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Effects of NaCl on pH, Peroxide Value, Oil Stability Index and Fatty Acid Composition of Buffalo's and Cow's Butter-Based Low-Fat Spreads.

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ABSTRACT

Buffaloe's butter-based low-fat spread (B-LFS) and Cow's butter-based low-fat spread (C-LFS) prepared by Buffaloe's and Cow's butter oil 40%, DIMODAN®HP-C distilled monoglyceride 0.5%, halal gelatin 2%, skim milk powder 1%, NaCl (0, 0.5, control (1), 1.5 and 2%), k-sorbate 0.1% and distilled water (100-ingredients together) and pH adjusted to 5.5. The treatments stored at 4 °C, and sampled after 3, 30, 60 and 90 days. The objective of this work was to evaluate the effects of NaCl on the pH, peroxide value (PV), oil stability index (OSI) and fatty acid composition (FAC). The pH values showed slightly differences among NaCl treatments (B-LFS and C-LFS) separately compared control samples. In addition, pH values of all treatments significantly decreased ($P < 0.05$) during storage periods. An increase of NaCl hadn't effects on PVs among B-LFS and CLFS separately. Furthermore, during storage periods, we noticed PVs increased noticeably ($P < 0.05$). The OSI values for all NaCl treatments declined with increasing of NaCl from 0 to 2%, and OSI values had decreased effects ($P < 0.05$) during storage periods. With regard to the FAC, we noticed changes in FAC between NaCl treatments and during the storage periods.

INTRODUCTION

Obviously, consumers have been reducing their fat consumption due to health concerns surrounding animal saturated fats and high-fat diets. This trend has affected several dairy ingredients including butter. In addition, Butter production has decreased from 596.6 to 563.5 million kg in the last 10 year ^[1] due to several reasons, mainly nutritional and economical. Gradually the people of Europe have responded to the call for a reduced intake of fat in diet, both because of health considerations and the desire to be slim and today reduced calorie products account for almost a tenth of the total food consumption.

Before, there was an opportunity for researchers to modify the formulation of staple dairy foods (such as butter) ideally without any major loss of the inherent sensory properties, because of obesity and dietary-related diseases ^[2].

The first low-fat spread products were composed of fat, water and emulsifiers only ^[3]. Such products had poor sensory qualities, with a pronounced fatty taste and poor flavor release characteristics, due to the ineffective breaking down of the products in the mouth. Improvements in sensory properties of low-fat spreads have been brought about by the use of hydrocolloids in the aqueous phase. However, as fat level is reduced, the influence of the aqueous phase on taste and emulsion stability becomes increasingly important ^[4, 5] and structuring agents are used in the aqueous phases of most commercial low-fat spreads.

Low fat butter spreads required to four types of structuring agents have been identified ^[6] to provide the required structure alongside a 'plastic' flow ^[7] as a following: 1) viscous (milk proteins or high-molecular weight polysaccharides), 2) gelling (hydrocolloids used to gel the aqueous phase), 3) phase-separating (with

thermodynamically incompatible hydrocolloids) and 4) synergistic (exploiting known synergistic interactions between hydrocolloids).

The present paper describes the effects of sodium chloride on PV, OSI, SFC and FAC of Buffalo's and Cow's butter-based low-fat spread.

MATERIALS AND METHODS

Materials

Buffaloe's butter (83.48% fat, solid not fat 2.91%, moisture 13.61% and peroxide value 0.145) was obtained from Department of Dairy Science, Faculty of Agriculture, Suez Canal University (Ismailia, Egypt). Cow's butter (82.68% fat, solid not fat 1.75%, moisture 15.57% and peroxide value 0.135), skim milk powder and sodium chloride (table salt) were purchased from a local market at Wuxi (Jiangsu, China). Halal gelatin (80-280 BLOOM) was purchased from Gelatin & Protein Co., Ltd. (Hangzhou, China). DIMODAN®HP-C distilled monoglyceride 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.

Preparation of Buffalo's and Cow's butter oil

Buffaloe's and Cow's butter were melted at 50 °C instead of 60 °C and the top oil layer was decanted and filtered through glass wool. The oil was then refiltered under vacuum (Whatman No.1) to obtain clear Buffalo's and Cow's butter oil [8].

Preparation of B-LFS and C-LFS with different NaCl concentrations

The recipe of making NaCl treatments (B-LFS and C-LFS) was according to Madsen [9] with some modifications. The treatments contained the following ingredients in percentage (w/w): Buffalo's and Cow's butter oil 40%, DIMODAN®HP-C distilled monoglyceride 0.5%, halal gelatin 2%, skim milk powder 1%, sodium chloride (0, 0.5, 1, 1.5 and 2%), k-sorbate 0.1% and distilled water (100-ingredients together). The steps of preparation B-LFS and C-LFS with different NaCl concentrations were as a following:

