.06^{aB}	0.09±0.03^{aB}	0.11±0.02^{aB}
C12	3	2.74±0.05^{aA}	2.68±0.19^{aAB}	1.89±0.18^{aC}	2.05±0.08^{aC}	2.42±0.26^{aB}
	90	1.53±0.05^{bB}	2.51±0.26^{aA}	1.69±0.22^{aB}	1.38±0.19^{bB}	1.50±0.21^{bB}
C13	3	0.17±0.03^{bA}	0.20±0.05^{aA}	0.24±0.04^{aA}	0.21±0.05^{aA}	0.20±0.04^{aA}
	90	0.32±0.04^{aA}	0.11±0.03^{aC}	0.22±0.06^{aB}	0.14±0.03^{aC}	0.29±0.05^{aAB}
C14	3	10.56±0.59^{aA}	10.55±0.57^{aA}	10.11±0.53^{aAB}	10.15±0.50^{aAB}	9.26±0.50^{aB}
	90	9.88±0.60^{aA}	10.11±0.56^{aA}	9.98±0.49^{aA}	9.89±0.61^{aA}	9.72±0.60^{aA}
C15	3	1.61±0.03^{bA}	1.69±0.16^{aA}	1.65±0.15^{aA}	1.69±0.17^{aA}	1.73±0.21^{aA}
	90	2.00±0.12^{aA}	1.61±0.06^{aB}	1.76±0.18^{aAB}	1.63±0.15^{aB}	1.55±0.17^{aB}
C16	3	35.45±0.80^{aAB}	35.70±0.83^{aA}	34.44±0.77^{aAB}	35.40±0.74^{aAB}	34.15±0.70^{aB}
	90	35.92±0.85^{aA}	34.88±0.72^{aAB}	34.56±0.60^{aB}	36.10±0.75^{aA}	35.40±0.77^{aAB}
C17	3	0.90±0.03^{aAB}	0.92±0.03^{aAB}	0.97±0.09^{aA}	0.89±0.09^{aAB}	0.81±0.03^{aB}
	90	0.95±0.02^{aA}	0.81±0.04^{bB}	0.71±0.04^{bC}	0.77±0.04^{aBC}	0.72±0.03^{bC}
C18	3	8.85±0.44^{aB}	10.11±0.38^{aA}	10.65±0.47^{aA}	10.66±0.37^{aA}	10.15±0.42^{aA}
	90	9.71±0.48^{aB}	9.64±0.45^{aB}	9.37±0.43^{bB}	10.88±0.38^{aA}	9.54±0.50^{aB}
US						
MUFA	3	27.09±0.69^{aB}	27.67±0.78^{aB}	29.17±0.76^{aA}	27.79±0.49^{aB}	27.97±0.79^{aAB}
	90	26.79±0.74^{aB}	28.75±0.44^{aA}	27.94±0.42^{aAB}	29.14±1.02^{aA}	28.24±1.25^{aAB}
C14:1	3	1.65±0.05^{aB}	1.77±0.17^{aAB}	1.95±0.16^{aA}	1.75±0.18^{aAB}	1.58±0.01^{aB}
	90	1.14±0.04^{bB}	1.53±0.23^{aA}	1.63±0.19^{aA}	1.53±0.17^{aA}	1.46±0.18^{aA}
C15:1	3	0.33±0.05^{aB}	0.26±0.12^{aB}	0.43±0.11^{aAB}	0.40±0.02^{aAB}	0.58±0.16^{aA}
	90	0.37±0.03^{aA}	0.44±0.05^{aA}	0.35±0.14^{aA}	0.41±0.11^{aA}	0.42±0.13^{aA}
C16:1	3	3.66±0.04^{aA}	3.65±0.17^{aA}	3.57±0.16^{aA}	3.67±0.18^{aA}	3.28±0.06^{aB}
	90	3.13±0.20^{bA}	3.16±0.07^{bA}	3.10±0.20^{bA}	3.20±0.17^{bA}	3.04±0.19^{aA}
C17:1	3	0.14±0.02^{bD}	0.24±0.05^{bB}	0.38±0.03^{aA}	0.22±0.04^{bBC}	0.16±0.04^{bCD}
	90	0.42±0.03^{aB}	0.60±0.06^{aA}	0.40±0.06^{aB}	0.47±0.03^{aB}	0.47±0.04^{aB}
C18:1	3	21.30±0.80^{aB}	21.76±0.67^{aAB}	22.84±0.62^{aA}	21.76±0.47^{bAB}	22.38±0.62^{aAB}
	90	21.72±0.85^{aB}	23.03±0.72^{aA}	22.45±0.63^{aAB}	23.53±0.56^{aA}	22.85±0.73^{aAB}
PUFA	3	1.75±0.19^{aAB}	1.84±0.09^{bAB}	1.48±0.14^{aB}	2.08±0.54^{aA}	1.98±0.25^{aAB}
	90	1.93±0.04^{aAB}	2.32±0.13^{aA}	2.30±0.58^{aAB}	2.18±0.10^{aAB}	1.80±0.22^{aB}
C18:2C	3	1.20±0.05^{aA}	1.21±0.04^{bA}	1.15±0.32^{aA}	1.39±0.37^{aA}	1.22±0.03^{bA}
	90	1.27±0.01^{aB}	1.53±0.05^{aAB}	1.63±0.40^{aA}	1.45±0.06^{aAB}	1.39±0.04^{aAB}
C18:3n3	3	0.55±0.14^{aAB}	0.63±0.05^{bAB}	0.47±0.04^{aB}	0.69±0.17^{aAB}	0.76±0.22^{aA}
	90	0.66±0.05^{aAB}	0.79±0.08^{aA}	0.68±0.19^{aA}	0.73±0.16^{aA}	0.41±0.18^{aB}

