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Evaluation of Physico-chemical Properties and Selected Antibiotic Residues in UHT Milk Marketed in Palestine

Bewertung physikalisch-chemischer Eigenschaften und ausgewählte Antibiotikarückstände in UHT-Milch, die in Palästina vermarktet wird

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Summary

Ultra-high temperature (UHT) milk products have the main market share in the Palestinian local dairy market. The aim of this study was to evaluate the sensory, physico-chemical and microbiological quality of UHT milk. In total 30 milk packs from Low and whole-fat UHT were selected from three different (two locals and one foreign labeled as A, B, and C) companies to evaluate physico-chemical properties (viscosity, density, freezing point, acidity, fat, protein, sensory traits, mineral composition, etc) and some microbiological parameters. Moreover, milk samples were tested for presence of residues of three commonly used antimicrobial drugs (Penicillin G, Sulfamethazine, and Tetracycline). Our study showed that there were significant differences ($P < 0.05$) in fat content among companies (3.56, 2.98, and 3.03% for A, B, and C companies, respectively). The non-compliance% in fat content with respect to labeled fat content ranged from –0.7% (for company B) to +18.7% (company A) in whole milk product, and +2.0% (company C) to +115% (company B) in low fat milk. Whole milk product from company B showed 4.75% of water addition which was significantly larger in comparison with other sources. Moreover, UHT milk products from company B exhibited 10% of positive results of antibiotic residues (+Sulfamethazine) which was significantly higher than other sources of milk products, and at the same time was higher than the Maximum Residue Level (MRL = 100 µg/L). In conclusion, this study showed that some of UHT milk products that are available in the Palestinian market do not fully meet the nutritional labeling (particularly in regard to fat content) and there were some violations in safety criteria.

Keywords: Physico-chemical properties, antibiotics, UHT, Fat content

Zusammenfassung

Ultra-Hoch-Temperatur Milchprodukte (UHT) haben den größten Anteil auf dem palästinensischen Markt. Ziel dieser Studie war es, die sensorische, physikalisch-chemische und mikrobiologische Qualität von UHT-Milch zu bewerten. Insgesamt wurden 30 Milchpackungen (fettarmer und vollfett Milch) aus zwei einheimischen (A und B) und einem ausländischen (C) Unternehmen ausgewählt, um physikalisch-chemische Eigenschaften (Viskosität, Dichte, Gefrierpunkt, Säuregehalt, Fett, Protein, sensorische Eigenschaften, Mineralzusammensetzung) und einige mikrobiologische Parameter zu untersuchen. Darüber hinaus wurden die Milchproben auf Rückstände von drei häufig verwendeten antimikrobiellen Medikamenten (Penicillin G, Sulfamethazin und Tetracyclin) getestet. Unsere Studie zeigte, dass es signifikante Unterschiede ($P < 0,05$) im Fettgehalt zwischen den Unternehmen gab (3,56%, 2,98% und 3,03% für Firma A, B und C). Die Nichteinhaltung des Fettgehalts in Bezug auf den gekennzeichneten Fettgehalt reichte von –0,7% (für Unternehmen B) bis +18,7% (Firma A) in Vollmilchprodukten und +2,0% (Firma C) bis +115% (Firma B) in fettarmer Milch. Das Vollmilchprodukt von Firma B zeigte 4,75% Wasserzugabe, was im Vergleich zu den anderen deutlich höher war. Darüber hinaus zeigten UHT-Milchprodukte der Firma B 10% positive Antibiotikarückstände (Sulfamethazin), die deutlich höher waren als bei anderen Milchprodukten und gleichzeitig über dem maximalen Rückstandsgehalt (MRL = 100 µg/L) lagen. Zusammenfassend zeigte diese Studie, dass einige der auf dem palästinensischen Markt erhältlichen UHT-Milchprodukte die Nährwertkennzeichnung (insbesondere in Bezug auf den Fettgehalt) nicht vollständig erfüllten und, dass einige Sicherheitskriterien verletzt wurden.

