



Alteration of Basic Quality Attributes of Set Yogurts as Added Sugar and Milk Fat Reduction

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Authors' contributions

This work was carried out in collaboration among all authors. Author ID designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and UR managed the analyses of the study and prepare the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Fat and sugar-free is a popular health claim for yogurts. However, eliminating these two components may alter the inherent quality attributes of the product. Knowledge of these alteration patterns is vital in the product development process. Present study was designed using general full factorial design, with two factors (and levels) as milk fat (0%, 1.5%, and 3.3%) and added sugar (0%, 3.5%, and 7%) to investigate individual and combined effects of fat and sugar levels on fermentation kinetics, total solid content, instrumental color values, lactic acid bacterial viability, texture profile, and rheology of set yogurt. Milk fat 3.3% and added sugar 7% yogurt was considered as the control. Results showed that fermentation kinetics not largely influenced by fat levels. Fermentation time not significantly differ with the fat/sugar alteration. Total solid content increase with milk fat and added sugar levels. Whiteness (L*) of the yogurt reduces with milk fat content but not significantly affected by added sugar levels. Yogurt bacteria counts were reduced with added sugar and milk fat levels. Full cream low sugar (3.3%, 3.5%) better texture profile over full cream high sugar (3.3%, 7%) yogurts. Texture integrity reduces with the reduction of fat content. The consistency index also increased with fat levels. Both milk fat and added sugar have interaction effects on tested quality parameters. Accordingly, the reduction of added sugar and milk fat is favorably influenced some quality attributes of yogurt.

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1. INTRODUCTION

Yogurt is the most popular dairy product worldwide which confer an array of health benefits to the consumers as provides a significant amount of calcium in a bioavailable form, improved lactose tolerance, a possible role in body weight and fat loss, and a variety of healthy features associated with probiotic bacteria [1]. To gain a competitive advantage in the market place, manufacturers make these yoghurts even healthier by altering its original composition by adding fiber, antioxidants, probiotics, etc. Yet, added sugar and milk fat with high-calorie count is there to increase palatability and consumer perception. Removing these two components make the yogurt super healthier. Therefore, low/no sugar; low/no fat, low/no calorie yoghurts getting popular day by day. However, added sugar, a bulking agent, adds viscosity, enhance flavor, provides texture, adds color, and is a preservative. Similarly, milk fat imparts good flavor, texture, and color to food [2–4]. Hence, before removing the above two components, food technologists should have a clear idea of the positive and negative impact of them towards yogurt quality.

Consequences on yogurt texture, rheology, microstructure and sensory perception as milk fat reduction were studied by several researchers [5–8]. Further, there are limited studies on the effect on different sugar levels on sensory perception and microbial viability [9]. However, the interaction impact on milk fat and added sugar is underexploited. The present study is intending to investigate the individual and interaction effect of milk fat and added sugar content on basic quality attributes of set yogurt.

2. MATERIALS AND METHODS

2.1 Experimental Design

¹The experiment was designed using general full factorial design, with factors (and levels) as fat (0%, 1.5% and 3.3%) and sugar (0%, 3.5% and 7%). The fat content of the milk and the yogurts were confirmed using the Gerber method [10].

¹ Abbreviations: NFNS, no fat no sugar; NFLS, no fat low sugar; NFHS, no fat high sugar; LFNS, low fat no sugar; LFLS, low fat low sugar; LFHS, low fat high sugar; FCNS, full cream no sugar; FCLS, full cream low sugar; FCHS, full cream high sugar yoghurt; AS, Added sugar; MF, Milk fat

The individual and combined effect of fat and sugar levels on the fermentation kinetics, rheology, texture profile, and lactic acid bacterial viability were tested. Nine yogurt samples with 27 total runs/triplicates were statistically evaluated except for fermentation kinetic values and rheological parameters (18 total runs/duplicates). The combination of fat and sugar amounts in each sample was as in Table 1. Skim milk powder and gelatine amounts per 100 ml of milk were constant for all samples as 3% and 0.5%. Milk fat 3.3% and added sugar 7% yogurt (FCHS) considered as the control. According to Sri Lankan Standards [11], the minimum fat content of full cream yogurt is 3% and according to EU, Regulation 1924/2006 [12] to claim as low sugar, added sugar content should less than 5%. Therefore, slightly higher values than these upper limits were selected for control combination since our expectation is to reduce milk fat and added sugar content up to maximum possible level.

2.2 Sample Preparation

Samples were prepared in the dairy processing laboratory, Department of Food Science and Technology, Sabaragamuwa University of Sri Lanka. All the utensils used for yogurt preparation were sterilized with boiling water. UHT treated milk (Kothmale dairy products (Pvt)ltd., Sri Lanka) were heated with continuous agitating in a medium flame. Pre-mixed sugar, skim milk powder and gelatine were added into the milk at 60°C. Yogurt formulations were heated at 85°C for 10 min. The mixture was allowed to cool down to 42°C and 0.01% (w/v) of YC-X11 (Chr. Hansen, Hoersholm, Denmark) starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was added into it. The mixture was homogenized using a homogenizer (Jainon, India) for 1-3 min in order to breakdown the fat globules into small particles and mix the constituents properly. The homogenized mixture was filled into sterilized plastic containers and allowed to incubate at 42°C until the pH of the mixture drops to 4.5.