Table 5a. (*continued*)

	Storage (days)	FAC (%)[a] B-LFS pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
Trans FA	3	2.43±0.03[aB]	2.39±0.15[aB]	2.37±0.08[aB]	2.67±0.12[aA]	2.76±0.11[aA]
	90	2.48±0.11[aAB]	2.38±0.34[aAB]	2.34±0.13[aB]	2.66±0.06[aA]	2.41±0.27[aAB]
C18:1T	3	1.87±0.03[aC]	1.78±0.10[aC]	1.92±0.21[aBC]	2.16±0.06[aA]	2.10±0.05[aAB]
	90	1.91±0.05[aAB]	1.72±0.30[aB]	1.77±0.26[aAB]	2.14±0.06[aA]	1.72±0.25[aB]
C18:2T	3	0.56±0.05[aABC]	0.61±0.05[aAB]	0.45±0.14[aC]	0.51±0.06[aBC]	0.66±0.06[aA]
	90	0.58±0.12[aABC]	0.66±0.04[aAB]	0.47±0.14[aC]	0.52±0.03[aBC]	0.70±0.03[aA]
Total FA	3	99.47±2.80[aA]	99.69±2.65[aA]	98.74±3.02[aA]	98.73±2.15[aA]	99.97±1.84[aA]
	90	98.37±2.81[aA]	98.39±2.35[aA]	99.38±2.54[aA]	99.64±2.80[aA]	99.96±2.29[aA]

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column with the same fatty acids. [a]mean±S.D; n=3.

Table 5b. Effect of different pH values on FAC of C-LFS

	Storage (days)	FAC (%)[a] C-LFS pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
SFA	3	75.14±1.67[aA]	69.32±1.83[aB]	70.75±1.54[aB]	68.77±2.06[aB]	72.01±2.41[aAB]
	90	74.29±2.38[aA]	70.64±2.54[aA]	71.00±2.28[aA]	72.28±1.44[aA]	70.97±3.55[aA]
C4	3	3.38±0.18[aA]	1.50±0.21[bC]	2.46±0.26[bB]	2.49±0.18[bB]	3.45±0.25[aA]
	90	3.68±0.11[aB]	2.04±0.06[aD]	3.24±0.17[aC]	4.25±0.10[aA]	3.51±0.19[aB]
C6	3	2.37±0.21[bAB]	0.91±0.02[bC]	2.22±0.05[bB]	1.01±0.04[bC]	2.65±0.30[aA]
	90	3.27±0.13[aA]	1.38±0.25[aD]	2.73±0.23[aB]	2.86±0.13[aB]	2.38±0.19[aC]
C8	3	1.76±0.07[aB]	0.80±0.07[aD]	1.51±0.07[aC]	0.69±0.09[bD]	2.27±0.11[aA]
	90	1.53±0.16[aC]	0.69±0.10[aD]	1.52±0.05[aC]	1.73±0.08[aB]	1.98±0.12[bA]
C10	3	3.07±0.08[bB]	2.75±0.17[aBC]	3.72±0.07[aA]	2.47±0.44[bC]	4.07±0.42[aA]
	90	3.52±0.17[aB]	2.52±0.34[aC]	3.72±0.04[aAB]	4.14±0.37[aA]	3.75±0.13[aAB]
C11	3	0.46±0.06[aA]	0.28±0.06[aB]	0.41±0.10[aA]	0.28±0.03[bB]	0.42±0.06[aA]
	90	0.48±0.04[aA]	0.26±0.05[aC]	0.39±0.06[aB]	0.41±0.05[aAB]	0.40±0.01[aB]
C12	3	6.21±0.27[aA]	4.58±0.25[aC]	4.70±0.08[aBC]	4.61±0.17[aC]	5.09±0.40[aB]
	90	5.31±0.08[bA]	3.82±0.33[bD]	4.22±0.11[bC]	4.69±0.14[aB]	4.17±0.22[bCD]
C13	3	0.36±0.01[aA]	0.24±0.04[aB]	0.24±0.10[aB]	0.23±0.04[bB]	0.30±0.06[bAB]
	90	0.37±0.04[aBC]	0.22±0.05[aC]	0.38±0.15[aB]	0.59±0.08[aA]	0.70±0.06[aA]
C14	3	13.00±0.74[aA]	12.12±0.68[aAB]	11.95±0.62[aAB]	11.89±0.67[aAB]	11.47±0.69[aB]
	90	12.36±0.73[aA]	11.83±0.68[aA]	11.75±0.68[aA]	11.56±0.65[aA]	12.04±0.73[aA]
C15	3	1.29±0.23[aA]	1.42±0.21[aA]	1.36±0.13[aA]	1.29±0.19[aA]	1.28±0.32[aA]
	90	0.90±0.08[bB]	1.74±0.27[aA]	0.83±0.10[bB]	0.94±0.16[aB]	1.10±0.26[aB]
C16	3	33.34±1.03[aA]	33.27±0.89[aA]	30.50±0.80[aBC]	31.84±0.86[aAB]	30.07±0.91[aC]
	90	32.93±1.02[aB]	34.73±0.95[aA]	32.17±0.95[aB]	32.04±0.89[aB]	32.05±1.05[aB]
C17	3	0.83±0.05[aA]	0.81±0.06[aA]	0.85±0.08[aA]	0.79±0.30[aA]	0.89±0.10[aA]
	90	0.73±0.04[bA]	0.69±0.42[aA]	0.22±0.05[bB]	0.13±0.03[bB]	0.23±0.03[bB]
C18	3	9.07±0.58[aC]	10.65±0.52[aAB]	10.83±0.50[aAB]	11.28±0.46[aA]	10.03±0.55[aB]
	90	9.21±0.52[aB]	10.72±0.55[aA]	9.83±0.51[aAB]	8.94±0.51[bB]	9.76±0.54[aB]
US						
MUFA	3	19.11±0.67[aC]	23.69±1.14[aA]	22.99±0.93[aAB]	24.16±1.04[aA]	21.94±0.54[aB]
	90	18.73±1.10[aB]	22.23±0.51[aA]	21.06±1.04[aA]	21.77±1.21[aA]	21.25±1.29[aA]