- The ingredients of water phase (halal gelatin, skim milk powder, NaCl and k-sorbate) were blended together into the distilled water at 70 °C for 10 min by JJ-1B Electric Blender (Changzhou Runhua Electric Appliance Co., Ltd, China).
- The temperature of water phase reduced from 70 to 40 °C, and the pH adjusted to 5.5 [with citric acid 20% (w/w)] with the stirring by JJ-1B Electric Blender.
- With regard to fat phase, a portion from melted butter oil (~5 × the weight of the emulsifier) was removed and heated to 70 °C with the stirring until dissolving of the emulsifier, and then added back to the melted Buffalo's and Cow's butter oil at 40 °C .
- The water phase was then slowly added to the fat phase and mixed using a homogenizer (IKA® T18 Basic ULTRA-TURRAX®, Germany) for 5 min at a speed No. 2.
- Pasteurization at 75 °C for 10 min in water bath and the mixture temperature decreased to 60 °C with the stirring by JJ-1B Electric Blender.
- The homogenization by laboratory Homogenizer (Model: GYB, Donghua High Pressure Homogenizer Factory, Shanghai, China) at a pressure 17 MPa and 60 °C (one step) has been done.
- The samples were kept in sterilized plastic cups (30 g) at room temperature for 15 h and moved to the refrigerator (4 °C).

pH values

The pH of NaCl treatments (B-LFS and C-LFS) were measured by pH meter (Mettler Toledo FE20, China). The samples moved out from the refrigerator and stayed 2 h at the room temperature, and then the pH measured. All of experiments were carried out in triplicate and mean results are reported.

PV

The PVs was modified from International Dairy Federation (IDF) Standard 74:1974 [10]. Briefly, samples of NaCl 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. Following 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

Whatman No. 4 filter paper to obtain clear fat. A 0.1 mL of melted fat was dissolved into 10 mL of chloroform/methanol (70:30) mixture, followed by addition of ammonium thiocyanate (0.05 mL) and ferrous chloride (0.05 mL) respectively. Using glass stoppers, the tubes were inverted and placed in dark cupboard for 10 min. Simultaneously, 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 experiments were carried out in triplicate and mean results are reported.

$$OD = Ab_{Ssample} - Ab_{Sstandard} \quad (1)$$

where, OD is the optical density.

OSI

The oxidation induction time (OIT) of extracted fat (see PV) was determined by the AOCS method Cd 12b-92^[11] with the Rancimat 743 apparatus (Metrohm AG, Herison, Switzerland). The samples were performed in duplicate 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/h. The induction time is defined as the time necessary to reach the inflection point of the conductivity curve.

FAC

Weighed 60 mg from the melted fat (extracted according to 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 into the test tube. The mixtures were vigorously shaken for 2 min, and then 1 g NaHSO₄ 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 in a high speed centrifuge (TGL-16B, Shanghai Anting scientific factory, China) for 10 min at 10,000 rpm. A 1 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 of injector and detector temperatures were set at 250 °C. The column oven temperature were as follows: 45 °C for 4 min, raised at 13 °C/min to 175 °C, held for 27 min, raised at 4 °C/min to 215 °C, held for 20 min. Nitrogen was the carrier gas. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analyzed under the same conditions. Results were expressed as w/w (%) total fatty acid.

Statistical analysis

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

RESULTS AND DISCUSSION

Effects of NaCl concentrations on pH of B-LFS and C-LFS

Effects of different NaCl concentrations on pH values of B-LFS and CLFS are presented in Table 1. The differences among B-LFS and C-LFS separately with concentrations of NaCl were slightly compared control samples. Also, we found pH values of NaCl treatments (B-LFS and C-LFS) significantly decreased during the storage periods. In addition, there are slight an increase in pH values of NaCl treatments from 3 to 30 days, but after 30 days until 90 days, we noticed pH values decreased. The declining in pH values of NaCl treatments, were no large between the beginning and end of storage periods, due to storing of NaCl treatments at 4 °C, preservative (k-sorbate) and the pasteurization. Our results are in agreement with Glibowski et al.^[12] who noticed that a slight decreasing in pH values, which resulted from microbiological growth during storage of O/W emulsions with inulin. Samet-Bali et al.^[13] found that during storage (~6 weeks) of traditional Tunisian butter at 4 and 10 °C, the pH decreased, due to the number and/or metabolic activity of acid-producing microorganisms. Furthermore, we noticed that the pH values significantly decreased during storage periods (3 to 90 days) of CaCl₂ and pH treatments (unpublished report). In contrary, Dalaly et al, Spurgeon et al, Balasubramanyam and Kulkarni, Patange et al.^[14-17] found that an increased pH in stored spread.

Table 1. Effects of different NaCl concentrations on pH values of B-LFS and C-LFS

Storage (Days)	pH values ^a									
	B-LFS					C-LFS				
	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%
3	5.68±0.02 ^{aA}	5.62±0.02 ^{bB}	5.53±0.02 ^{bC}	5.57±0.03 ^{bC}	5.64±0.02 ^{aB}	5.65±0.03 ^{bA}	5.57±0.02 ^{bB}	5.52±0.02 ^{bC}	5.55±0.04 ^{abBC}	5.54±0.03 ^{bBC}
30	5.70±0.02 ^{aA}	5.67±0.02 ^{aAB}	5.59±0.02 ^{aC}	5.65±0.01 ^{aB}	5.67±0.02 ^{aB}	5.77±0.02 ^{aA}	5.62±0.02 ^{aB}	5.58±0.01 ^{aC}	5.57±0.03 ^{aC}	5.62±0.02 ^{aB}
60	5.67±0.05 ^{aA}	5.63±0.02 ^{abA}	5.53±0.03 ^{bBC}	5.48±0.03 ^{cC}	5.55±0.01 ^{bB}	5.62±0.02 ^{bA}	5.51±0.01 ^{cB}	5.51±0.02 ^{bB}	5.52±0.02 ^{bB}	5.51±0.04 ^{bB}
90	5.50±0.03 ^{bA}	5.41±0.03 ^{cB}	5.43±0.04 ^{cB}	5.44±0.04 ^{cAB}	5.41±0.04 ^{cB}	5.48±0.03 ^{cA}	5.42±0.02 ^{dC}	5.39±0.02 ^{cC}	5.42±0.02 ^{cBC}	5.46±0.02 ^{cAB}