2.3 Fermentation Kinetics

The changes in pH during fermentation were monitored continuously by means of a glass electrode pH meter (Hanna Instruments, Padova,

Italy) with a slight modification of the method described by Guggisberg et al. (2009) [5]. The electrode was standardized carefully by means of three buffers (pH 10.0, 7.0 and 4.0), disinfected with 70% (v/v) alcoholic solution, rinsed with sterilized distilled water and pH of the milk sample in the incubator was recorded manually at 15 min intervals. (a) Maximum acidification rates (V_{max}) were calculated from the pH–time curves according to the equation $V_{max} = (dpH/dt)_{max}$ and expressed in 10^{-3} pH units per min. (b) Time at which the maximum acidification rate was observed (T_{max}), (c) time to reach pH 5.0 ($T_{pH5.0}$), pH near to the isoelectric point of casein and (d) the time at which pH 4.5 ($T_{pH4.5}$) was observed after the completion of fermentation also were recorded in hours. Two independent batch fermentations were carried out in duplicate on different days at 42°C up to pH 4.5.

2.4 Total Solid, Color and Post Acidification

Determination of total solids in milk bases and titratable acidity in yogurts were made according to AOAC (1995) [13]. The post acidification was determined as pH after 1, 7, 14 and 28 days of cold storage using a pH meter, model Q-400M1 (Quimis, Sao Paulo, Brazil). The results were expressed as the means of three replicates.

The color of the samples was measured using a Hunter Lab color meter (CR 400, Konica Minolta, Japan). Measurements were taken directly at three different locations, after standardization with a white calibration plate ($L^* = 94.12$, $a^* = 0.29$, and $b^* = 2.73$). Colour was expressed in Hunter Lab units L^* , a^* and b^* , where L^* indicates lightness, a^* indicates hue on a green (–) to red (+) axis, and b^* indicates hue on a blue (–) to yellow (+) axis [14]. A transparent

polythene film was used to cover the yogurt while taking the readings.

2.5 Microbial Analysis

Bacterial enumerations were carried out after 1, 7, 14 and 28 days of cold storage in three replicates of each sample. Yogurts (1 ml) were diluted with sterile distilled water (9 mL). Afterward, serial dilutions were carried out, and bacteria were counted, applying the pour plate technique. All media were obtained from HiMedia Laboratory Pvt. Ltd., Bombay, India. In co-cultures, *Streptococcus thermophilus* colonies were enumerated in M17 agar, while those of *Lactobacillus delbrueckii* subsp. *bulgaricus* in MRS agar (pH 5.4), both under aerobic incubation at 37°C for 24 h [15]. Total colonies were counted by colony counter (Rocker, Galaxy 230) and expressed in CFU/mg.

Moreover, Viability proportion index (VPI) of yogurt microorganism at the end of storage time were calculated as the following equation;

$$VPI = \text{Final cell population (CFU/ml)} / \text{Initial cell population (CFU/ml)} (g^{-1})$$

2.6 Syneresis and Texture Profile Analysis

Syneresis was measured according to the filtration method described by Harwalkar & Kalab (1983) [16]. Unstirred yogurt (30 g) was spread evenly on a Whatman No. 1 filter paper (Whatman Ltd., Maidstone, UK) in a funnel, which was placed on top of a 50 ml graduated cylinder. The graduated cylinder was then held at 4°C in a refrigerator for 5 h and the volume of liquid collected was recorded. Syneresis index (%) was calculated as follows.

$$\text{Syneresis index \%} = (\text{volume of whey/volume of yoghurt}) * 100$$

Table 1. Combination of fat and sugar percentages in each treatment

Fat%	Sugar %	Description	Abbreviation
0	0	No fat no sugar	NFNS (T1)
0	3.5	No fat low sugar	NFLS (T2)
0	7	No fat high sugar	NFHS (T3)
1.5	0	Low fat no sugar	LFNS (T4)
1.5	3.5	Low fat low sugar	LFLS (T5)
1.5	7	Low fat high sugar	LFHS (T6)
3.3	0	Full cream no sugar	FCNS (T7)
3.3	3.5	Full cream low sugar	FCLS (T8)
3.3	7	Full cream high sugar (control)	FCHS (T9)

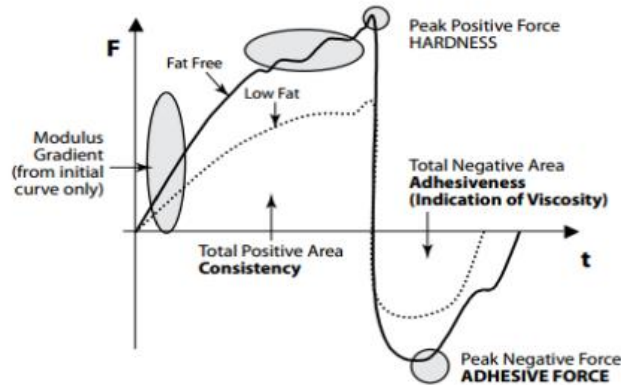


Fig. 1. An illustration of a graph of time (t) vs. force (F) during texture analyzing

Source: "Brookfield CT3 Texture Analyser User Manual | 56 pages," n.d.[17]

Two-cycle texture profile analysis was conducted by Brookfield CT3 texture analyzer by using the TA4/1000 cylindrical probe and TA-BT-KIT fixture. Target distance, hold time, trigger load, recovery time, pre-test speed, return speed, and load cell were set as 20 mm, 0, 4.5 g, 5 sec, 2 mm/s, 1 mm/s, and 1500 g, respectively. The Uniform sample size (80 ml) was tested after 24 h cold storage at 4 °C. All the samples were in the same pH (4.5) and temperature (20 °C) at the testing point. Calculations were made using a graph of time (t) vs. force (F) and texture pro CT V1.8 software (Fig. 1).