Schlüsselwörter: Physikalisch-chemische Eigenschaften, Antibiotika, UHT, Fettgehalt

Introduction

The dairy industry is one of the main food industries in Palestine where the annual milk production reached more than 172,000 m^3 (Palestinian Central Bureau Statistics, 2006; Ministry of National Economy, 2006). UHT milk is widely consumed in Palestine and has a higher market share than fresh and pasteurized milk (Palestinian Central Bureau Statistics, 2006; Ministry of National Economy, 2006). Ultra-high-temperature (UHT) treatment has several adverse implications on the quality of products such as modification of color and flavor as well as consistency (gelation). These modifications are usually attributed to protein denaturation, fat/protein oxidation, and Maillard reactions (Valero et al., 2001; Baumgartner et al. 2010; Jansson et al., 2014). Losses in nutrients may also occur to some extent during storage of UHT processed milk (Guzman et al., 2003; Rehman et al., 2005). Moreover, the presence of virulence factors in milk have been evaluated (Gundogan et al., 2012).

Different studies have been conducted on the evaluation of physico-chemical properties and the safety aspects of UHT milk marketed in different countries around the world. The exposure of consumers to chloramphenicol (CAP) at Northern Parana (Brazil) has been evaluated (Sifuentes et al., 2016). Karim and Dey (2013) showed that the microbiological quality of UHT milk was not accepted after three months of production. In Switzerland, the presence of Cronobacter *(Enterobacter sakazakii)* has been evaluated in big milk processing companies (Baumgartner et al., 2010). For the microbiological analysis, 37.5%, 62.5% and 12.5% of samples acquired from Brazil, Argentina, and Paraguay, respectively, showed counts above the established limits for mesophilic microorganisms (Domareski et al., 2010).

The information and studies about the physico-chemical and safety aspects of UHT milk commercialized in Palestine are very limited. Accordingly, the present study was conducted with the aim of investigating the physico-chemical parameters as well as some safety aspects of UHT milk and their compliance with regulations.

Materials and Methods

Collection of samples

In total, 30 milk samples were collected from the local market in Toulkarem city (north of the West Bank, Palestine). Five packs of 1% fat UHT milk and five packs of 3% UHT milk were randomly selected from each of three companies with the largest share in the Palestinian market (labeled hereafter as A, B, and C). Selected milk packs were of one-liter size and all were from different batches. It was taken into consideration that all samples have similar production dates (maximum difference of two weeks).

Microbiological analysis

Total aerobic mesophile microorganisms were determined. Two dilutions (-1,-2) were prepared in buffer peptone water. Samples were inoculated into plate count agar and incubated at 30ºC for 72 h. The total count of aerobic spore-forming bacteria was estimated by exposing milk samples to heat treatments at 80ºC for 12 minutes (Evelise et al., 1992). One ml samples of treated milk were inoculated by pour technique on plate count agar, then incubated at 32ºC for 48 h. The total anaerobic spore-forming bacteria count was evaluated in the same way as aerobic spore forms except that the plates were incubated under anaerobic conditions.

Physical-chemical analysis

The volume of milk samples was determined in order to compare them with the labeled values. The weights of milk samples including packaging were recorded. Then, the milk packs were emptied and the empty dry weight of package was recorded. The net weight of milk was determined by difference. The volume was then estimated by dividing the milk weight on measured milk density at room temperature.

The pH values were measured by using a digital pH-meter (pH meter 3310, Jenway, UK). Samples of 10 ml of homogenous milk were used to measure titratable acidity by titration with 0.1 N NaOH to the phenolphthalein end point (Feldsine et al., 2002).

The viscosity (expressed in mPa.s) was determined at constant temperature (5ºC) by using rotational viscometer (Portable viscometer VT-03/04, Rion CO, Tokyo, Japan).

Proximate chemical composition (fat, protein, lactose, ash, and SNF) and physical traits (density and freezing point) of the milk samples were measured using milk lactoscan analyzer (Milkoscope; Julie C8 Automatic, Scope Electric, Germany Regensburg).