Table 5b. (*continued*)

	Storage (days)	FAC (%)[a] C-LFS pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
C14:1	3	1.03±0.05[aB]	1.50±0.18[aA]	1.37±0.34[aA]	1.42±0.09[aA]	1.31±0.08[aAB]
	90	0.95±0.15[aBC]	0.45±0.06[bD]	1.09±0.05[aB]	0.91±0.08[bC]	1.32±0.12[aA]
C15:1	3	0.33±0.03[aA]	0.32±0.05[aAB]	0.23±0.04[aB]	0.34±0.09[aA]	0.23±0.04[bB]
	90	0.32±0.10[aB]	0.25±0.03[aB]	0.26±0.04[aB]	0.37±0.07[aB]	0.60±0.09[aA]
C16:1	3	1.68±0.25[aB]	2.63±0.06[aA]	2.49±0.35[aA]	2.64±0.26[aA]	2.62±0.36[aA]
	90	1.47±0.15[aB]	2.17±0.30[aA]	1.63±0.13[bB]	1.45±0.21[bB]	2.10±0.34[aA]
C17:1	3	0.13±0.04[aB]	0.14±0.06[bB]	0.13±0.01[bB]	0.18±0.03[aB]	0.38±0.05[aA]
	90	0.16±0.01[aB]	0.27±0.04[aA]	0.27±0.02[aA]	0.19±0.01[aB]	0.26±0.04[bA]
C18:1	3	15.94±1.03[aC]	19.11±0.89[aA]	18.78±0.82[aAB]	19.58±0.77[aA]	17.40±0.89[aBC]
	90	15.84±0.92[aC]	19.08±0.86[aA]	17.82±0.80[aAB]	18.85±0.85[aA]	16.97±0.95[aBC]
PUFA	3	1.92±0.07[aAB]	2.15±0.39[aAB]	1.64±0.27[aB]	2.31±0.43[aA]	2.00±0.37[aAB]
	90	2.02±0.11[aAB]	2.19±0.14[aA]	1.86±0.31[aB]	2.18±0.07[aA]	1.78±0.08[aB]
C18:2C	3	1.17±0.05[aBC]	1.70±0.29[aA]	0.97±0.09[aC]	1.66±0.54[aAB]	1.36±0.17[aABC]
	90	1.26±0.03[aB]	1.36±0.09[aAB]	1.04±0.19[aC]	1.50±0.09[aA]	1.23±0.14[aBC]
C18:3n3	3	0.75±0.06[aA]	0.45±0.09[bB]	0.67±0.19[aAB]	0.65±0.12[aAB]	0.64±0.20[aAB]
	90	0.76±0.14[aA]	0.83±0.05[aA]	0.82±0.12[aA]	0.69±0.03[aAB]	0.55±0.06[aB]
Trans FA	3	3.15±0.38[aB]	4.05±0.37[aA]	3.77±0.41[aA]	3.85±0.20[aA]	3.51±0.19[aAB]
	90	3.74±0.15[aB]	4.59±0.65[aA]	4.06±0.10[aAB]	3.56±0.06[aB]	3.65±0.18[aB]
C18:1T	3	2.59±0.33[aB]	3.67±0.33[aA]	3.46±0.45[aA]	3.43±0.10[aA]	3.19±0.49[aAB]
	90	3.18±0.20[aB]	4.16±0.40[aA]	3.81±0.05[aA]	3.24±0.09[aB]	3.16±0.14[aB]
C18:2T	3	0.56±0.05[aA]	0.37±0.04[aA]	0.31±0.04[aA]	0.42±0.10[aA]	0.32±0.30[aA]
	90	0.56±0.05[aA]	0.43±0.25[aABC]	0.25±0.05[aC]	0.31±0.03[aBC]	0.48±0.04[aAB]
Total FA	3	99.32±1.91[aA]	99.21±2.98[aA]	99.16±2.64[aA]	99.09±3.71[aA]	99.45±2.40[aA]
	90	98.78±3.52[aA]	99.64±3.56[aA]	97.98±3.73[aA]	99.80±2.64[aA]	97.65±5.03[aA]

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column with the same fatty acids. [a]mean±S.D; n=3.

trend during storage. The differences in our study could be explained by the experimental samples of previous authors were stored and measured at 20 and 30 °C without the tempering by heating at 100 °C·15 min^{-1}, and then 60 °C·15 min^{-1} before measuring by pNMR. Fatouh *et al*. (2003) determined the SFC of buffalo butter oil as a follows: 41.7, 34.6, 28.0, 18.6, 11.9, 9.6, 3.3 and 1.4 g·100g^{-1} at 0, 5, 10, 15, 20, 25, 30 and 35 °C; however, the differences between our results and those observed by previous authors may be due to seasonal variation, geographical location, the ratio of solid to liquid fat present, and the shape and size of the fat crystals.

The highest and lowest decreasing trends in the SFC values of each B-LFS and C-LFS with pH values were at 15–20 °C and 0–5 °C respectively; however, our results were in agreement with those observed by Nahid (2007). Furthermore, the decreasing rates in the SFC values of the pH treatments (C–LFS) at (0–5 °C), (10–15 °C), (20–25 °C) and (25–30 °C) were higher than in the B-LFS.