2.7 Rheology

The rheological parameters were determined as the method described by Zubairi (2010)[18] using a rotational viscometer (model RV, DV-III ULTRA, BROOKFIELD). The temperature of the system was set and maintained at ambient temperature (25°C) for the flow curves. The viscosities of the yogurts were determined at the rotational speeds of 0, 2, 4, 5, 10, 20, 50 and 100 rpm.

Flow behavior index (n) was calculated as the slope of the graph log torque vs. log rotational speed. The consistency index (K) was calculated as the intercept of the log shear stress (σ) vs. log shear rate ($\dot{\gamma}$) graph. The rheological properties were fitted to the power law model.

2.8 Statistical Analysis

Effect of reducing milk fat and added sugar on quality attributes of yogurt was analyzed by two

way ANOVA (general linear model) in Minitab 17 statistical software. Tukey's test was used to check the significant difference ($p > 0.05$) among samples.

3. RESULTS AND DISCUSSION

3.1 pH and Kinetics Parameters of Acidification

Initial pH was not significantly changed ($p > 0.05$) by alteration of both MF and AS (Table 2,3, and 4). No individual or Interaction effects on initial pH of yoghurt mixture as MF and AS alteration within the selected range (MF: 0-3.3%; AS: 0-7%).

Findings of Shah & Ravula (2000) [19] showed that high sugar contents tend to reduce the water activity of yogurt and that can be a hurdle for the growth and viability of lactic acid bacteria. The pH of yoghurt mixture reduces as a result of lactic acid production from those bacteria by fermenting lactose in milk. Accordingly, if the high sugar contents will reduced the lactic acid bacteria count, that will be a reason for delaying the fermentation process and reduce the acidification rate or fermentation kinetics.

The individual effect of added sugar content shown to be in agreement with the above explanation. T_{max} (Time required to reach maximum acidification rate), and T_{pH5} (time required to attain the pH of 5) were highest in 7% sugar added yoghurt. However, no significant difference observed in these parameters of 3.5%

and 0% sugar added yoghurts. They have comparatively less T_{max} and T_{pH5} than 7% sugar added yoghurts. Yet, no significant difference observed in $T_{pH4.5}$ (time required to attain the pH of 4.5) with the sugar content (Table 3).

No individual effect was observed by MF content on T_{max} , T_{pH5} , and $T_{pH4.5}$. Findings of Espirito Santo et al. (2012)[20] demonstrated that skim milk yogurt needs higher fermentation time (5.30 to 6.30 h) than whole milk (4.30 to 5.50 h). The results of the present study were not in line with those findings (Table 2).

Interaction effects of MF and AS contents on T_{max} and V_{max} (Maximum acidification rate) were observed. T_{max} highest in NFHS sample and lowest in NFLS yogurt. Highest V_{max} observed in the FCLS combination while lowest in the LFNS sample. No significant interaction effects on T_{pH5} and $T_{pH4.5}$. In general, 0% MF and 3.5% AS combination showed comparatively less fermentation times which is cost effective (Table 4). All yogurt samples completed the fermentation process within 5.30 to 6.30 hours.

Table 2. Individual effect of milk fat content on initial pH and kinetic parameters

Fat %	Initial pH	T_{max}	V_{max}	T_{pH5}	$T_{pH4.5}$
0	6.43±0.03 ^a	1.87±0.29 ^a	16.95±0.50 ^b	2.71±0.17 ^a	6.03±0.28 ^a
1.5	6.50±0.01 ^a	2.10±0.03 ^a	17.17±1.50 ^{ab}	2.93±0.10 ^a	6.03±0.17 ^a
3.3	6.40±0.01 ^a	2.10±0.03 ^a	19.00±0.44 ^a	2.81±0.12 ^a	5.97±0.19 ^a

Mean ± SE (n=2). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 3. Individual effect of added sugar content on initial pH and kinetic parameters

Sugar %	Initial pH	T_{max}	V_{max}	T_{pH5}	$T_{pH4.5}$
0	6.45±0.02 ^a	2.00±0.11 ^{ab}	15.81±0.94 ^b	2.63±0.12 ^b	6.30±0.00 ^a
3.5	6.42±0.03 ^a	1.82±0.19 ^b	17.89±0.49 ^{ab}	2.75±0.14 ^{ab}	5.87±0.18 ^a
7.0	6.46±0.03 ^a	2.27±0.15 ^a	19.42±0.97 ^a	3.07±0.08 ^a	5.87±0.29 ^a

Mean ± SE (n=2). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 4. Interaction effect of milk fat and added sugar content on initial pH and kinetic parameters