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

Table 2. Effects of different NaCl concentrations on PVs of B-LFS and C-LFS

Storage (Days)	PV values ^a									
	B-LFS					C-LFS				
	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%
3	0.222±0.024 ^{dA}	0.226±0.016 ^{cA}	0.239±0.011 ^{dA}	0.228±0.025 ^{dA}	0.233±0.021 ^{dA}	0.197±0.021 ^{cA}	0.182±0.009 ^{cA}	0.180±0.010 ^{cA}	0.189±0.024 ^{cA}	0.193±0.022 ^{cA}
30	0.295±0.012 ^{cAB}	0.284±0.019 ^{bB}	0.325±0.014 ^{cA}	0.279±0.024 ^{cB}	0.285±0.019 ^{cB}	0.246±0.013 ^{bB}	0.262±0.017 ^{bAB}	0.289±0.017 ^{bA}	0.277±0.023 ^{bAB}	0.291±0.016 ^{bA}
60	0.385±0.019 ^{bA}	0.381±0.024 ^{aA}	0.391±0.026 ^{bA}	0.387±0.012 ^{bA}	0.398±0.011 ^{bA}	0.275±0.021 ^{bB}	0.288±0.016 ^{bAB}	0.300±0.016 ^{bAB}	0.310±0.026 ^{bA}	0.317±0.014 ^{bA}
90	0.420±0.016 ^{aA}	0.409±0.024 ^{aA}	0.428±0.011 ^{aA}	0.435±0.021 ^{aA}	0.441±0.017 ^{aA}	0.351±0.021 ^{aA}	0.342±0.021 ^{aA}	0.358±0.026 ^{aA}	0.365±0.010 ^{aA}	0.370±0.010 ^{aA}

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

Table 3. Effects of NaCl concentrations on OSI values of B-LFS and C-LFS

Storage (Days)	OSI values (h) ^a									
	B-LFS					C-LFS				
	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%
3	4.64±0.14 ^{aA}	4.45±0.15 ^{aA}	4.37±0.18 ^{aA}	4.09±0.17 ^{aB}	3.99±0.09 ^{aB}	5.73±0.16 ^{aA}	5.62±0.14 ^{aA}	5.24±0.18 ^{aB}	4.70±0.08 ^{aC}	4.45±0.17 ^{aC}
30	4.56±0.13 ^{aA}	4.40±0.11 ^{aAB}	4.25±0.16 ^{abB}	4.02±0.04 ^{abC}	3.96±0.12 ^{aC}	5.53±0.11 ^{bA}	5.42±0.16 ^{abA}	5.04±0.10 ^{abB}	4.54±0.08 ^{aC}	4.35±0.10 ^{aC}
60	4.45±0.08 ^{aA}	4.25±0.12 ^{abB}	4.21±0.09 ^{abB}	3.91±0.09 ^{abC}	3.86±0.10 ^{abC}	5.33±0.06 ^{cA}	5.24±0.10 ^{bC}	4.88±0.08 ^{bC}	4.29±0.11 ^{bC}	4.24±0.10 ^{abC}
90	4.44±0.12 ^{aA}	4.14±0.06 ^{bB}	4.02±0.12 ^{bBC}	3.84±0.16 ^{bCD}	3.66±0.17 ^{bD}	5.16±0.07 ^{cA}	5.08±0.14 ^{cA}	4.68±0.18 ^{cB}	4.17±0.12 ^{bC}	4.07±0.15 ^{bC}

