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Influence of Adding Mucilage as a Fat Replacer on the Characteristics of Yoghurt

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Abstract

The objective of this study was to examine the effects of substituting milk fat with different levels of mucilage as a fat replacer on the chemical, microbiological and sensory characteristics of yoghurt. Raw cow milk was divided into two parts of whole fat milk and another part of skimmed milk with 0.0%, 0.01% and 0.025% and 0.05%, respectively. The experimental yoghurt was compared with the control yoghurt produced from the whole milk and yoghurt control made from the skim milk without the addition of the mucilage. Samples of yoghurt were kept in a refrigerator at (5± 2Cº) for7 day. These samples were examined when fresh, and after 2, 3 and 7th the obtained results indicated that the addition of mucilage resulted in a significant increase in titratable acidity%. The total solids content of yoghurt fortified with mucilage decrease gradually by increasing the concentration of added mucilage. Also, the total solids content of yoghurt in all of the treatments slightly increased during the storage period. On the other hand, less whey syneresis was observed during the storage of the yoghurt samples. On the other hand, replacing milk fat by mucilage was of no significant effect on fat content.

Addition of mucilage stimulated the growth of lactic acid bacteria. The colony forming units of total bacterial counts, *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* increased up to the 3rd day of the storage period, followed by a decrease up to the end of the storage period. Sensory evaluations were almost stable during the first 3rd days of storage, then decreased slightly until the end of storage the period.

Yoghurt samples containing 0.01% of mucilage showed no different in the sensory characteristics compared with control and it the most acceptable sample. In conclusion, mucilage could be successfully used as a fat replacer in making of low fat yoghurt with additional nutritional benefits without affecting the physicochemical properties of yoghurt.

Keywords: *Fat replacers, mucilage, quality of yoghurt.*

Introduction

Yoghurt is among the most popular of dairy products due to various health claims and therapeutic values (Serafeimidou *et al.,* 2012). In addition, yoghurt is a fermented dairy product with specific rheological and textural characteristics. Its texture results from the development of a three-dimensional network of milk proteins due to the aggregation of casein micelles with denatured whey proteins through hydrophobic and electrostatic bonds (Paseephol *et al.,* 2008).

Current research tends to use fat replacers in the manufacture of dairy products. Fat replacers, which decrease the calorific value of food, can be used to solve some physical and organoleptic problems originating from low-fat levels in the final products. Fat replacers consist of mixtures of lipid originated fat substitutes, protein- or carbohydrate originated fat mimetics, or their combinations (Huyghebaert *et al.*, 1996).

Okra is botanically known as *Abelmoschus esculentus* (L.) Moench, previously known as *Hibiscus esculentus* (L.), and belongs to the Malvaceae family. In 2010, its worldwide production was estimated close to 7 million metric tons (Dimopoulou *et al.,* 2015). Okra is a perishable fruit and its skin is very susceptible to browning caused by the loss of water, thereby depressing its economic value for consumption in natural. The rejected fruit has a potential utilization due to the mucilaginous substances usually concentrated in the pod walls. Okra mucilage is a natural polysaccharide made up galactose, rhamnose, and galacturonic acid. It is a renewable and inexpensive source of biodegradable material. Its physical and chemical properties include high water solubility, plasticity, elasticity and viscosity (Monique *et al.,* 2016).

Mucilage is a plant hydrocolloid, which is a polymer of a monosaccharide or mixed monosaccharide. In fact, polysaccharide mucilage is highly hydrophilic substances with high molecular weight molecules. The polysaccharides are soluble and dispersible in water due to their ability to interact with water and swell. The swelling properties are characterized by the entrapment of a large amount of water between the polymer chains and branches. Thus, mucilage can be used as one of the food additives, to modify the food quality in terms of food stability, texture and appearance properties by acting as emulsifiers, thickeners, and gelling agents or texture modifiers (Deogade *et al.*, 2012).

The objective of this study was to investigate the possibility of using mucilage in the manufacture of low-fat yoghurt and study the effect of the addition of mucilage on chemical, microbiological and sensory properties of yoghurt during storage at refrigerator temperature.