Sample	Initial pH	T_{max} (h)	V_{max}	T_{pH5} (h)	$T_{pH4.5}$ (h)
NFNS	6.45±0.05 ^a	1.73±0.28 ^{bc}	17.16±0.16 ^{abc}	2.45±0.00 ^a	6.30±0.00 ^a
NFLS	6.38±0.00 ^a	1.23±0.08 ^c	18.00±0.00 ^{ab}	2.45±0.00 ^a	5.30±0.00 ^a
NFHS	6.48±0.07 ^a	2.65±0.35 ^a	15.67±1.00 ^{bc}	3.23±0.23 ^a	6.50±0.05 ^a
LFNS	6.47±0.01 ^a	2.15±0.00 ^{ab}	12.84±0.17 ^c	2.73±0.28 ^a	6.30±0.00 ^a
LFLS	6.52±0.03 ^a	2.08±0.07 ^{abc}	19.67±0.34 ^{ab}	3.10±0.07 ^a	6.07±0.08 ^a
LFHS	6.50±0.01 ^a	2.08±0.00 ^{abc}	19.00±1.67 ^{ab}	3.00±0.00 ^a	5.72±0.58 ^a
FCNS	6.43±0.01 ^a	2.08±0.07 ^{abc}	17.42±0.09 ^{abc}	2.76±0.28 ^a	6.30±0.00 ^a
FCLS	6.39±0.02 ^a	2.15±0.07 ^{ab}	20.59±0.09 ^a	2.77±0.28 ^a	6.23±0.07 ^a
FCHS	6.40±0.01 ^a	2.08±0.07 ^{abc}	19.00±1.67 ^{ab}	3.00±0.00 ^a	5.37±0.08 ^a

Mean ± SE (n=2). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 5. Individual effect of milk fat content on TSS and color values

Fat %	TSS %	L*	a*	b*
0	17.87±0.97 ^c	95.59±1.24 ^b	-4.03±0.21 ^b	14.74±1.12 ^b
1.5	19.08±1.20 ^b	102.92±0.20 ^a	-3.84±0.30 ^{ab}	15.76±0.43 ^a
3.3	23.07±0.17 ^a	103.42±0.13 ^a	-3.50±0.09 ^a	16.38±0.37 ^a

Mean ± SE (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

3.2 Total Soluble Solid (TSS) and Color Values

As shown in Table 7, the least TSS content observed in NFNS yogurt. This might leads to the highest whey separation of this yogurt sample. There was no significant differences ($P>0.05$) in. TSS of LFHS yogurt and full cream yogurt with 0%, 3.5%, and 7% sugar. TSS increased with fat content (Tables 5,7). Similarly, TSS increased with sugar content, except for 3.3% fat (full cream) yogurt series (Tables 6, 7).

Previous studies revealed that yogurt color changed with milk fat content. According to the findings of Yazici & Akgun (2004)[21], Lab color values of 0.5% fat yogurt is higher than 2%. In contrast, a review by Tamime & Deeth (1980)[22] concluded that an increase in the number of fat globules effects lights reflectance and scattering. In the present study, 0% fat samples have the least color values (Tables 5 and 7). Sugar content did not affect L^* but b^* significantly lower ($p<0.05$) in the 0% sugar yogurt series (Table 6). Yet, the highest a^* observed in 0% sugar yogurts.

3.3 Post Acidification and Titratable Acidity

The pH values were between 4.60 and 4.89 on day 1 for the different treatments. Statistically significant ($P<0.05$) pH reduction was observed over storage time except for T1, T2, T3, and T5. In those treatments, augmentation of pH on day 28 was observed with compare to the day 14 may be due to the alterations of microflora. pH values of day 28 were between 4.29 and 4.61 (Fig. 2).

The acidity of all 9 treatments increased with time (Fig. 3). Titratable acidity of most yogurts

was significantly lower at 7% sugar treatments compare to 0% and 3.5% at 25 °C (Fig. 3).

In the case of sugar reduction, a significant increase of acidity was observed in 1st day.

3.4 Microbiological Analysis

Counts and viability of *streptococcus thermophilus* and *Lactobacillus bulgaricus* were tested after 1, 7, 14 and 28 days. Findings of Espirito Santo et al. (2012)[20] showed skimmed yogurts have higher counts of lactic acid bacteria than whole yogurts. Further, previous studies showed added sugar reduces the water activity of foods and beverages, which makes water unavailable for bacterial and fungal growth [23]. In the same way, this study showed high added sugar and milk fat tend to reduce *streptococcus thermophilus* and *Lactobacillus bulgaricus* counts.

For *Streptococcus thermophilous* least counts observed in the highest fat samples (Table 8). Added sugar content has no significant effect on day 1 and day 14. However, the highest sugar content tends to decrease bacterial counts on day 7 and day 28 (Table 9). For *Lactobacillus bulgaricus*, the least counts observed in the highest fat samples except day 7 (Table 10). Added sugar content has no significant effect on day 1 and day 14. Similarly, the highest sugar content tends to decrease bacterial counts except day 14 (Table 9).

Microbial counts getting increase over time and sometimes tend to decrease after 2nd week. However, all the counts were in the biotherapeutic level, >6 logs (CFU/ml) (6.5 – 11.5 log (CFU/ml) (Fig. 4 and Fig. 5).