3.6. Rheological properties

3.6.1. Effects of pH values on the hardness of B-LFS and C-LFS

The effects of pH values on the hardness of the pH treatments are shown in Table 7. The texture evaluation showed that the differences in the hardness of each B-LFS and C-LFS with pH 5 compared to the control samples were slight, while the differences between other pH treatments compared to the control samples were noticeably decreasing ($P<0.05$); the decline in hardness was in the following order: pH 6>6.5>7. Furthermore, all the pH treatments had an increasing effects ($P<0.05$) during the storage periods, due to the slow post-crystallization processes and development of bonds within the fat crystal network that took place during storage, which resulted in an increase in the solidness of samples at 4 °C (Alexa *et al*. 2010). Kolanowski *et al*. (2004) noticed a slight increasing trend in the

TABLE 6A. Effect of different pH values on SFC of B-LFS

Temperature (°C)	Storage (days)	SFC (%)^{a}				
		B-LFS				
		pH 5.00	pH 5.50 (control)	pH 6.00	pH 6.50	pH 7.00
0	3	58.67±1.00^{bC}	58.97±0.60^{cBC}	59.50±1.22^{aABC}	60.97±0.78^{bA}	60.27±0.34^{bAB}
	30	60.37±0.52^{aA}	61.17±0.42^{bA}	60.77±0.78^{aA}	60.77±0.96^{bA}	60.13±0.38^{bA}
	60	58.80±0.82^{bC}	63.43±0.78^{aA}	60.03±0.76^{aBC}	60.10±0.40^{bB}	60.77±0.62^{abB}
	90	57.83±0.50^{bD}	59.10±0.38^{cC}	60.80±0.76^{aB}	62.60±0.68^{aA}	61.63±0.86^{aAB}
5	3	58.00±0.74^{bcB}	58.27±0.78^{bB}	57.80±0.34^{bB}	60.47±0.64^{bA}	59.40±0.30^{bA}
	30	60.03±0.60^{aAB}	60.57±0.36^{aA}	59.53±0.72^{aBC}	59.83±0.42^{bABC}	59.40±0.64^{bC}
	60	58.90±0.42^{abB}	61.47±0.60^{aA}	58.90±0.80^{abB}	59.70±0.32^{bB}	59.47±0.54^{bB}
	90	57.27±0.88^{cC}	58.07±0.76^{bC}	59.77±0.36^{aB}	62.07±1.00^{aA}	61.20±0.74^{aA}
10	3	51.43±0.74^{abC}	52.07±0.80^{bBC}	51.70±0.38^{bC}	54.23±0.78^{bA}	53.13±1.02^{abAB}
	30	52.67±0.74^{aA}	53.17±0.88^{abA}	52.50±1.06^{abA}	53.23±0.48^{bcA}	52.57±0.62^{bA}
	60	51.93±0.98^{abC}	54.43±0.62^{aA}	52.90±0.88^{abBC}	52.40±0.46^{cBC}	53.33±0.44^{abAB}
	90	50.60±0.80^{bD}	52.23±0.62^{bC}	53.93±0.70^{aB}	55.83±0.28^{aA}	54.47±0.98^{aB}
15	3	40.17±0.92^{aB}	40.97±0.36^{bAB}	40.20±0.72^{abB}	42.03±1.02^{abA}	40.90±0.84^{bAB}
	30	40.33±0.76^{aA}	40.47±0.38^{bcA}	40.00±0.60^{bA}	40.90±0.70^{bA}	40.27±0.62^{bA}
	60	39.90±0.74^{aB}	42.00±0.32^{aA}	41.03±0.40^{abA}	40.93±0.58^{bAB}	41.17±0.86^{abA}
	90	39.97±0.38^{aD}	40.13±0.60^{cC}	41.37±0.74^{aB}	43.33±0.84^{aA}	42.43±0.40^{aAB}
20	3	22.87±0.55^{aCD}	23.67±0.37^{aBC}	22.33±0.72^{bD}	25.17±0.62^{bA}	24.57±0.26^{abAB}
	30	23.17±0.65^{aA}	23.87±0.50^{aA}	23.40±0.71^{bA}	23.93±0.42^{cA}	23.37±0.35^{cA}
	60	23.83±0.54^{aB}	24.30±0.35^{aAB}	24.77±0.71^{aAB}	25.23±0.52^{bA}	24.37±0.43^{bAB}
	90	23.61±0.56^{aC}	23.83±0.40^{aC}	25.35±0.58^{aB}	26.48±0.44^{aA}	25.04±0.27^{aB}
25	3	14.13±0.45^{aB}	14.17±0.38^{aB}	13.17±0.63^{cC}	15.37±0.35^{aA}	14.50±0.28^{aB}
	30	14.07±0.59^{aAB}	14.17±0.43^{aAB}	13.47±0.50^{bcB}	14.37±0.56^{bA}	13.53±0.34^{bAB}
	60	14.23±0.58^{aC}	14.47±0.47^{aBC}	15.43±0.54^{aA}	15.37±0.52^{aAB}	14.50±0.45^{aBC}
	90	13.90±0.54^{aC}	14.00±0.46^{aC}	14.50±0.71^{abBC}	15.97±0.40^{aA}	15.07±0.30^{aAB}
30	3	6.77±0.55^{aC}	7.20±0.34^{aBC}	7.30±0.58^{bcBC}	8.23±0.35^{abA}	7.90±0.22^{aAB}
	30	7.23±0.35^{aAB}	7.27±0.34^{aAB}	6.67±0.31^{cB}	7.40±0.44^{cA}	6.67±0.19^{bB}
	60	7.43±0.26^{aB}	7.77±0.29^{aB}	8.47±0.44^{aA}	8.57±0.36^{aA}	7.63±0.26^{aB}
	90	7.07±0.35^{aB}	7.67±0.34^{aAB}	7.53±0.33^{bAB}	7.63±0.49^{bcAB}	8.03±0.39^{aA}
35	3	1.53±0.33^{aA}	1.57±0.29^{aA}	1.50±0.38^{aA}	2.00±0.27^{aA}	1.73±0.37^{aA}
	30	1.30±0.34^{aAB}	1.20±0.29^{abAB}	1.47±0.10^{abA}	1.13±0.27^{bAB}	0.93±0.24^{bB}
	60	1.43±0.33^{aB}	1.67±0.34^{aAB}	1.87±0.29^{aAB}	2.23±0.33^{aA}	1.63±0.32^{aB}
	90	1.43±0.20^{aB}	0.93±0.17^{bC}	1.00±0.13^{bC}	2.30±0.21^{aA}	1.63±0.24^{aB}