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

Table 4. Effects of different NaCl concentrations on FAC of B-LFS and LFS

Storage (Days)		FAC ^a									
		B-LFS					C-LFS				
		NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%	NaCl 0%	NaCl 0.5%	NaCl 1% (Control)	NaCl 1.5%	NaCl 2%
S	3	67.26±3.15 ^{AB}	65.70±0.49 ^{AB}	68.89±1.56 ^{AB}	69.68±0.96 ^{AB}	66.73±1.43 ^{AB}	71.55±0.86 ^{AB}	69.30±1.31 ^{AB}	68.68±1.35 ^{AB}	69.67±1.48 ^{AB}	69.93±1.63 ^{AB}
	90	67.80±2.22 ^{AB}	65.53±2.33 ^{AB}	66.62±2.38 ^{AB}	68.24±2.28 ^{AB}	68.17±1.50 ^{AB}	71.27±0.78 ^{AB}	69.38±2.38 ^{AB}	71.15±1.56 ^{AB}	69.11±1.42 ^{AB}	69.08±1.98 ^{AB}
C4	3	3.01±0.07 ^{AB}	1.72±0.20 ^{BD}	3.95±0.28 ^{AB}	3.28±0.24 ^{AB}	2.21±0.14 ^{BC}	1.20±0.22 ^{BC}	1.61±0.33 ^{BC}	1.91±0.24 ^{AB}	1.36±0.21 ^{BC}	2.29±0.23 ^{AB}
	90	3.52±0.12 ^{AB}	2.31±0.19 ^{BD}	2.87±0.34 ^{BC}	3.02±0.14 ^{BC}	3.96±0.17 ^{AB}	3.10±0.21 ^{AB}	1.98±0.16 ^{BC}	3.19±0.15 ^{AB}	2.08±0.27 ^{BC}	2.42±0.20 ^{AB}
C6	3	1.08±0.05 ^{AB}	0.87±0.23 ^{AB}	1.50±0.31 ^{AB}	1.12±0.07 ^{AB}	0.80±0.16 ^{AB}	1.19±0.25 ^{AB}	1.28±0.09 ^{AB}	0.79±0.10 ^{BC}	1.16±0.10 ^{AB}	1.75±0.13 ^{AB}
	90	1.46±0.08 ^{AB}	0.91±0.09 ^{AB}	1.38±0.25 ^{AB}	1.58±0.17 ^{AB}	1.36±0.20 ^{AB}	2.14±0.25 ^{AB}	1.37±0.19 ^{AB}	2.18±0.17 ^{AB}	1.36±0.12 ^{AB}	1.54±0.10 ^{AB}
C8	3	0.70±0.18 ^{AB}	0.49±0.06 ^{BC}	0.89±0.12 ^{AB}	0.79±0.12 ^{AB}	0.46±0.06 ^{BC}	1.12±0.07 ^{AB}	1.02±0.08 ^{ABC}	0.90±0.15 ^{BC}	0.92±0.05 ^{ABC}	1.08±0.08 ^{AB}
	90	0.82±0.22 ^{AB}	0.49±0.11 ^{AB}	0.63±0.03 ^{AB}	0.76±0.06 ^{AB}	0.68±0.12 ^{AB}	1.36±0.19 ^{AB}	0.94±0.07 ^{AB}	1.37±0.17 ^{AB}	0.95±0.11 ^{AB}	1.07±0.10 ^{AB}
C10	3	1.83±0.13 ^{AB}	1.50±0.30 ^{AB}	1.73±0.11 ^{AB}	2.10±0.35 ^{AB}	1.07±0.11 ^{AB}	3.38±0.32 ^{AB}	2.65±0.14 ^{AB}	3.42±0.20 ^{AB}	2.62±0.32 ^{AB}	3.44±0.12 ^{AB}
	90	1.51±0.17 ^{AB}	1.09±0.28 ^{BC}	1.29±0.11 ^{BC}	2.34±0.23 ^{AB}	1.40±0.28 ^{BC}	3.11±0.34 ^{AB}	2.44±0.24 ^{AB}	3.08±0.23 ^{AB}	2.43±0.12 ^{AB}	2.67±0.31 ^{AB}
C11	3	0.09±0.02 ^{AB}	0.11±0.04 ^{AB}	0.13±0.02 ^{AB}	0.08±0.01 ^{AB}	0.07±0.02 ^{AB}	0.39±0.12 ^{AB}	0.33±0.02 ^{AB}	0.36±0.07 ^{AB}	0.32±0.07 ^{AB}	0.36±0.05 ^{AB}
	90	0.10±0.03 ^{AB}	0.08±0.02 ^{AB}	0.09±0.02 ^{AB}	0.11±0.04 ^{AB}	0.08±0.02 ^{AB}	0.37±0.07 ^{AB}	0.28±0.07 ^{AB}	0.37±0.05 ^{AB}	0.25±0.09 ^{AB}	0.28±0.06 ^{AB}
C12	3	2.04±0.13 ^{ABC}	1.86±0.29 ^{BC}	2.18±0.38 ^{AB}	2.34±0.25 ^{AB}	1.65±0.20 ^{BC}	4.28±0.28 ^{AB}	4.21±0.37 ^{AB}	4.64±0.19 ^{AB}	4.76±0.35 ^{AB}	4.76±0.35 ^{AB}
	90	1.67±0.17 ^{AB}	1.43±0.27 ^{AB}	1.52±0.09 ^{AB}	1.50±0.22 ^{AB}	1.63±0.27 ^{AB}	4.41±0.33 ^{AB}	3.84±0.17 ^{AB}	3.88±0.22 ^{AB}	3.89±0.21 ^{AB}	3.68±0.30 ^{AB}
C13	3	0.19±0.04 ^{AB}	0.35±0.08 ^{AB}	0.15±0.03 ^{AB}	0.23±0.04 ^{AB}	0.20±0.06 ^{AB}	0.27±0.11 ^{AB}	0.22±0.03 ^{AB}	0.30±0.07 ^{AB}	0.21±0.03 ^{AB}	0.23±0.02 ^{AB}
	90	0.08±0.03 ^{BC}	0.17±0.04 ^{AB}	0.09±0.01 ^{BC}	0.23±0.04 ^{AB}	0.23±0.05 ^{AB}	0.30±0.07 ^{AB}	0.19±0.06 ^{AB}	0.21±0.05 ^{AB}	0.19±0.02 ^{AB}	0.19±0.02 ^{AB}