Table 12 showed the viability proportion index of two yogurt microorganisms. Highest VPI observed in 0% sugar and 3.3%, full-fat yogurt.

Table 6. Individual effect of added sugar content on TSS and color values

Sugar %	TSS %	L^*	a^*	b^*
0	17.20±1.38 ^c	101.70±1.94 ^a	-3.53±0.01 ^a	14.44±0.30 ^b
3.5	20.44±0.70 ^b	100.16±0.22 ^a	-4.05±0.43 ^b	16.37±1.02 ^a
7.0	22.35±0.42 ^a	100.05±0.89 ^a	-3.79±0.38 ^{ab}	16.07±0.79 ^a

Mean ± SE (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 7. Interaction effect of milk fat and added sugar content on TSS and color values

Sample	TSS%	L*	a*	b*
NFNS	14.14±0.00 ^d	93.96±0.00 ^{bc}	-3.52±0.00 ^{ab}	11.60±0.00 ^b
NFLS	18.72 ± 0.20 ^c	93.90±2.50 ^c	-4.43±0.47 ^b	17.41±1.19 ^a
NFHS	20.67±0.12 ^b	98.89±0.52 ^{ab}	-4.15±0.05 ^{ab}	15.21±0.35 ^a
LFNS	14.76±0.79 ^d	102.71±0.31 ^a	-3.54±0.09 ^{ab}	15.69±0.16 ^a
LFLS	19.44±0.18 ^c	103.10±0.31 ^a	-4.14±0.08 ^{ab}	15.37±0.04 ^a
LFHS	23.02±0.27 ^a	102.95±0.03 ^a	-3.85±0.04 ^{ab}	16.23±0.10 ^a
FCNS	22.70±0.11 ^a	103.50±0.13 ^a	-3.52±0.02 ^a	16.03±0.09 ^a
FCLS	23.16±0.49 ^a	103.50±0.38 ^a	-3.58±0.01 ^{ab}	16.35±0.14 ^a
FCHS	23.03±0.46 ^a	103.27±0.00 ^a	-3.39±0.04 ^a	16.77±0.18 ^a

Mean ± SE (n=3). Values followed by different letters in the same column are significantly different (p < 0.05) according to Tukey's test

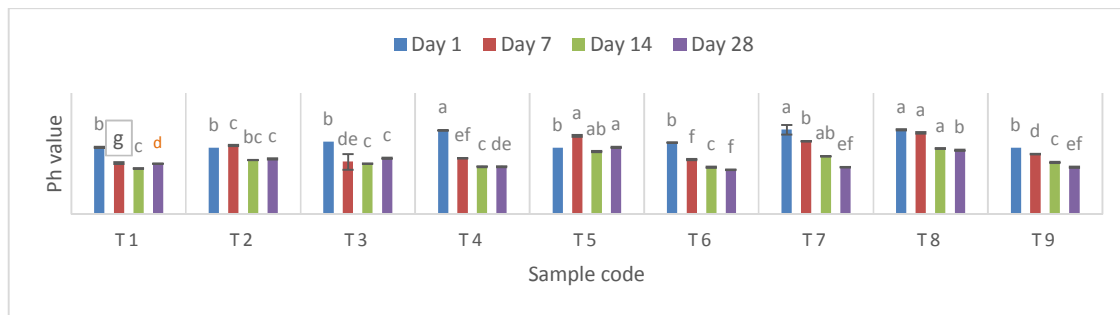


Fig. 2. Post acidification (pH change) of yogurt formulations

Values followed by different letters in the same color bars are significantly different (p < 0.05) according to Tukey's test, Error bars represent standard error (n=3)

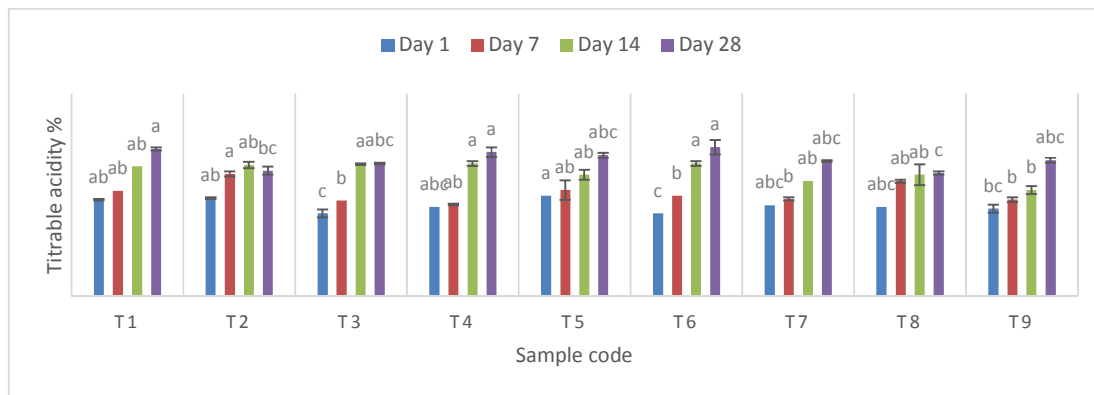


Fig. 3. Titratable acidity (TA) lactic acid change with time

Values followed by different letters in the same color bars are significantly different (p < 0.05) according to Tukey's test, Error bars represent standard error (n=2)