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. ^{a}mean±S.D; n=3.

hardness of the control sample and fish oil-enriched spreadable fat during storage, which is explained by the changes in the β-structure of fat crystals during storage. Furthermore, Glibowski *et al*. (2011) found that a slight increasing in the hardness during the storage of O/W emulsions with inulin. Although the hardness in all the pH treatments during storage was increased, it was shown that the hardness was decreased with an increase in pH from 5 to 7. This can be explained by the fact that the stability of B-LFS and C-LFS began to decrease at pH 6 to pH 7 (Fig. 1).

From 0 to 15 °C, the SFC of pH treatments (C-LFS) was higher than B-LFS [Table 6 (a and b)], whereas the C-LFS samples were softer than B-LFS from 30 to 35 °C, thus the SFC values at the 30–35 °C range affected the hardness of pH treatments more than in range of 0–15 °C. Therefore, the hardness of the pH treatments (B-LFS) were slightly higher than C-LFS. However, we also noticed that the pH treatments

Table 6b. Effect of different pH values on SFC of C-LFS

Temperature (°C)	Storage (days)	SFC (%)^{a}				
		C-LFS				
		pH 5.00	pH 5.50 (control)	pH 6.00	pH 6.50	pH 7.00
0	3	67.63±0.60^{bAB}	66.80±0.82^{bBC}	65.70±0.52^{bC}	68.47±0.98^{aA}	67.83±0.80^{bcAB}
	30	64.90±0.80^{cB}	66.30±0.34^{bAB}	66.37±1.08^{abA}	67.03±0.86^{bA}	66.67±0.68^{cA}
	60	68.33±0.62^{abBC}	70.00±0.92^{aA}	67.37±0.56^{aC}	69.33±0.32^{aAB}	69.33±0.94^{aAB}
	90	69.07±0.84^{aA}	68.87±0.58^{aA}	67.57±0.72^{aB}	69.23±0.38^{aA}	68.97±0.70^{abA}
5	3	63.67±0.90^{bBC}	63.30±0.72^{bBC}	62.67±0.84^{cC}	65.27±0.62^{bcA}	64.47±0.46^{bcAB}
	30	62.20±0.72^{bC}	63.67±0.54^{bAB}	63.23±0.66^{bcBC}	64.57±0.78^{cA}	63.77±0.64^{cAB}
	60	65.40±0.70^{aAB}	66.43±0.44^{aA}	64.40±0.74^{abB}	66.43±0.36^{abA}	65.57±0.84^{abA}
	90	66.30±0.88^{aAB}	66.17±0.46^{aAB}	64.93±0.78^{aB}	66.77±0.64^{aA}	66.03±0.98^{aAB}
10	3	57.37±1.04^{bB}	56.57±0.38^{bB}	56.67±0.66^{bcB}	58.83±0.60^{bA}	57.67±0.60^{bcAB}
	30	55.53±1.26^{cC}	57.10±0.66^{bABC}	56.23±0.98^{cBC}	58.43±0.40^{bA}	57.53±0.80^{cAB}
	60	58.60±0.80^{abBC}	59.93±0.76^{aAB}	57.97±0.66^{abC}	60.23±0.82^{aA}	59.03±0.62^{abABC}
	90	59.50±0.66^{aAB}	59.63±0.54^{aAB}	58.37±0.38^{aB}	60.30±0.86^{aA}	59.33±1.00^{aAB}
15	3	42.77±0.82^{bB}	43.20±0.80^{bcB}	43.00±0.64^{aB}	44.63±0.56^{abA}	43.03±0.90^{abB}
	30	41.07±0.58^{cC}	42.53±0.70^{cB}	40.80±0.22^{bC}	43.83±0.38^{bA}	42.73±0.72^{bB}
	60	43.93±0.56^{abB}	44.30±0.76^{abAB}	42.43±0.46^{aC}	45.23±0.86^{aA}	43.87±0.70^{abB}
	90	44.47±0.66^{aA}	45.10±0.38^{aA}	42.80±0.58^{aB}	45.57±0.94^{aA}	44.47±0.88^{aA}
20	3	24.33±0.50^{aB}	24.37±0.42^{abB}	23.20±0.57^{aC}	25.40±0.66^{abA}	24.20±0.53^{aB}
	30	22.53±0.44^{bC}	23.63±0.62^{bB}	22.90±0.39^{aBC}	24.77±0.47^{bA}	23.33±0.62^{aBC}
	60	24.27±0.52^{aB}	24.23±0.50^{abB}	23.40±0.44^{aB}	25.57±0.49^{abA}	24.13±0.54^{aB}
	90	25.14±0.48^{aB}	25.11±0.49^{aB}	23.67±0.43^{aC}	26.21±0.56^{aA}	24.27±0.45^{aBC}
25	3	14.47±0.41^{aBC}	15.17±0.40^{aAB}	14.37±0.40^{aC}	15.40±0.42^{aA}	14.43±0.52^{abBC}
	30	13.50±0.46^{bC}	14.47±0.40^{aAB}	14.17±0.41^{aBC}	15.30±0.51^{aA}	13.53±0.56^{bC}
	60	14.53±0.55^{aBC}	15.10±0.40^{aAB}	13.83±0.40^{aC}	15.73±0.53^{aA}	14.30±0.47^{abBC}
	90	14.47±0.58^{aB}	14.43±0.47^{aB}	13.93±0.47^{aB}	16.07±0.43^{aA}	14.50±0.50^{aB}
30	3	6.33±0.39^{aA}	6.57±0.44^{abA}	6.30±0.33^{aA}	7.03±0.50^{aA}	6.50±0.39^{aA}
	30	6.17±0.48^{aAB}	6.10±0.29^{bAB}	5.77±0.54^{aB}	6.80±0.41^{aA}	5.97±0.32^{aB}
	60	6.23±0.42^{aB}	6.40±0.22^{abB}	6.03±0.51^{aB}	7.27±0.34^{aA}	6.23±0.43^{aB}
	90	6.63±0.51^{aA}	6.67±0.20^{aA}	5.87±0.49^{aB}	7.00±0.41^{aA}	6.47±0.36^{aAB}
35	3	–	–	–	–	–
	30	–	–	–	–	–
	60	–	–	–	–	–
	90	–	–	–	–	–