Materials and Methods

Materials:

Fresh cow's milk used in this study was obtained from the Collection Center, Arab El-awamer, Abnoub, Assiut. Okra was obtained from the local market in Assiut city. *Lactobacillus delbrueckii ssp. bulgaricus LB340 and Streptococcus thermophilus* were obtained from Dairy Enzymes Applications, Danisco, France. Metabisulphite and pure acetone were obtained from Sigma- Aldrich, UK.

Methods:

Preparations and Manufacture

Extraction of Okra mucilage:

The okra was washed to remove any unnecessary dirt from their surfaces. The cleaned pods without seeds were then immediately frozen and kept at -20°C to prevent any colour changes resulting from natural oxidation and browning process.

The extraction of okra mucilage was carried out according to the method done by Ameena *et al.* (2010)

with some modifications. Initially, one kg of cleaned and sliced okra with different maturity indices was blended using ~5°C distilled water containing 1% (w/v) of sodium metabisulphite. Then, the slurry was centrifuged at 3000 rpm for 5 minutes and precipitated from the supernatant using pure acetone (99.5%). The precipitated mucilage was washed several times with acetone and was freezing dried (Christ Alpha 1-4LD Plus, Germany). Then the freeze-dried okra mucilage was ground into powder form using a commercial dry blender.

Yoghurt manufacture:

All whole milk and the skim milk parts were heated to 90ºC for 5 min. and then cooled immediately to 40±1ºC. The milk was inoculated at a rate of 2% with yoghurt starters then the general control (C1) made from whole fresh cow's and the three-skim milk were supplemented with Okra mucilage at different levels 0.01, 0.025 and 0.05%, respectively. While the fourth parts were used as a control of skim milk yoghurt. Inoculated milk was dispensed in plastic cups of 100 ml volume and incubated at the fermentation temperature (40°C) until coagulation. Fermentation was stopped by rapid cooling to 5-6°C. Yoghurt samples were analyzed when fresh, 2, 3 and 7 days of storage (Tamime and Robinson, 1999). All experiments were carried out in triplicate.

Chemical analyses:

Titratable acidity and total solids of the experimental samples were determined according to A.O.A.C. (2000). Percentage of fat content and moisture had been determined according to Ling (1963). Percentage of the total nitrogen (T.N) content and soluble nitrogen (S.N) content was determined by using the Kjeldahl method according to A.O.A.C. (2000).

Rheological properties:

Syneresis was determined by measuring the volume of separated whey (ml whey/50 ml yoghurt) collected after 30 min at room temperature (Abd-EL-Salam *et al.*, 1991).

Microbiological analysis:

Total bacterial count (T.B.C.) in the samples was determined by using the standard plate count technique. Appropriate dilutions of yoghurt samples were plated in duplicate on an agar medium (FIL/IDF, 1991). Lactic acid bacteria (L.A.B.) in the samples were according to the International Standard FIL/IDF 117A (1988). Determination of coliform bacteria had been carried out according to the International Standard FIL/IDF 65 (1971). Counts of yeasts and moulds had been carried out according to the International Standard FIL/IDF 67 (1971).

Organoleptic properties:

Yoghurt samples were assessed according to Abou- Donia *et al.* (1991) when fresh and after 2, 3 and 7 days of storage by ten panellists of staff members at Department of Dairy Science, Food Technology Research Institute.

Statistical analyses:

The obtained data were subjected to statistical analysis, using "F-test". Means were compared using the least significant difference (L.S.D. test). Data were performed in the computer using the SPSS package (SPSS, 1998).

Results and Discussion

Chemical analyses:

The data in Table (1) show that developed titratable acidity (TA %) of yoghurt treatments increased significantly ($p \leq 0.05$) as the storage period increased.

Replacement of milk fat with Mucilage caused a significant increase in TA% (Table 1). These results might be due to the higher water holding capacity of Mucilage. Also, it has been claimed that Mucilage stimulates the growth of lactic acid bacteria**.** These results are in agreement with those of Nikoofar *et al.* (2013). Also, it could be observed in Table (1), that yoghurt made from whole milk (C_1) had significantly ($p \leq 0.05$) higher total solids (TS) when fresh and during storage, compared with other yoghurt treatments.