Table 8. Individual effect of milk fat content on the growth of *Streptococcus thermophilus*

Fat %	Counts of <i>Streptococcus thermophilus</i> log(CFU/ml)			
	Day 1	Day 7	Day 14	Day 28
0	8.46 ^a	8.81 ^b	10.10 ^a	10.32 ^a
1.5	8.33 ^a	10.59 ^a	10.28 ^a	9.94 ^a
3.3	7.03 ^b	9.38 ^b	9.09 ^b	8.49 ^b

Mean (n=3). Values followed by different letters in the same column are significantly different (p < 0.05) according to Tukey's test

Table 9. Individual effect of added sugar content on the growth of *Streptococcus thermophilus* counts

Sugar %	Counts of <i>Streptococcus thermophilus</i> log(CFU/ml)			
	Day 1	Day 7	Day 14	Day 28
0	7.87 ^a	9.60 ^a	9.73 ^a	9.83 ^a
3.5	8.34 ^a	9.96 ^a	9.92 ^a	9.82 ^a
7	7.60 ^a	8.88 ^b	9.82 ^a	9.11 ^b

Mean (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 10. Individual effect of milk fat content on the growth of *Lactobacillus bulgaricus* counts

Fat %	Counts of <i>Lactobacillus bulgaricus</i> log(CFU/ml)			
	Day 1	Day 7	Day 14	Day 28
0	8.27 ^a	9.36 ^a	10.43 ^a	10.54 ^a
1.5	8.52 ^a	9.21 ^a	10.13 ^a	10.84 ^a
3.3	7.09 ^b	8.75 ^a	9.04 ^b	8.66 ^b

Mean (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 11. Individual effect of added sugar content on the growth of *Lactobacillus bulgaricus* counts

Sugar%	Counts of <i>Lactobacillus bulgaricus</i> log(CFU/ml)			
	Day 1	Day 7	Day 14	Day 28
0	8.40 ^a	9.77 ^a	9.66 ^b	10.57 ^a
3.5	8.16 ^a	8.80 ^b	10.20 ^a	10.04 ^{ab}
7	7.31 ^b	8.75 ^b	9.75 ^{ab}	9.44 ^b

Mean (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

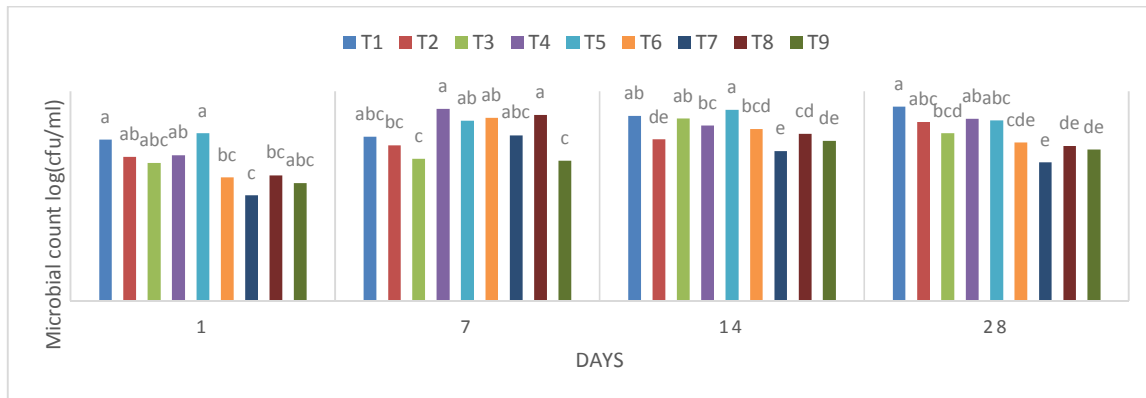


Fig. 4. Interaction effect of milk fat and added sugar content on the growth of *Lactobacillus bulgaricus*

T = treatment, Values followed by different letters in the bars on the same day are significantly different ($p < 0.05$) according to Tukey's test

3.5 Syneresis and Texture Profile Analysis

Texture characteristics are critical parameters in sensory evaluation and in the consumer acceptability of yogurt [24].

Hardness/firmness increased with the fat level, comply with the findings of Guggisberg et al. (2009)[5]. Similarly, adhesiveness, fracturability, and springiness also highest in full cream yogurts. Moreover, syneresis considerably increased with milk fat reduction (Table 13). Reduction in fat

content can cause a fragile texture due to the weaker network of the protein gel in yogurts [20], [25]. In the present study, cohesiveness was not altered by milk fat content (Table 13), is contrary to the finding of Espirito Santo et al. (2012)[20] which showed higher cohesiveness in whole milk yogurts than the respective skim ones ($P < 0.05$).

Hardness, adhesiveness, fracturability, and gumminess were lowest in 7% sugar added yogurts while the syneresis was highest (Table 14). This trend was upturned with fat content. The main effect of 0% and 3.5% sugar levels have not a significant difference ($P > 0.05$) on tested texture parameters except syneresis (Table 14).

Interaction effects on milk fat and added sugar levels on texture parameters showed that

hardness of full cream, 7% sugar was less than the hardness of full cream, 3.5% sugar (Table 15). That means the firmness of yogurt can reduce at high sugar levels. All the texture parameters have interaction effects ($p < 0.05$) except springiness (Table 15 and 16).