Capital letters mean average values with different letters are statistically significant ($p<0.05$) within each row. Small letters mean average values with different letters are statistically significant ($p<0.05$) within each column. ^{a}mean±S.D; n=3.

(B-LFS) began to crystallize at a higher temperature than C-LFS (data not shown). Therefore, it is clear that both the solidifying point and the crystallization are responsible for the hardness test results. The range of the solidifying point for buffalo milk fat (16.0–28.0 °C) was higher than for cow milk fat (15.0–23.5 °C) (Patel *et al.* 2002), thus the fat phase affected the texture evaluation of B-LFS and C-LFS.

3.6.2. Effects of pH values on the viscosity of B-LFS and C-LFS

Table 8 shows the effects of pH values on the viscosity of B-LFS and C-LFS. The differences in viscosity of the treatments (B-LFS and C-LFS) with pH 5 were negligible; while with pH 6, 6.5 and 7 they were noticeably decreased ($P<0.05$) as compared

TABLE 7. Effect of different pH values on the hardness (g)[a] of B-LFS and C-LFS

Storage periods (days)	pH 5	pH 5.5 (control)	pH 6	pH 6.5	pH 7
			B-LFS		
3	54.96±0.61^{dA}	53.07±0.84^{dB}	37.21±0.35^{dC}	37.85±0.38^{cC}	35.48±0.26^{dD}
30	58.42±0.61^{cA}	55.33±0.85^{cB}	38.89±0.34^{cC}	38.24±0.30^{cCD}	37.36±0.29^{cD}
60	61.43±0.71^{bA}	62.21±1.00^{bA}	41.06±0.80^{bB}	39.16±0.28^{bC}	39.00±0.36^{bC}
90	66.70±0.61^{aA}	64.96±0.93^{aB}	43.02±0.38^{aC}	40.85±0.48^{aD}	39.65±0.26^{aE}
			C-LFS		
3	51.17±0.59^{dA}	51.70±0.61^{bA}	38.07±1.03^{bB}	36.77±0.96^{cB}	34.69±0.57^{cC}
30	54.26±0.65^{cA}	52.25±0.51^{bB}	38.08±0.57^{bC}	37.38±0.72^{bcC}	36.23±0.43^{bD}
60	58.66±0.71^{bB}	62.04±0.55^{aA}	39.13±0.71^{abC}	38.54±0.71^{bC}	37.97±0.58^{aC}
90	64.22±0.51^{aA}	62.87±0.47^{aB}	40.56±0.73^{aC}	40.11±0.77^{aC}	38.05±0.47^{aD}

Capital letters mean average values with different letters are statistically significant (p<0.05) within each row. Small letters mean average values with different letters are statistically significant (p<0.05) within each column. [a]mean±S.D; n=3.

TABLE 8. Effect of different pH values on the apparent viscosity [η_{app} (Pa s) at 100 γ^{s-1}][a] of B-LFS and C-LFS

Storage periods (days)	pH 5	pH 5.5(control)	pH 6	pH 6.5	pH 7
			B-LFS		
3	0.34±0.04^{cA}	0.36±0.06^{cA}	0.21±0.03^{bB}	0.18±0.03^{bB}	0.14±0.03^{cB}
30	0.41±0.05^{bcA}	0.43±0.07^{bcA}	0.23±0.03^{bB}	0.20±0.05^{bB}	0.19±0.04^{bcB}
60	0.49±0.06^{abA}	0.50±0.04^{abA}	0.28±0.06^{bB}	0.30±0.07^{aB}	0.25±0.03^{bB}
90	0.59±0.07^{aA}	0.58±0.06^{aA}	0.37±0.04^{aB}	0.37±0.04^{aB}	0.33±0.05^{aB}
			C-LFS		
3	0.27±0.04^{cA}	0.26±0.02^{cA}	0.20±0.01^{bB}	0.16±0.04^{cB}	0.16±0.05^{bB}
30	0.39±0.06^{Ba}	0.39±0.07^{bA}	0.26±0.06^{abB}	0.22±0.06^{bcB}	0.18±0.05^{bB}
60	0.47±0.07^{abA}	0.46±0.04^{abA}	0.28±0.04^{abB}	0.27±0.03^{abB}	0.25±0.05^{abB}
90	0.52±0.02^{aA}	0.52±0.03^{aA}	0.34±0.06^{aB}	0.32±0.06^{aB}	0.30±0.08^{aB}