Whereas the lowest TS content was observed in yoghurt, samples made from skim milk (C_2). Also, the TS content of yoghurt in all treatments slightly increased with the progress of the storage period. TS content of yoghurt fortified with mucilage decrease gradually by increasing the percentage of added Mucilage ($p \leq 0.05$). These results might be due to the higher water holding capacity of Mucilage. These results are in agreement with those of Tamime and Robinson (1999).

Results also showed slight changes were observed in the total protein and soluble nitrogen of yoghurts from different treatments during storage. Where found that the total protein and soluble nitrogen contents decreased by increasing Mucilage levels. On the other hand, The total protein and soluble nitrogen contents increased in all treatments as the storage period progressed. These results are in agreement with those of Abd EL-Salam *et al.* (1996), Kebary and Hussein (1999), Tamime and Robinson (1999) and Kebary *et al.* (2004).

The obtained results also showed that the fortification of low-fat yoghurt with Mucilage as fat replacers did not affect the fat content of the yoghurt. These results are in agreement with those reported by Kebary *et al.* (1996), Kebary and Hussein (1999), Hussein *et al*. (2004) and Badawi *et al*. (2008).

Table 1. Changes in the chemical composition of yoghurt as affected by the addition of Mucilage through the storage at 5- 7ºC for 7th days

Components (%)	Treatments	Storage Period (Days)			
		0	2	3	7
Titratable acidity	C_1	0.97^{A}	1.04^{B}	1.07^{BC}	1.12^{CD}
	C_2	0.92^{B}	1.00^{C}	1.04^{C}	1.09^{D}
	T_1	0.98^{A}	1.07^{B}	1.09^{B}	1.13^{C}
	T_2	0.99^{A}	1.11^{A}	1.15^{A}	1.25^{B}
	T_3	0.99^{A}	1.11^{A}	1.18^{A}	1.31^{A}
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.037	0.036	0.036	0.036
Total Solids	C_1	12.27^{A}	12.38^{A}	12.43^{A}	12.50^{A}
	C_2	10.47^{E}	10.50^{E}	10.53^{E}	10.88^{C}
	T_1	11.42^{B}	11.59^{B}	11.68^{B}	12.50^{A}
	T_2	11.38^{C}	11.45^{C}	11.57^{C}	12.47^{A}
	T_3	11.26^{D}	11.35^{D}	11.40^{D}	12.22^{B}
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.036	0.036	0.037	0.037
Total protein	C_1	2.43^{D}	2.55^{D}	2.92^{C}	3.09^{B}
	C_2	2.79^{B}	2.79^{C}	3.26^{A}	3.29^{A}
	T_1	2.97^{A}	2.98^{A}	3.04^{B}	3.0^{B}
	T_2	2.79^{B}	2.88^{B}	3.03^{B}	3.06^{BC}
	T_3	2.54^{C}	2.54^{D}	3.01^{B}	3.04^{C}
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.039	0.036	0.036	0.036
Water soluble nitrogen	C_1	0.045^{D}	0.105^{C}	0.126^{C}	0.213^{B}
	C_2	0.052^{C}	0.182^{A}	0.210^{A}	0.252^{A}
	T_1	0.098^{A}	0.112^{B}	0.154^{B}	0.213^{B}
	T_2	0.098^{A}	0.112^{B}	0.125^{C}	0.203^{C}
	T_3	0.063^{B}	0.098^{D}	0.112^{D}	0.126^{D}
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.036	0.036	0.036	0.036
Fat	C_1	2.5^{A}	2.5^{A}	2.7^{A}	3.1^{A}
	C_2	0.7^{B}	0.7^{B}	0.8^{B}	1.1^{B}
	T_1	0.7^{B}	0.7^{B}	0.8^{B}	1.1^{B}
	T_2	0.6^{B}	0.6^{B}	0.6^{B}	0.8^{B}
	T_3	0.6^{B}	0.6^{B}	0.7^{B}	0.7^{B}
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.48	0.48	0.41	0.29

Each value in the Table was the mean of three replicates.
A, B, C and D: Means having different superscripts within each column are significantly different ($P \leq 0.05$).
C_1: Control yoghurt made from whole cow milk.
C_2: Control yoghurt made from cow skim milk without the addition of Mucilage.
T_1: Yoghurt made from cow skim milk + 0.01% Mucilage.
T_2: Yoghurt made from cow skim milk + 0.025% Mucilage.
T_3: Yoghurt made from cow skim milk + 0.05% Mucilage.