C14	3	9.98±0.78 ^{BC}	10.83±0.61 ^{AB}	9.40±0.66 ^{BC}	11.34±0.58 ^{AB}	9.63±0.67 ^{BC}	13.43±0.67 ^{AB}	11.72±0.64 ^{AB}	12.16±0.73 ^{AB}	12.62±0.53 ^{AB}	11.77±0.61 ^{AB}
	90	10.15±0.69 ^{AB}	9.39±0.74 ^{AB}	9.42±0.68 ^{AB}	9.02±0.59 ^{AB}	9.28±0.59 ^{AB}	11.78±0.59 ^{AB}	11.27±0.58 ^{AB}	11.99±0.56 ^{AB}	11.29±0.56 ^{AB}	11.15±0.51 ^{AB}
C15	3	1.63±0.11 ^{AB}	0.98±0.24 ^{AB}	1.62±0.33 ^{AB}	1.79±0.25 ^{AB}	1.61±0.17 ^{AB}	1.51±0.26 ^{AB}	1.39±0.08 ^{AB}	1.33±0.10 ^{AB}	1.36±0.10 ^{AB}	1.34±0.07 ^{AB}
	90	1.49±0.14 ^{AB}	1.55±0.23 ^{AB}	1.45±0.12 ^{AB}	1.71±0.18 ^{AB}	1.61±0.22 ^{AB}	1.51±0.27 ^{AB}	1.18±0.20 ^{BC}	1.12±0.10 ^{BC}	1.44±0.11 ^{AB}	1.27±0.07 ^{ABC}
C16	3	35.86±1.05 ^{AB}	35.91±0.80 ^{AB}	34.86±0.87 ^{AB}	36.04±0.79 ^{AB}	37.09±0.97 ^{AB}	32.64±0.98 ^{AB}	32.47±0.92 ^{AB}	31.29±0.99 ^{AB}	32.13±0.54 ^{AB}	31.80±0.82 ^{AB}
	90	36.29±0.99 ^{AB}	36.72±1.06 ^{AB}	36.09±0.96 ^{AB}	36.39±1.03 ^{AB}	36.20±0.80 ^{AB}	32.26±0.79 ^{AB}	33.56±0.80 ^{AB}	32.47±0.73 ^{AB}	33.10±0.70 ^{AB}	32.67±0.65 ^{AB}
C17	3	1.00±0.11 ^{AB}	0.68±0.14 ^{AB}	1.06±0.11 ^{AB}	0.89±0.09 ^{AB}	1.05±0.10 ^{AB}	0.93±0.06 ^{AB}	0.87±0.10 ^{AB}	0.88±0.10 ^{AB}	0.84±0.09 ^{AB}	0.49±0.08 ^{AB}
	90	0.76±0.20 ^{BC}	0.60±0.32 ^{BC}	1.00±0.10 ^{AB}	1.33±0.11 ^{AB}	0.73±0.07 ^{BC}	0.83±0.08 ^{AB}	0.80±0.07 ^{AB}	0.21±0.04 ^{BC}	0.78±0.06 ^{AB}	0.76±0.06 ^{AB}
C18	3	9.86±0.62 ^{BC}	10.41±5.0 ^{ABC}	11.41±0.46 ^{AB}	10.02±0.47 ^{BC}	10.89±0.52 ^{AB}	10.14±0.47 ^{AB}	11.46±0.48 ^{AB}	11.13±0.61 ^{AB}	11.44±0.40 ^{AB}	10.62±0.50 ^{AB}
	90	9.96±0.57 ^{AB}	10.77±5.6 ^{AB}	10.78±0.55 ^{AB}	10.19±0.56 ^{AB}	11.01±0.43 ^{AB}	10.19±0.38 ^{AB}	11.54±0.43 ^{AB}	11.08±0.49 ^{AB}	11.38±0.40 ^{AB}	11.38±0.42 ^{AB}
US Mono	3	26.78±1.36 ^{AB}	28.15±1.60 ^{AB}	26.80±1.41 ^{AB}	25.97±1.09 ^{AB}	27.84±1.33 ^{AB}	22.60±0.78 ^{BC}	23.89±0.59 ^{ABC}	25.03±1.04 ^{AB}	24.45±1.12 ^{AB}	22.39±1.40 ^{BC}
	90	27.56±1.36 ^{AB}	27.96±1.65 ^{AB}	27.16±1.00 ^{AB}	27.49±1.09 ^{AB}	27.03±1.36 ^{AB}	22.79±0.63 ^{AB}	23.75±1.32 ^{AB}	22.31±0.97 ^{AB}	24.14±1.50 ^{AB}	24.00±1.15 ^{AB}
C14:1	3	1.70±0.08 ^{AB}	1.37±0.26 ^{AB}	1.53±0.35 ^{AB}	2.00±0.31 ^{AB}	1.73±0.18 ^{AB}	1.75±0.15 ^{AB}	1.38±0.17 ^{ABC}	1.61±0.15 ^{AB}	1.59±0.11 ^{ABC}	1.35±0.10 ^{BC}
	90	1.55±0.15 ^{AB}	1.63±0.24 ^{AB}	1.44±0.43 ^{AB}	1.74±0.20 ^{AB}	1.39±0.24 ^{AB}	1.54±0.14 ^{AB}	1.19±0.21 ^{BC}	1.41±0.11 ^{AB}	1.32±0.11 ^{AB}	1.06±0.06 ^{BC}
C15:1	3	0.41±0.09 ^{AB}	0.47±0.19 ^{AB}	0.42±0.26 ^{AB}	0.45±0.22 ^{AB}	0.46±0.13 ^{AB}	0.31±0.04 ^{AB}	0.22±0.02 ^{AB}	0.28±0.04 ^{AB}	0.22±0.02 ^{AB}	0.21±0.03 ^{AB}
	90	0.41±0.11 ^{AB}	0.38±0.19 ^{AB}	0.44±0.06 ^{AB}	0.39±0.13 ^{AB}	0.45±0.16 ^{AB}	0.41±0.04 ^{AB}	0.21±0.02 ^{AB}	0.24±0.03 ^{AB}	0.22±0.03 ^{AB}	0.22±0.07 ^{AB}
C16:1	3	3.56±0.12 ^{AB}	3.18±0.27 ^{AB}	3.43±0.11 ^{AB}	3.64±0.31 ^{AB}	3.64±0.18 ^{AB}	2.90±0.28 ^{AB}	2.71±0.20 ^{AB}	2.51±0.33 ^{AB}	2.67±0.15 ^{AB}	1.28±0.11 ^{AB}
	90	3.01±0.15 ^{AB}	3.19±0.25 ^{AB}	3.91±0.44 ^{AB}	3.80±0.24 ^{AB}	3.07±0.24 ^{AB}	2.68±0.30 ^{AB}	2.37±0.21 ^{AB}	1.70±0.20 ^{BC}	2.29±0.13 ^{AB}	2.25±0.15 ^{AB}