Capital letters mean average values with different letters are statistically significant (p<0.05) within each row. Small letters mean average values with different letters are statistically significant (p<0.05) within each column. [a]mean±S.D; n=3.

to the control samples. The trends of pH from 7 to 5 were accompanied by a reduction in the negative charge on the protein, which results in a large increase in the viscosity of B-LFS and C-LFS due to partial aggregation (Keogh 2006). Furthermore, phase separation occurred with pH 6, 6.5 and 7 in both B-LFS and C-LFS, and the phase separation was increased with an increase in the pH from 6 to 7, therefore the phase separation affected the viscosity values. Moreover, the differences in all pH treatments during the storage periods were increased significantly (P<0.05) due to the post-crystallization processes (Alexa *et al*., 2010), and to the changes in the β-structure of fat crystals during the storage periods (Kolanowski *et al*., 2004). However, Glibowski *et al*. (2011) found that the viscosity of O/W emulsions with inulin increased during storage due to the conformational changes in the inulin chains.

The SFC values at 25 °C in each B-LFS and C-LFS with pH values were similar, while both C-LFS and B-LFS samples were completely melted at 30 and 35 °C respectively [Table 6 (a and b)]; therefore the pH treatments (B-LFS) were greater than C-LFS in total high melting species, which was in correlation with the hardness and viscosity results (Aguedo *et al*. 2008), and the viscosity values of the pH treatments (B-LFS) were slightly higher than C-LFS. However, our results have shown that the samples, which had the same SFC values, various crystal types and/or network structures that are formed upon crystallization of hard fats can result in variability in hardness and therefore the viscosity (Braipson-Danthine and Deroanne, 2004). The viscosity was highly correlated with the hardness of samples (Glibowski *et al*., 2008); however, there are studies that reported an increase in viscosity during storage, for instance Glibowski *et al*. (2011).

3.7. Effects of pH values on the melting behavior of B-LFS and C-LFS

The melting thermogram of the samples before and after 90 days from the storage periods are presented in Fig. 2. The differences in temperatures of the endothermic zones of each B-LFS and C-LFS were negligible with an increase in pH from 5 to 7, while the endothermic zones of F were only detected with pH 6.5 (C-LFS at 3 days) but disappeared after 90 days of storage.

The differences in the temperatures of the deepest peaks (A and G) for pH treatments (B-LFS and C-LFS separately and together) were slight with an increase pH values and during the storage periods. In addition, we noticed that the intermediate zones of C (at 3 days) were shifted to the endothermic zones of B and D after 90 days from the beginning of storage. Furthermore, the endothermic zones of K were detected with pH 5.5, 6 and 7 with C-LFS at 90 days from the beginning of storage. With regard to the intermediate zones of C and H (at 3 days) we found that the temperatures were with in C and H and together were close to each other. Moreover, the differences in the temperature ranges of high melting zones (E and L separately) among the pH treatments and during the storage periods were slight; however, the temperature ranges for the endothermic zones of E (B-LFS samples) were greater than L (C-LFS samples). This mean a slight increase in total high melting species (Aguedo *et al*. 2008), which was in total agreement with the hardness and viscosity. The temperature ranges of the high melting zones in our experiments resembled those observed for the melting behavior of the $CaCl_2$ and NaCl treatments (data not shown).

4. CONCLUSIONS

The sensory evaluation scores showed that B-LFS and C-LFS with pH values were accepted by panelists. The pH values did not have any influence on protecting of samples against oxidation. No significant differences were observed in the OIT between the pH treatments (B-LFS and C-LFS separately) and the control samples, while the OIT significantly decreased ($P<0.05$) during the storage periods. An increase in the pH values were accompanied by changes in the FAC of the pH treatments. In addition, the pH values did not affect the SFC among B-LFS and C-LFS separately or during the storage periods. An increase in the pH values led to a decrease in both the hardness and viscosity, and in turn had an increased effect during the storage periods. The changes in the melting profile between pH treatments (B-LFS and C-LFS separately) and during the storage periods were slight; however, there were differences in the melting behavior between B-LFS and C-LFS together.

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REFERENCES

Aguedo M, Hanon E, Danthine S, Paquot M, Lognay G, Thomas A, Vandenbol M, Thonart P, Wathelet J.-P, Blecker C. 2008. Enrichment of anhydrous milk fat in polyunsaturated fatty acid residues from linseed and rapeseed oils through enzymatic interesterification. *J. Agric. Food Chem*. **56**, 1757–1765. http://dx.doi.org/10.1021/jf0722203.

Alexa RI, Mounsey JS, O'Kennedy BT, Jacquier JC. 2010. Effect of k-carrageenan on rheological properties, microstructure, texture and oxidative stability of water-in-oil spreads. *LWT- Food Sci. Technol*. **43**, 843–848.

Basaran T, Coupland J, McClements D. 1999. Monitoring molecular diffusion of sucrose in xanthan solutions using ultrasonic velocity measurements. *J. Food Sci*. **64**, 125–128. http://dx.doi.org/10.1111/j.1365-2621.1999.tb09874.x.

Bot A, Vervoort S. 2006. Gums and stabilisers for the food industry 13. In P. Williams, G. Phillips, eds. *Royal Society of Chemistry*, Cambridge pp. 381–394.