Rheological properties:

Syneresis:

The syneresis of yoghurt was affected by the concentration of added Mucilage used as shown in Table (2). Replacement of milk fat with Mucilage caused a significant ($p \leq 0.05$) reduction of whey syneresis from curd and this reduction was proportional to the rate of replacement. Similar results were reported by Farooq and Haque (1992), Kebary and Hussein (1999) and Nikoofar *et al.,* (2013).

These results might be due to the addition of Mucilage leads to form a complex with casein micelles and prevent them from excessive fusion and from a fine meshed gel network, which is less susceptible to whey separation and/or increasing the water holding capacity (Danneberg and Kessler, 1988). Syneresis from all yoghurt treatments decreased gradually ($p \leq 0.05$) as the storage period progressed and reached their minimum values on the seventh day of storage period. These results are in agreement with those reported by Farooq and Haque (1992), Kebary and Hussein (1999) and Kebary *et al.* (2009).

Microbiological characteristics:

Data presented in Table (3) illustrated that the total bacterial count and lactic acid bacteria followed similar results. Total bacteria and lactic acid bacteria counts increased as the storage period progressed up to the third day, and then decreased up to the end of the storage period. These results might be due to the development of acidity. These results are in harmony with those finding of Kebary *et al.* (1996), Kebary and Hussein (1999), Kebary *et al.* (2004), Kebary *et al.* (2007), Badawi *et al.* (2008), Naji *et al.* (2012) and Mohamed *et al.* (2013) and EL-Sonbaty *et al.* (2008). The obtained results also showed that the count of lactic acid bacteria of all treatments with Mucilage had a higher count than the control at any time.

Table 2. Changes of syneresis in yoghurt as affected by the addition of mucilage through the storage at 5- 7 ºC.

Treatments	Storage Period (Days)			
	0	2	3	7
C_1	29^{BC}	27^{BC}	26	25
C_2	33^{A}	32^{A}	30	27
T_1	32^{AB}	31^{AB}	28	27
T_2	30^{AB}	29^{ABC}	27	26
T_3	26^{C}	26^{C}	25	24
F-Test	*	*	N.S	N.S
$LSD_{0.05}$	3.638	3.844	-	-

Each value in the Table was the mean of three replicates.

A,B,C and D: Means having different superscripts within each column are significantly different ($P \leq 0.05$).

C_1: Control yoghurt made from whole cow milk.

C_2: Control yoghurt made from cow skim milk without the addition of Mucilage.

T_1: Yoghurt made from cow skim milk + 0.01% Mucilage.

T_2: Yoghurt made from cow skim milk + 0.025% Mucilage.

T_3: Yoghurt made from cow skim milk + 0.05% Mucilage.

The count of total bacterial counts and lactic acid bacteria increased by increasing the rate of adding mucilage. These results might be due to the stimulation effect of mucilage on the growth of microflora (Gibson and Roberfroid, 1995, Kebary *et al.,* 2005, Donkor *et al.,* 2007 and Oliveira *et al*., 2009). Also, results showed that all samples were free of the count of yeast and moulds and coliform bacteria during the storage period.

These results were disagreement with what was found by Mohamed (2004), who found that yeasts and moulds free during storage at 5°C or 25°C. While moulds and yeasts were not detected in yoghurt containing cress seeds in fresh and through the storage period.

Table 3. Changes in microbiological characteristics yoghurt manufactured by a mucilage through the storage at 5-7ºC. (Cfu/gm).