3.6 Rheology

Yogurt is a non-Newtonian substance and yogurt viscosity is an indication of a network of casein-particle aggregation leading to gelation. Consistency index (k) and flow behavior index (n) are two parameters which use to explain non-Newtonian flow behavior. They can be calculated using the graphs made by log rotational speed vs. log torque and log shear rate ($\dot{\gamma}$) vs. log shear stress (σ), respectively by a viscometer.



Fig. 5. Interaction effect of milk fat and added sugar content on the growth of *Streptococcus thermophilus*

T = treatment, Values followed by different letters in the bars on the same day are significantly different ($p < 0.05$) according to Tukey's test

Table 12. Viability proportion index of two yogurt producing microorganisms as a function of milk fat and added sugar content

Fat %	Sugar%	Viability proportion index (VPI)	
		<i>Streptococcus thermophiles</i>	<i>Lactobacillus bulgaricus</i>
0	0	1.20	1.23
0	3.5	1.24	1.29
0	7	1.22	1.31
1.5	0	1.25	1.36
1.5	3.5	1.08	1.23
1.5	7	1.28	1.30
3.3	0	1.31	1.34
3.3	3.5	1.24	1.18
3.3	7	1.29	1.26

Table 13. Individual effect of milk fat content on texture parameters

Fat %	Hardness/firmness (g)	Adhesiveness (mJ)	Fracturability (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Syneresis index %
0	126.80 ^c	3.48 ^c	68.53 ^c	0.44 ^a	19.19 ^b	54.99 ^c	45.92 ^a
1.5	154.98 ^b	7.49 ^b	149.07 ^b	0.44 ^a	19.58 ^{ab}	70.07 ^b	12.44 ^b
3.3	286.72 ^a	14.92 ^a	282.20 ^a	0.44 ^a	20.24 ^a	121.58 ^a	1.58 ^c

Mean (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 14. Individual effect of added sugar contents on texture parameters

Sugar %	Hardness/firmness (g)	Adhesiveness (mJ)	Fracturability (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Syneresis index %
0	205.90 ^a	10.31 ^a	188.47 ^a	0.43 ^b	19.70 ^a	90.3 ^a	17.97 ^c
3.5	197.30 ^a	9.55 ^a	179.20 ^a	0.43 ^b	19.84 ^a	84.19 ^a	19.19 ^b
7	165.30 ^b	6.02 ^b	132.13 ^b	0.45 ^a	19.47 ^a	72.14 ^b	22.77 ^a

Mean (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 15. Interaction effect of milk fat and added sugar content of texture parameters (Part I)

Sugar %	Fat %	Hardness/ firmness (g)	Adhesiveness (mJ)	Fracturability (g)	Cohesiveness
0	0	148.33±8.40 ^{cd}	5.15±0.45 ^{cd}	116.40±3.40 ^{cd}	0.42±0.00 ^b
3.5	0	124.67±5.28 ^{de}	3.07±0.35 ^d	75.50±1.30 ^d	0.41±0.01 ^b
7	0	107.40±5.97 ^e	2.21±0.16 ^d	13.70±1.30 ^e	0.47±0.00 ^a
0	1.5	174.20±10.80 ^c	9.87±1.00 ^b	163.50±13.90 ^c	0.42±0.02 ^b
3.5	1.5	149.73±9.10 ^{cd}	7.16±0.35 ^{bc}	148.70±7.30 ^c	0.47±0.00 ^a
7	1.5	141.00±4.80 ^{cde}	5.44±0.34 ^{cd}	135.00±10.90 ^c	0.45±0.00 ^b
0	3.3	295.17±5.11 ^a	15.92±0.02 ^a	285.50±4.50 ^{ab}	0.44±0.00 ^{ab}
3.5	3.3	317.90±11.10 ^a	18.43±0.97 ^a	313.40±9.80 ^a	0.42±0.01 ^b
7	3.3	247.50±12.50 ^b	10.40±1.22 ^b	247.70±12.50 ^b	0.44±0.01 ^{ab}

Mean ± SE (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Table 16. Interaction effect of milk fat and added sugar content of texture parameters (part II)

Sugar %	Fat %	Springiness (mm)	Gumminess (g)	Syneresis Index %
0	0	19.26±0.01 ^a	62.93±4.13 ^{cd}	43.30± 0.46 ^b
3.5	0	19.32±0.09 ^a	51.37±2.13 ^d	46.40±0.83 ^a
7	0	19.23±0.31 ^a	50.93±2.57 ^d	48.24±0.09 ^a
0	1.5	19.46±0.29 ^a	78.17±3.98 ^c	8.31±0.35 ^d
3.5	1.5	19.68±0.16 ^a	69.63±3.82 ^c	10.81±0.42 ^d
7	1.5	19.62±0.03 ^a	62.40±2.90 ^{cd}	18.19±0.95 ^c
0	3.3	20.39±0.57 ^a	129.80±0.20 ^a	2.32±0.33 ^e
3.5	3.3	20.53±1.12 ^a	131.85±4.15 ^a	0.37±0.31 ^e
7	3.3	19.80±0.19 ^a	103.25±4.05 ^b	2.05±0.17 ^e

Mean ± SE (n=3). Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test

Several mathematical models have been developed to describe this relationship between σ and $\dot{\gamma}$. These models are used to characterize flow properties in an effort to determine the ability of a fluid to perform specific functions. Power Law is the simplest model use to describe the flow behavior of non-Newtonian fluids. However, power law model has a limitation that it is valid over only a limited range of shear rates. Therefore the values of consistency index (k) and flow behavior index (n) are dependent on the range of shear rates taken into account. Yet, 'Power Law' is the most common and widely used model dealing with process engineering applications.