C17:1	3	0.25±0.04 ^{AB}	0.31±0.07 ^{AB}	0.39±0.14 ^{AB}	0.25±0.09 ^{AB}	0.28±0.06 ^{AB}	0.39±0.04 ^{AB}	0.24±0.03 ^{ABC}	0.27±0.06 ^{AB}	0.18±0.02 ^{BCD}	0.13±0.03 ^{BD}
	90	0.43±0.06 ^{AB}	0.28±0.09 ^{AB}	0.41±0.15 ^{AB}	0.49±0.03 ^{AB}	0.46±0.04 ^{AB}	0.21±0.03 ^{BC}	0.27±0.06 ^{AB}	0.33±0.03 ^{AB}	0.26±0.03 ^{AB}	0.27±0.06 ^{AB}
C18:1	3	20.87±1.05 ^{ABC}	22.83±0.82 ^{AB}	21.03±0.77 ^{ABC}	19.63±0.78 ^{BC}	21.73±0.78 ^{AB}	17.25±0.71 ^{AB}	19.34±0.76 ^{AB}	20.36±0.89 ^{AB}	19.79±0.57 ^{AB}	19.42±0.77 ^{AB}
	90	22.16±0.89 ^{AB}	22.47±0.88 ^{AB}	20.94±0.91 ^{AB}	21.08±0.92 ^{AB}	21.66±0.72 ^{AB}	17.94±0.61 ^{BC}	19.71±0.70 ^{AB}	18.63±0.75 ^{BC}	20.05±0.61 ^{AB}	20.29±0.63 ^{AB}
Poly	3	1.72±0.13 ^{AB}	2.05±0.40 ^{AB}	1.68±0.13 ^{AB}	1.64±0.19 ^{AB}	1.78±0.16 ^{AB}	1.71±0.05 ^{AB}	2.07±0.13 ^{AB}	2.21±0.21 ^{AB}	2.05±0.12 ^{AB}	2.07±0.10 ^{AB}
	90	1.77±0.13 ^{AB}	1.99±0.05 ^{AB}	1.94±0.14 ^{AB}	1.90±0.16 ^{AB}	1.03±0.10 ^{BC}	1.82±0.10 ^{AB}	2.05±0.25 ^{AB}	2.31±0.20 ^{AB}	2.23±0.10 ^{AB}	2.18±0.12 ^{AB}
C18:2C	3	1.15±0.24 ^{AB}	1.56±0.29 ^{AB}	1.26±0.14 ^{AB}	1.20±0.11 ^{AB}	1.03±0.26 ^{AB}	1.25±0.01 ^{BC}	1.58±0.21 ^{AB}	1.41±0.11 ^{BC}	1.37±0.02 ^{BC}	1.68±0.11 ^{AB}
	90	1.10±0.02 ^{AB}	1.23±0.02 ^{AB}	1.22±0.04 ^{AB}	1.18±0.03 ^{AB}	0.31±0.06 ^{BC}	1.27±0.12 ^{AB}	1.55±0.20 ^{AB}	1.51±0.14 ^{AB}	1.55±0.10 ^{AB}	1.60±0.12 ^{AB}
C18:3n3	3	0.57±0.11 ^{AB}	0.49±0.25 ^{AB}	0.42±0.05 ^{BC}	0.45±0.05 ^{BC}	0.75±0.17 ^{AB}	0.46±0.04 ^{BC}	0.49±0.08 ^{BC}	0.80±0.03 ^{AB}	0.68±0.05 ^{BC}	0.39±0.05 ^{BC}
	90	0.67±0.15 ^{AB}	0.76±0.07 ^{AB}	0.72±0.07 ^{AB}	0.72±0.08 ^{AB}	0.72±0.22 ^{AB}	0.55±0.02 ^{BCD}	0.50±0.05 ^{BD}	0.81±0.02 ^{AB}	0.68±0.05 ^{BC}	0.57±0.02 ^{BC}
Trans	3	2.63±0.09 ^{AB}	2.42±0.20 ^{AB}	2.21±0.16 ^{AB}	2.28±0.44 ^{AB}	2.38±0.27 ^{AB}	3.32±0.21 ^{AB}	3.93±0.32 ^{AB}	3.98±0.06 ^{AB}	3.41±0.19 ^{AB}	3.93±0.18 ^{AB}
	90	2.85±0.12 ^{AB}	2.92±0.24 ^{AB}	2.95±0.21 ^{AB}	2.34±0.03 ^{BC}	3.08±0.31 ^{AB}	3.84±0.23 ^{AB}	4.35±0.26 ^{AB}	4.02±0.21 ^{BC}	4.28±0.23 ^{AB}	4.55±0.27 ^{AB}
C18:1T	3	2.21±0.16 ^{AB}	2.12±0.12 ^{AB}	1.91±0.16 ^{AB}	2.05±0.41 ^{AB}	2.13±0.24 ^{AB}	3.08±0.12 ^{AB}	3.56±0.20 ^{AB}	3.57±0.03 ^{AB}	3.14±0.12 ^{AB}	3.57±0.15 ^{AB}
	90	2.28±0.02 ^{BC}	2.61±0.16 ^{AB}	2.60±0.19 ^{AB}	2.12±0.02 ^{BC}	2.78±0.32 ^{AB}	3.60±0.16 ^{AB}	4.15±0.20 ^{AB}	3.67±0.27 ^{AB}	3.82±0.13 ^{AB}	4.08±0.18 ^{AB}
C18:2T	3	0.42±0.06 ^{AB}	0.30±0.23 ^{AB}	0.30±0.03 ^{AB}	0.23±0.04 ^{AB}	0.25±0.04 ^{AB}	0.24±0.03 ^{AB}	0.37±0.04 ^{AB}	0.41±0.03 ^{AB}	0.27±0.03 ^{AB}	0.36±0.05 ^{AB}
	90	0.57±0.14 ^{AB}	0.31±0.02 ^{BC}	0.35±0.01 ^{BC}	0.22±0.02 ^{BC}	0.30±0.01 ^{BC}	0.24±0.01 ^{BC}	0.19±0.03 ^{BC}	0.35±0.06 ^{BC}	0.46±0.05 ^{AB}	0.48±0.06 ^{AB}
Total	3	98.39±4.29 ^{AB}	98.33±2.96 ^{AB}	99.58±3.25 ^{AB}	99.57±2.63 ^{AB}	98.72±3.45 ^{AB}	99.18±2.29 ^{AB}	99.19±2.34 ^{AB}	99.90±2.33 ^{AB}	99.91±2.73 ^{AB}	98.32±2.45 ^{AB}
	90	99.98±3.34 ^{AB}	98.39±3.64 ^{AB}	98.66±4.39 ^{AB}	99.97±3.52 ^{AB}	99.31±2.85 ^{AB}	99.72				