Braipson-Danthine S, Deroanne C. 2004. Influence of SFC, microstructure and polymorphism on texture (hardness) of binary blends of fats involved in the preparation of industrial shortenings. *Food Res. Int*. **37**, 941–948. http://dx.doi.org/10.1016/j.foodres.2004.06.003.

Britton G. 1995. Structure and properties of carotenoids in relation to function. *The FASEB J*. **9**, 1551–1558.

Cheng L, Lim B, Chow K, Chong S, Chang Y. 2008. Using fish gelatin and pectin to make a low-fat spread. *Food hydrocolloids* **22**, 1637–1640. http://dx.doi.org/10.1016/j.foodhyd.2007.10.006.

Fatouh A, Singh R, Koehler P, Mahran G, El-Ghandour M, Metwally A. 2003. Chemical and thermal characteristics of buffalo butter oil fractions obtained by multi-step dry fractionation. *LWT- Food Sci. Techno*l. **36**, 483–496.

Firestone D. 2004. *Official methods and recommended practices of the American Oil Chemists' Society, 5th edn*. American Oil Chemists' Society, Champaign.

GB/T 17376. 2008. *Animal and vegetables fats and oils: Preparation of methyl esters of fatty acids*. pp. 5–6.

Glibowski P, Kordowska-Wiater M, Glibowska A. 2011. Effect of storage on texture and microbiological stability of O/W emulsions with inulin. *Czech J. Food Sci*. **29**, 137–144.

Glibowski P, Zarzycki P, Krzepkowska M. 2008. The rheological and instrumental textural properties of selected table fats. *Int. J. Food Prop*. **11**, 678–686. http://dx.doi.org/10.1080/10942910701622599.

Haggag H, Hamzawi L, Shahin Y. 1987. Fatty acid composition of globule core lipids from Egyptian cow, buffalo and goat's milk. *Egypt. J. Dairy Sci*. **15**, 25–30.

Janssens L, Muyldermans G. 1994. Gelatin-a modern food ingredient in food technology international europe. In A. Turner, ed. *London Sterling Publications Ltd*, pp. 133–137.

Keogh M. 2006. Chemistry and technology of butter and milk fat spreads. In Advanced Dairy Chemistry Volume 2 Lipids. Springer, pp. 333–363.

Kolanowski W, Swiderski F, Jaworska D, Berger S. 2004. Stability, sensory quality, texture properties and nutritional value of fish oil-enriched spreadable fat. *J. Sci. Food Agric*. **84**, 2135–2141. http://dx.doi.org/10.1002/jsfa.1770.

Krause A, Miracle R, Sanders T, Dean L, Drake M. 2008. The effect of refrigerated and frozen storage on butter flavor and texture. *J. Dairy Sci*. **91**, 455–465. http://dx.doi.org/10.3168/jds.2007-0717.

Kristensen D, Boesen M, Jakobsen U, Månsson L, Erichsen L, Skibsted L. 2000. Oxidative and colour stability of salted sour cream dairy spread compared to salted sweet cream dairy spread. *Milchwissenschaft* **55**, 504–507.

Laia O, Ghazalia H, Cho F, Chong C. 2000. Physical and textural properties of an experimental table margarine prepared from lipase-catalysed transesterified palm stearin: Palm kernel olein mixture during storage. *Food Chem*. **71**, 173–179. http://dx.doi.org/10.1016/S0308-8146(00)00084-4.

Madsen F. 2000. Substitution of gelatin in low-fat spread: a rheological characterization. *Sp. Pub. Roy. Soc. Chem*. **251**, 411–420.

Mageean P, Jones S. 1989. Low-fat spread products. *Food Sci. Technol. Today* **3**, 162–164.

Mallia S. 2008. Oxidative stability and aroma of UFA/CLA (unsaturated fatty acids/conjugated linoleic acid) enriched butter. In Diss Eidgenössische Technische Hochschule ETH Zürich, Nr. 18020.

Moran D. 1991. Developments in yellow fat spreads. *Chem. Ind*. **3**, 379–383.

Nahid A. 2007. Modeling Heat Transfer in Butter Products: A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy (Ph. D.) in Bioprocess Engineering, Institute of Technology and Engineering, Massey University, Palmerston North, New Zealand. Massey University, Palmerston North.

Patange D, Patel A, Singh R, Patil G, Bhosle D. 2013. Storage related changes in ghee-based low-fat spread. *J. Food Sci. Technol*. **50**, 346–352. http://dx.doi.org/10.1007/s13197-011-0339-7.

Patel A, Gupta V, Singh S, Singh A. 2002. Advances in fat-rich dairy products. National Dairy Research Institute (ICAR), Karnal-132001.

Richardson D. 1990. Food fortification. *Proc. Nutr. Soc*. **49**, 39–50. http://dx.doi.org/10.1079/PNS19900007.

Samet-Bali O, Ayadi M, Attia H. 2009. Traditional Tunisian butter: Physicochemical and microbial characteristics and storage stability of the oil fraction. *LWT-Food Sci. Technol*. **42**, 899–905.

Stathopoulos CE, Chockchaisawasdee S, Doyle J, O'Kennedy BT, Mounsey JS. 2009. Effect of mineral fortification on textural and oxidative stability of reduced-fat spreads. *Int. J. Food Prop*. **12**, 368–378. http://dx.doi.org/10.1080/10942910701799231.

Varricchio M, Di Francia A, Masucci F, Romano R, Proto V. 2007. Fatty acid composition of Mediterranean buffalo milk fat. *Ital. J. Anim. Sci*. **6**, 509–511.