In the present study, shear stress became constant (nearly independent from shear rate) at elevated shear rate values. Therefore, k, n, and R^2 values were separately calculated by including that constant shear stress values (Table 17) and by omitting those values (Table 18). The calculation method explained by Mitschka (1982)[26] was used for the calculations as explained by Zubairi (2010)[18].

Both results showed, all the yogurt samples exhibited a non-Newtonian and pseudoplastic flow behavior because the flow index (n) is less than 1 for all the samples. If the regression coefficient is near 1 ($R^2 > 0.95$), the power-law model is suitable to describe the parameters. In that sense, lower shear rates are far better describing the power law model (Table 18). The Power-law model for these yogurts is only valid in low shear rate values (0.3 to 20 s^{-1}). Therefore, values obtained through Table 17 used to describe the rheology of yogurt samples.

Consistency coefficient, K, from the power model reflects the magnitude of viscosity in terms of consistency. Highest consistencies observed in full-fat yogurt samples. Flow index (n) was comparatively less than the results of the previous studies [27–29] for yogurts, but did not largely change with added sugar or milk fat level. The consistency index is matched with the values obtained by those studies to the yogurts. Low K values of no-fat yogurts demonstrated they have weak gel networks. Therefore, optimization need to increase the gel strength of no-fat yogurts for better consumer acceptability.

Table 17. Power-law parameters of yogurt samples (part I)

Sample Code	Fat %	Sugar %	n-flow index	log K – log consistency index	R ²	K Pa s ⁿ
NFNS	0	0	0.24±0.00	3.55±0.01	0.84±0.00	3624±39.64
NFLS	0	3.5	0.31±0.01	3.45±0.01	0.92±0.00	2782±44.50
NFHS	0	7	0.22±0.00	3.54±0.01	0.89±0.01	3452±50.47
LFNS	1.5	0	0.24±0.00	3.69±0.01	0.84±0.01	4974±54.50
LFLS	1.5	3.5	0.18±0.00	3.62±0.00	0.82±0.00	4154±13.50
LFHS	1.5	7	0.20±0.00	3.61±0.00	0.75±0.01	4101±64.50
FCNS	3.3	0	0.10±0.00	3.74±0.00	0.52±0.00	5478±49.50
FCLS	3.3	3.5	0.24±0.00	3.78±0.00	0.67±0.17	6061±20.24
FCHS	3.3	7	0.24±0.00	3.65±0.01	0.69±0.17	5663±65.40

Mean ± SE (n=3)

Table 18. Power-law parameters of yogurt samples (part II)

Sample Code	Fat %	Sugar %	n-flow index	log K – log consistency index	R ²	K Pa s ⁿ
NFLS	0	0	0.40±0.00	3.64±0.00	0.97±0.01	4365±0.00
NFLS	0	3.5	0.34±0.01	3.47±0.01	0.94±0.01	2914±105.00
NFHS	0	7	0.24±0.02	3.55±0.01	0.91±0.02	3508±40.00
LFNS	1.5	0	0.35±0.00	3.74±0.01	0.96±0.01	5516±107.74
LFLS	1.5	3.5	0.27±0.05	3.64±0.00	0.91±0.01	4345±20.00
LFHS	1.5	7	0.31±0.01	3.65±0.00	0.96±0.01	4467±0.00
FCNS	3.3	0	0.29±0.00	3.82±0.00	0.92±0.00	6622±456.50
FCLS	3.3	3.5	0.39±0.00	3.85±0.00	0.97±0.00	7120±41.00
FCHS	3.3	7	0.36±0.01	3.88±0.06	0.94±0.01	7559±111.00

Mean ± SE (n=3)

4. CONCLUSIONS

Both added sugar and milk fat levels have individual and interaction effects on tested parameters. Some parameters showed clearly identifiable positive or negative relationships with the fat/sugar level and others have not shown such clear relationships.

Fermentation kinetics (acidification rates) not largely influenced by fat levels. Fermentation time not significantly differ with the fat/sugar alteration. As expected TSS content increase with milk fat and added sugar content. Whiteness (L*) of the yogurt reduces with milk fat content but no effect by added sugar levels. Results showed that added sugar and milk fat content can act as hurdles for lactic acid-producing bacteria growth.

FCLS yogurts have the highest springiness, gumminess, hardness, adhesiveness, fracturability and lowest syneresis over FCHS yogurts. Therefore, reducing sugar content up to 3.5% from 7% gave even better texture attributes

particularly in full cream yogurts. Texture integrity reduces with the reduction of fat content.

Accordingly, the reduction of added sugar and milk fat is favorably influenced by some quality parameters of yogurt. Further, studies need to find the optimum added sugar and milk fat combination. Consumer perception also should consider in this sense.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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