Effects of NaCl concentrations on PV of B-LFS and C-LFS

The oxidative stability in dairy products is influenced by many factors including concentration of oxygen, metals (Cu^{+2} , Fe^{+3}), antioxidants, water activity, etc., [18]. Therefore, there is a difficulty in interpreting the results regarding PV [19]. Effects of NaCl concentrations on PVs of B-LFS and C-LFS are shown in Table 2. An oxidation of NaCl treatments (B-LFS) were more than C-LFS samples, due to the fat phase of C-LFS samples was contained color agent (β -carotene), while fat phase of B-LFS samples was without β -carotene, therefore β -carotene reported-antioxidative [20-22]. In addition, β -Carotene has been shown to protect lipids from free radical autooxidation by reacting with peroxy radicals, thus inhibiting propagation and promoting termination of the oxidation chain reaction [23].

The differences in PVs of control samples and other treatments was slightly, and an increase of NaCl hadn't effects on PVs of B-LFS and C-LFS, however our results were in agreement with Al-Ismael and Humeid [24] who found that no significant differences between NaCl 0, 1.07 and 2.14% with model of butter fat/water during 3 months, but the differences became significant with NaCl 4.6 and 6.6% during the storage for 6 months. On the other hand, during storage periods, we noticed PVs increased noticeably ($P < 0.05$), consequently all NaCl treatments were be accepted in an industrial setting, because the highest PV was 0.441 (B-LFS with NaCl 2% at 90 days). Moreover, the samples are considered rancid and unacceptable when the PV are over 5, while the ideally PV should be below 1-1.5 [25].

The viscosity increased with an increase of NaCl in B-LFS and C-LFS (data not shown), however the viscosity not delaying the proceed of oxidation during the storage [26]. In addition, the oxidation was promoted in our treatments, may be due to incorporation of air and commencement of oxidation during prepared of butter oil [10]. Furthermore, the heat treatments and light exposure causes the oxidation in the treatments during storage periods [22].

Effect of NaCl concentrations on OSI values of B-LFS and C-LFS

The OIT of NaCl treatments (B-LFS and C-LFS) are shown in Table 3. The OIT of B-LFS and C-LFS separately with NaCl 1.5 and 2% were slightly lower; while with NaCl 0 and 0.5% were slightly higher than control samples. In addition, the consequence of an increasing NaCl from 0 to 2%, we found OIT of NaCl treatments (B-LFS and C-LFS) declined may be due to the electrical conductivity increases proportionally with increasing ionized NaCl concentrations i.e. the electrons' mobility would be enhanced, facilitating the oxidation process [24].