Sensory analysis

The 9-point hedonic rating scale (9 = excellent; 1 = extremely poor) was used. The sensory traits evaluated (color, flavor, taste, and overall acceptability) were assessed by 31 randomly selected consumer panelists.

Mineral analysis

Ash content was determined by the difference in weight after incineration at 525°C of 5 g of milk samples for 4 h. The ash was dissolved by boiling in 10 M HCl, then filtrated and diluted by deionized water. Potassium, calcium, and sodium were measured by using atomic emission spectroscopy (410 Flame photometer, Sherwood, UK). For each element, a standard curve was prepared in the range of good linearity (R^2= 0.995-0.997).

Antibiotic analysis

IDEXX kit for ß-Lactam, gentamicin, and sulfamethazine SNAP test were used to analyze antibiotic residues in milk samples. After homogenization by shaking, 450 µL (±50 µL) samples were drawn by pipette and loaded in 1 ml plastic tubes. Samples were gently shaken side to side three to four times until the reagent pellet was completely dissolved. Samples were preheated and incubated for a specific time depending on the type of antibiotic on a heating block adjusted at 45°C ±5°C. The entire content of the milk sample was poured into the well of a SNAP device. The SNAP device was activated after milk flows across the edge of the blue activation circle. Incubation time was 5 minutes for Tetracycline, and 2 minutes for Gentamicin and Sulfamethazine. The results were read within 30 seconds after incubation using the SNAP shot* DSR Reader.

Statistical analysis

The differences between the mean values of physico-chemical characteristics within the same fat category were

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evaluated by one-way ANOVA using Tukey's test for multiple comparisons with differences declared significant at the 0.05 level. Sensory data were analyzed using the PROC GLM procedure of SAS. The analysis model included the effects of panelist, product (low fat or whole fat), company (three levels) and the interaction between product and company. Tukey's test was used to adjust P values for multiple comparisons.

Results and discussion

Physico-chemical properties of whole and low-fat UTH milk are shown in Table 1 and 2, respectively. Several indigenous (compositional) and exogenous (such as temperature and post milk treatment) factors usually affect the physical and chemical properties of milk) Mc Sweeney and Fox 2003; Mccarthy and Singh, 2009). The results of the study showed that in both low and whole milk groups, significant differences between companies were found in fat content. The range of fat content in whole milk was (2.98–3.56%) which is a little far from the normal rage (3.5–4.7%). In addition, non-compliance percentage in fat content compared to the labeled value was very high. In particular in company B, the fat content was higher than the labeled value by 115% in low-fat milk. This variability can be considered as an indicator of improper fat standardization protocols. For protein content, there were no significant differences between companies for both low and whole milk. The non-compliance percentages of measured protein content compared to the labeled values were less than those for fat content. The maximum value of protein non-compliance was for company B where it exceeded the labeled value by 16.9% in the low-fat milk group.

Lactose content did not show any significant differences between companies in both low and whole fat milk. Lactose content was compared with labeled carbohydrates content because most of milk carbohydrates content is lactose. In general, there were slight differences between measured lactose and labeled values of carbohydrates (was less by 6.9% for low-fat milk from company C). Lactose content can be used as an indicator of animal health, particularly mastitis.

Total solids and total non-fat solids did not exhibit any significant differences between companies in low and whole milk (Table 1 and 2).