Determination	Treatments	Storage Period /Days			
		0	2	3	7
Total plate count	C_1	$16.9\times10^{6\ E}$	$24.65\times10^{6\ D}$	$26.15\times10^{6\ D}$	$15.9\times10^{6\ E}$
	C_2	$22.95\times10^{6\ D}$	$24.95\times10^{6\ D}$	$27.4\times10^{6\ D}$	$18.55\times10^{6\ D}$
	T_1	$84.55\times10^{6\ A}$	$91.5\times10^{7\ A}$	$97.0\times10^{7\ A}$	$86.5\times10^{6\ A}$
	T_2	$56.05\times10^{6\ B}$	$58.2\times10^{7\ B}$	65.55×10^{7B}	$52.75\times10^{6\ B}$
	T_3	45.40×10^{6C}	$48.50\times10^{7\ C}$	$53.0\times10^{7\ C}$	$42.35\times10^{6\ C}$
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.045	0.042	0.041	0.045
Lactic acid bacteria	C_1	$40.5\times10^{5\ D}$	$66.0\times10^{5\ C}$	$84.5\times10^{5\ B}$	$41.7\times10^{5\ C}$
	C_2	$13.9\times10^{6\ A}$	$15\times10^{6\ A}$	$15.85\times10^{6\ A}$	$11.55\times10^{5\ D}$
	T_1	$51.5\times10^{5\ C}$	$62.5\times10^{6\ CD}$	$63.0\times10^{6\ C}$	$52.0\times10^{5\ A}$
	T_2	$50.0\times10^{5\ C}$	$59.0\times10^{6\ D}$	$62.0\times10^{6\ C}$	$50.85\times10^{5\ A}$
	T_3	$74.4\times10^{5\ B}$	$81.0\times10^{6\ B}$	$87.0\times10^{6\ B}$	$46.2\times10^{5\ B}$
	F-Test	**	**	**	**
	$LSD_{0.05}$	0.041	0.041	0.043	0.037
Yeast and moulds	C_1	Nil	Nil	Nil	Nil
	C_2	Nil	Nil	Nil	Nil
	T_1	Nil	Nil	Nil	Nil
	T_2	Nil	Nil	Nil	Nil
	T_3	Nil	Nil	Nil	Nil
	F-Test	-	-	-	-
	$LSD_{0.05}$	-	-	-	-
Coliform bacteria	C_1	Nil	Nil	Nil	Nil
	C_2	Nil	Nil	Nil	Nil
	T_1	Nil	Nil	Nil	Nil
	T_2	Nil	Nil	Nil	Nil
	T_3	Nil	Nil	Nil	Nil
	F-Test	-	-	=	-
	$LSD_{0.05}$	-	-	-	-

Each value in the Table was the mean of three replicates.
A, B, C and D: Means having different superscripts within each column are significantly different ($P\leq 0.05$).
C_1: Control yoghurt made from whole cow milk.
C_2: Control yoghurt made from cow skim milk without the addition of Mucilage.
T_1: Yoghurt made from cow skim milk + 0.01% Mucilage.
T_2: Yoghurt made from cow skim milk + 0.025% Mucilage.
T_3: Yoghurt made from cow skim milk + 0.05% Mucilage.

This is due to the presence of cress seeds, which contain several compounds as possess antimicrobial against food spoilage organisms. Behrouzian *et al.* (2014) found that coliform and aerobic spore-forming bacteria were not detected in all yoghurt treatments either when fresh or during the storage period. This may be due to the high hygienic condition during the preparation.

Organoleptic properties:

Scores of organoleptic properties (flavour, body & texture, appearance, acidity and total scores) followed almost similar trends (Table 4). Yoghurt made from skim milk without adding Mucilage (C_2) gained the lowest scores for organoleptic properties and was different from other yoghurt treatments. Fortification of skim milk with Mucilage improved the organoleptic properties of yoghurt. Treatment that made from skim milk fortified with 0.01% was not different from yoghurt that made from whole milk (C_1). Storage up to the 3rd day did not affect the scores of organoleptic properties, then scores of all yoghurt treatments decreased gradually up to the end of the storage period. These results are in agreement with those reported by Kebary and Hussein (1999) and Kebary *et al.* (2004).