On the other hand, during the storage, the OIT for all treatments had decreased effects ($P < 0.05$). However, our results were in agreement with that determined by Krause et al. [27] because they found that the OSI values of stick butter (Cow's butter) were decreased during storage times at refrigeration conditions. The correlation between OSI values and PVs were reversible, but not linear, due to trends of PVs (Table 2) were fluctuated with an increase of NaCl, while trends of OSI values were decreased with an increasing of NaCl, however NaCl treatments (B-LFS) had relatively short OIT compared C-LFS samples, because Cow's butter was contained β -carotene, while Buffalo's butter wasn't β -carotene (see PV), therefore β -carotene led to prolong of the OIT for NaCl treatments (C-LFS) than B-LFS samples.

Furthermore, Läubli and Bruttel [28] they reported that the OIT for cooking butter at 100, 110 and 120 °C were 20.88, 9.33 and 5.03 respectively. Also, Mathäus [29] determined the OIT for walnut oil at 110, 120 and 130 °C, the OSI values were 156, 84 and 45 min respectively. Fatouh et al [30] found that the OIT for Buffalo's butter oil at 110 °C was 8.2 h. likewise, Krause et al. [27] noticed that the OIT for Cow's butter at 110 °C and the air rate 0.05 mL/min was 2 times approximately during the storage from 0 to 3 months.

Effect of NaCl concentrations on FAC of B-LFS and C-LFS

All of the changes in FAC of Buffalo's and Cow's milk fat due to the breed, lactating stage, season and diet. Nevertheless the differences are present not only among species but also within species [31]. Table 4 shows FAC of different NaCl concentrations with B-LFS and C-LFS from 3 to 90 days. The major fatty acids in NaCl treatments (B-LFS and C-LFS) were C16, C18:1, C14:0 and C18. The results among NaCl treatments (B-LFS) revealed that the saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) (at 90 days) and trans fatty acids (TFA) (at 90 days) were significantly different with the control samples, while monounsaturated fatty acids (MUFA), PUFA (at 3 days), TFA (at 3 days) and total FA were without significant. Moreover, during storage periods of B-LFS samples we found the PUFA (NaCl 2%), and TFA (control and NaCl 2%) were only significant.

On the other hand, among C-LFS samples, we found that the SFA (at 3 days), MUFA (at 3 days) PUFA and TFA had significant differences, whereas SFA (at 90 days), MUFA (at 90 days) and total FA were without significant. In addition the differences during storage periods were only significant with MUFA (control) and TFA (NaCl 0, 1.5

and 2%). Therefore, we can say the changes in FAC during the storage periods, due to degradation of fat under pasteurization and oxidation [13].

Proportions of SFA, C6, C8, C10, C11, C12, C14, C18:2C and TFA in NaCl treatments (B-LFS) were lower than C-LFS, whereas proportions of C4, C15, C16, MUFA, C15:1, C16:1, C17:1 and C18:1 for NaCl treatments (C-LFS) were lower than B-LFS. Moreover, the percentages of PUFA in B-LFS and C-LFS with NaCl 0 and 0.5% were close to each others, but with NaCl 1, 1.5 and 2%, the PUFA were lower in B-LFS than C-LFS samples. In contrary Varricchio et al., Blasi et al., Ménard et al. [32,33,34] reported that Buffalo's milk fat contained a higher amounts of SFA and lower amounts of unsaturated fatty acids than Cow's milk fat, but results of the previous authors came from other breeds were different than breed of Egyptian Buffalo's Animals. Furthermore, Samet-Bali et al. [13] reported that milk fatty acid composition depends on several factors as animal species, nutrition, climate and environmental conditions. Additionally the differences in the FAC of the Buffalo's and Cow's milk fat due to the genetics of the two animal species considered [34]. However our results were in agreement with Haggag et al. [35] who reported that an unsaturated fatty acids for Egyptian Buffalo's milks were higher than Egyptian Cow's milks.

On the other hand, Ahmad et al. [31] reported that the proportions of C4, C16, C17, and C18 were higher, but C6, C8, C10, C12, C14, and C14:1 were lower in Buffalo's than in Cow's milk fat. Moreover, Patel et al. [36] found that the averages of C4, C16, C17 and C18 in Buffalo's milk fat were higher than Cow's milk fat, while C6, C8, C10, C10:1, C12, C14, C14:1 and C18:1 in Cow's milk fat were higher than Buffalo's milk fat. Although, Talpur et al. [37] found that the averages of four seasons as percentages of C4, C8 and C12 were higher in Buffalo's than Cow's milk fat which reared under the traditional feeding system of Sindh, Pakistan.

The changes in FAC of NaCl treatments during the storage were slightly, however our results were in agreement with Mallia [22]. In addition, our results between and within NaCl treatments were approximately resemble with that observed for FAC of pH and CaCl₂ treatments (data not shown). Furthermore, the differences in FAC between NaCl treatments (B-LFS and C-LFS) and during storage periods, presumably attributed to chemical changing, degradation by non-enzymatic, pasteurization, or by metabolic conversions mediated by enzymes produced by microorganisms introduced during processing of B-LFS and C-LFS.

CONCLUSIONS

An increase of sodium chloride hadn't effects on pH values of B-LFS and C-LFS separately. The differences in PVs of control samples and other treatments was slightly, while within NaCl treatments, there are significant differences during the storage periods. In addition, an increase of NaCl was accompanied with decreasing of OSI values in both B-LFS and C-LFS separately. Likewise, we noticed OSI values of all NaCl treatments decreased during the storage. The changes in FAC between NaCl treatments and during the storage, attributed to the chemical changing, degradation, pasteurization.

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