The results showed an average of 4.75% added water in the whole milk samples from company B but no added water was detected for the other two companies, while the quantity of added water in the low-fat milk was small and not significant between companies. The addition of water in low-fat milk can be easily detected at a low concentration while in whole fat milk it is more difficult to detect. The obtained result of added water for company B was in agreement with result of freezing point where the freezing point for company B was less than regulations (–0.516 versus –0.5258)

The titratable acidity was determined in both low and whole-fat milk. The results showed that the acidity did not vary significantly between companies. Acidity is usually used as a quality indicator for the microbial level (freshness of milk) which is affected by hygienic conditions, handling, and transportation temperature. The increase of milk acidity is mainly due to fermentation of lactose by lactic acid bacteria or due to high lipase activity that produces free fatty acids from fat (Walstra and Jennes, 1984). The findings showed that the range of acidity in low (0.202–0.216%) and whole (0.17–0.195%) milk was consistent with the normal range for milk (0.08–0.25%) and also consistent with the most common range (0.14–0.17%) (Moussa et al., 2013). No significant differences in ash content were found between companies for whole milk and low-fat milk.

The results showed that there were no significant differences between companies in freezing point. Freezing point can be used as a good indicator for the level of total soluble solids in milk as well as the added water. The addition of water to milk decreases the concentration of solutes in milk, which increases the freezing point. The range of freezing points for all companies was –0.516 to –0.534 C. The usual range of freezing point for cow milk is –0.512 to –0.551 but the most common range is –0.520 to –0.530 C (Mc Sweeney and Fox, 2009). This variability is usually due to differences in the concentration of individual solutes of milk components. In this context, the freezing point is highly affected by the osmotic molality of milk which is

TABLE 1: *Physico-chemical properties of ultra-high-temperature whole milk (3% fat) from three companies with the highest share in the Palestinian market.*

Parameters	Company A			Company B			Company C			P
	MC±SEM[1]	N.V[2]	N.C%[3]	M.C±SEM	N.V	N.C%	M.C±SEM	N.V	N.C%	Value
Fat	3.56±0.08[a]	3	+18.7	2.98±0.16[b]	3	–0.7	3.03[b]±3.03	3	+1.0	<0.05
Protein	3.13±0.03	2.9	+7.9	3.00±0.08	2.9	+3.4	3.16±0.05	3.3	–4.2	0.156
Lactose	4.67±0.05	4.6	+1.5	4.40±0.14	N.A	N.A	04.7±2.01	4.95	+4.6	0.082
Total solids	11.69±0.19	N.A[4]	N.A	11.09±0.23	N.A	N.A	11.23±0.22	N.A	N.A	0.167
SNF	8.54±0.09	N.A	N.A	8.02±0.25	8.5	–5.6	8.53±0.03	N.A	N.A	0.094
Added water	0.00[b]±0.00	N.A	N.A	4.75±2.1[a]	N.A	N.A	0.00[b]±0.00	N.A	N.A	<0.05
Acidity	0.19±0.01	N.A	N.A	0.19±0.01	N.A	N.A	0.17±0.01	N.A	N.A	0.234
Ash	0.716±0.002	N.A	N.A	0.716±0.004	N.A	N.A	0.725±0.002	N.A	N.A	0.148
Freezing point	–0.532±0.006	N.A	N.A	–0.516±0.019	N.A	N.A	–0.534±0.008	N.A	N.A	0.553
Density	1.029±0.001	N.A	N.A	1.028±0.001	N.A	N.A	1.030±0.001	N.A	N.A	0.132
pH	6.82±0.02	N.A	N.A	6.76±0.04	N.A	N.A	6.78±0.01	N.A	N.A	0.390
Viscosity	4.0±0.1	N.A	N.A	3.8±0.3	N.A	N.A	4.0±0.1	N.A	N.A	0.632

Means within a row followed by different superscript letters differ significantly (P≤0.05). [1]: M.C measured content; SEM standard error of mean. [2]: N.V is nutritional value that is labeled on the container. [3]: N.C non compliance percentage calculated as the difference between measured and labeled value. [4]: N.A: not available. +: when measured values are higher than labeled values. –: when measured values are less than labeled values.

TABLE 2: *Physico-chemical properties of ultra-high-temperature low-fat milk (1 and 1.5% fat) from three different companies from the Palestinian market.*

Parameters	Company A			Company B			Company C			P
	MC±SEM[1]	N.V[2]	N.C%[3]	M.C±SEM	N.V	N.C%	M.C±SEM	N.V	N.C%	Value
Fat	1.95±0.14[b]	1.5	+30.0	2.15±0.15[a]	1	+115.0	1.02±.032[c]	1	+2.0	<0.05
Protein	3.39±0.14	2.9	+16.9	3.21±0.05	2.9	10.7	3.18±0.07	3.35	−5.1	.237
Lactose	4.71±0.09	4.6	+2.4	4.70±0.06	N.A	N.A	4.75±0.11	5.1	−6.9	.940
Total solids	11.02±0.61	N.A	N.A	10.62±0.40	N.A	N.A	10.84±0.58	N.A	N.A	.876
SNF	8.58±0.16	N.A	N.A	8.69±0.08	8.5	2.2	8.65±0.20	N.A	N.A	.875
Added water	0.44±0.44	N.A	N.A	0.60±0.60	N.A	N.A	0.32±.21	N.A	N.A	.907
Acidity	0.20±0.01	N.A	N.A	0.21±0.02	N.A	N.A	0.22±0.02	N.A	N.A	.796
Ash	0.755±0.003	N.A	N.A	0.728±0.004	N.A	N.A	0.688±.030	N.A	N.A	.068
Freezing point	−0.517±0.008	N.A	N.A	−0.519±.005	N.A	N.A	0.519−±0.006	N.A	N.A	.483
Density	1.030±0.001	N.A	N.A	1.030±0.001	N.A	N.A	1.031±0.001	N.A	N.A	.694
pH	6.77±0.03	N.A	N.A	6.70±0.057	N.A	N.A	6.76±0.052	N.A	N.A	.590
Viscosity	3.65±0.33	N.A	N.A	3.73±0.12	N.A	N.A	3.6±0.18	N.A	N.A	.927
Volume	996.1±0.7[b]	1000	−0.39	990.6±1.1[c]	1000	−0.94	1000.0±3.1[a]	1000	0	<0.05

Means within a row followed by different superscript letters differ significantly (P≤0.05). [1]: M.C measured content; SEM standard error of mean. [2]: N.V is nutritional value that is labeled on the container. [3]: N.C non compliance percentage calculated as the difference between measured and labeled value. [4]: N.A: not available. +: when measured values are higher than labeled values. −: when measured values are less than labeled values.

normally controlled by the mammary gland. In general, the effect of farming (feed, lactation stage, water intake, and breed of cow), environmental (climate and season), and pathological factors (mastitis) on freezing point is very limited (Mc Sweeney and Fox, 2009). In addition, processing conditions (in particular heat treatment) has an effect on freezing point. During heating, some of the soluble salts are transformed into the colloidal state while lactose interacts with proteins which raise the freezing point of milk. Several studies showed that these changes have a significant impact on freezing point while others did not show any difference. Different regulations have been issued regarding the standard freezing point. In general, milk with freezing point more than –0.5258 can be considered as adulterated (Henningson, 1969). Our results showed that all samples had freezing points less than –0.5258 indicating no adulteration, except one group of low-fat samples from company B where the freezing point was –0.516, the results showed that this group had on average 4.7% of added water.

The density ranged from 1.0301 to 1.0309 g/ml for low-fat and 1.0276 to 1.0296 g/m for whole fat milk. These results are in agreement with previous studies where the density ranged from 1.028 to 1.033 g/ml. The density of milk can be sharply affected by added water. Density may give an indication of composition differences (solid content) in the milk which can be affected by lactation stage, feed composition, and patho-physiological conditions (Mccarthy and Singh, 2009). Fat content is the most important component that influences the density of milk because it is the lowest in density (Walstra and Jenness, 1984). The measurement of density should be at a constant temperature because the differences in temperature cause different expansion coefficients for fat and water. In this context, the expansion coefficient for fat is higher than for water, therefore the effect of fat on density is higher than the effect of water (Walstra and Jenness, 1984). In general, the range of measured pH values (6.7–6.81) in all groups was within the normal range of pH for milk (6.7–6.8). The pH-values were not significantly different among companies in both low and whole-fat milk. The pH plays an important role in the quality of milk, particularly during processing, and also can be used as an indicator of milk freshness. Moreover, pH may be used as an indicator of the health status of the animals as milk from cows with mastitis has higher pH than milk from normal cows. This is due to the release of sodium and chloride ions in the milk as well as the decrease of soluble inorganic phosphate in the milk when the mammary cells lose permeability. In addition, it can be used as an indicator of microbial quality and quantity of milk (McCarthy and Singh, 2009).

The viscosity of milk for both low and whole-milk was measured. The results did not show any significant differences. The viscosity of milk may be affected by some native enzymes such as heat-stable proteinases that resist the sterilization process (Kelly and Foley, 1997; Datta and Deeth, 2003). The absolute values of viscosity were higher than in previous studies (Saini et al., 2011) but there were no significant differences between companies. High values of viscosity may be attributed to measuring the viscosity at low temperature (4°C) which increased the viscosity.

Three milk minerals (Na, Ca, and K) have been determined. There were no significant differences in mineral content between companies for both low and whole fat milk. These results can be explained by high variability in mineral contents from lot to lot in the same company as indicated by the large standard errors. Moreover, different factors may affect the mineral content such as lactation stage, nutritional composition of feed, and genotype.

TABLE 3: *Mineral analysis for low and high-fat UHT milk.*

Parameters	Company A (mg/100 ml)	Company B (mg/100 ml)	Company C (mg/100 ml)	P Value
High fat 3%				
Ca	140.04±20.75	112.59±18.57	139.92±15.78	0.557
K	183.38±52.35	130.88±4.51	136.78±8.95	0.479
Na	64.05±13.72	41.39±8.83	38.89±2.20	0.201
Low fat 1–1,5%				
Ca	136.53±16.98	165.29±20.88	116.14±14.00	0.172
K	110.85±14.99	168.859±20.65	118.79±18.27	0.113
Na	39.85±8.01	62.57±8.42	31.83±5.38	0.130

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TABLE 4: *Microbiological analysis for low and high-fat UHT milk.*

Parameters	Company A	Company B	Company C
High-fat milk (3%)			
TPC	0%	0%	0%
Anaerobic	0%	0%	0%
Spore	0%	*20%	0%
Low-fat milk (1%)			
TPC	0%	0%	0%
Anaerobic	0%	0%	0%
Spore	0%	30%*	0%

* All positive results were in the range of 10–500 cfu/ml

TABLE 5: *Antibiotics residues in low and whole milk products.*

Parameters	Company A	Company B	Company C
High-fat milk (3%)			
Penicillin G	-ve*	-ve	-ve
Sulfamethazine	-ve	-ve	-ve
Tetracycline	-ve	-ve	-ve
Low-fat milk (1%)			
Penicillin G	-ve	-ve	-ve
Sulfamethazine	-ve	%10	-ve
Tetracycline	-ve	-ve	-ve

*-ve: means negative results

The ranges of calcium (112.59–165.29 mg/100 g), sodium (31.83–64.05 mg/100 g) and potassium (110.8–168.8 mg/100 g) were in agreement with previous studies (Zamberlin et al., 2012).

Low and whole-fat milk products from different sources did not show any significant differences in total mesophilic aerobic and anaerobic microbial counts. All types of milk samples were free from aerobic vegetative bacteria. This type of analysis may give an indication of the initial microbial load of milk before sterilization which depends mainly on milking hygiene, cleanliness of equipment, and transportation/storage temperatures (Muir, 1996). Our results indicated that the quality of raw milk that has been used for UHT treatment had the required specifications from the microbiological viewpoint.

Low and whole fat milk products from company B showed that the percentage of positive spore-forming tests were 20 and 30%, respectively; whereas company A and C did not show any positive tests. Determination of spore-forming bacteria load in the finished products is very important to evaluate the initial count (related to cleanliness of cow teats and bedding) as well as the success of the UHT process. The count and resistance of microbes are critical to choosing the right UHT conditions necessary to eliminate them.

Our results showed that all samples were free from antibiotic residues (penicillin G, sulfamethazine, and tetracycline) except low fat-milk samples from company B where the positive tests for sulfamethazine were 10%.

The results of the sensory analysis are in Table 6. By considering the main effect of fat content on sensory traits, low-fat milk exhibited lower values of taste (4.78 vs. 5.49) and overall acceptance (4.85 vs 5.60) when compared to whole-milk fat. There were no significant differences in color and flavor values. The main effect of the company on sensory traits was evaluated. It was found that company B showed the lowest average values in all sensory traits (color, taste, flavor, and overall acceptance) when compared to company A and C while there were no differences between company A and C. These results for company B may be explained by the lower microbiological quality where 20 to 30% of low and whole-milk samples from company B were positive.

By considering the interaction effect of fat and company on the sensory traits, it was found that low fat-milk group from company B showed the lowest values in all sensory traits while other groups of low and whole milk fat from the remaining companies did not exhibit any differences.

Organoleptic properties of milk can be affected by hydrolysis and oxidation of milk-fat and proteins by thermostable bacterial lipases and proteinases (Choi and Jeon, 1993; Coolbear et al., 2003). In addition, organoleptic properties such as color and taste may be affected by Maillard reaction during sterilization, where different browning compounds (such as pyrazines and melanoidinis) are usually produced (Van Boekel, 1998). The difference in sensory traits in our findings may be explained by the effect of the severity of heat treatment rather than due to the effect of fat and protein oxidations.

Conclusion

The results showed that UHT milk products that are available in the Palestinian market were not similar in the quality traits. The most cases of non-compliance were in fat content, in particular for local companies. Low fat-milk products did not meet the nutritional labeling in some compositional traits. Therefore, it is necessary for Palestinian regulation authorities to increase the quality surveillance on milk products that are sold in the Palestinian market.

TABLE 6: *Least square means of sensory characteristics of two milk products from three different companies.*

Effects in the model		Least squares mean ±SEM of studied product sensory characteristics Color	Taste	Flavor	Acceptance
Main effects					
Fat					
1 %		5.28±0.21^{a,*}	4.78±0.22^{b}	4.94±0.23^{a}	4.85±0.22^{b}
3 %		5.77±0.21^{a}	5.49±0.22^{a}	5.32±0.23^{a}	5.60±0.22^{a}
Company					
A		6.08±0.26^{a}	5.74±0.27^{a}	5.63±0.29^{a}	5.87±0.28^{a}
B		4.58±0.26^{b}	4.23±0.27^{b}	4.27±0.29^{b}	4.52±0.28^{b}
C		5.92±0.26^{a}	5.45±0.27^{a}	5.48±0.29^{a}	5.29±0.28^{a,b}
Fat% x Company					
Fat%	**Company**				
1%	A	6.16±0.37^{a}	5.77±0.38^{a}	5.61±0.40^{a}	5.84±0.39^{a}
	B	3.77±0.37^{b}	3.26±0.38^{b}	3.55±0.40^{b}	3.52±0.39^{b}
	C	5.90±0.37^{a}	5.32±0.38^{a}	5.65±0.40^{a}	5.19±0.39^{a}
3%	A	6.00±0.37^{a}	5.71±0.38^{a}	5.65±0.40^{a}	5.90±0.39^{a}
	B	5.39±0.37^{a}	5.19±0.38^{a}	5.00±0.40^{a,b}	5.52±0.39^{a}
	C	5.94±0.37^{a}	5.58±0.38^{a}	5.32±0.40^{a}	5.39±0.39^{a}

*: Means in the same column with different superscripts are significantly different ($P < 0.05$) based on Tukey's adjustment for multiple comparisons. SEM: standard error of mean

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Conflict of interest

The authors declare that they have no conflict of interests

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