Table 4. Effect of mucilage ratios on organoleptic properties of low-fat yoghurt during storage period at 5-7°C.

Treatment	Storage period (days)	Properties				
		Flavor (45)	Body& texture (30)	Appearance (15)	Acidity (10)	Total (100)
C1	0	38.50	25.00	12.88	8.69	85.07
	2	37.55	27.23	12.91	7.77	85.46
	3	36.83	27.83	12.00	7.83	84.49
	7	30.63	21.13	9.50	8.13	69.39
C2	0	34.33	23.33	10.33	4.67	72.67
	2	35.67	21.33	9.67	6.33	73.00
	3	39.50	26.83	12.67	7.50	88.67
	7	39.33	25.67	12.67	9.00	86.67
T1	0	36.67	26.67	11.33	6.67	81.33
	2	41.67	26.00	13.67	8.33	89.33
	3	37.67	25.33	12.67	8.00	83.67
	7	31.33	23.33	10.33	6.67	71.67
T2	0	35.00	20.00	6.67	6.00	67.67
	2	37.00	23.33	11.33	6.33	78.00
	3	39.50	26.83	12.67	7.50	88.67
	7	38.00	25.33	12.67	8.67	84.67
T3	0	37.67	23.33	9.33	7.33	77.67
	2	41.00	27.33	13.50	8.33	95.00
	3	40.67	25.33	12.67	8.00	86.67
	7	39.33	25.67	11.67	8.67	85.33

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تأثير الميوسيلاج كبديل للدهن على خصائص الزبادي

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الملخص

الهدف من هذه الدراسة هو معرفة تأثير استبدال دهن اللبن بمستويات مختلفة من الميوسيلاج على الخصائص الكيميائية والميكروبيولوجية والحسية للزبادي حيث تم تقسيم اللبن البقري الخام إلى قسمين من اللبن كامل الدسم وجزء آخر من اللبن الفرز الذي أضيف له الميوسيلاج بنسبة ٠,٠ و٪ ٠,٠١ و٪ ٠,٠٢٥ ٪ ٠,٠٥٪ على التوالي. تمت مقارنة الزبادي المضاف له الميوسلاج مع الزبادي الكنترول المنتج من اللبن الكامل والزبادي الكنترول المنتج من اللبن الفرز دون إضافة الميوسيلاج . تم تخزين الزبادي من المعاملات المختلفة في الثلاجة وتم أخذ عينات منها طازجا وبعد ٣،٢، ٧ أيام من التخزين وتحليلها لكل من التركيب الكيميائي والخصائص الميكروبيولوجية والحسية. وأظهرت النتائج أن إضافة الميوسيلاج تسبب في زيادة معنوية في نسبة الحموضة ويتناقص محتوى المواد الصلبة الكلي من الزبادي المدعم بالميوسيلاج تدريجيا بزيادة تركيز الميوسيلاج المضافة. أيضا، زاد إجمالي محتوى المواد الصلبة في الزبادي في جميع المعاملات بشكل طفيف خلال فترة التخزين، بينما انخفض التشرش أثناء تخزين المعاملات الناتجة عن اضافة الميوسلاج. ومن ناحية أخرى، لم يكن لاستبدال دهن اللبن بالميوسيلاج أي تأثير معنوي على محتوى الدهن. إضافة الميوسيلاج شجع نمو بكتيريا حامض الاكتيك حيث زاد العدد الكلي للبكتيريا وعدد كل من الـ *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* حتى اليوم الثالث من فترة التخزين ثم حدث تناقص حتى نهاية فترة التخزين. وكانت نتائج التقييم الحسي لم تتأثر تقريبا خلال أول ٣ أيام من فترة التخزين ثم انخفضت قليلا حتى نهاية فترة التخزين. الزبادي المضافة له ٠,٠١٪ من الميوسيلاج لا يختلف في الخصائص الحسية مقارنة مع الكنترول وهي العينة الأكثر قبولا. توصي الدراسة باستخدام الميوسيلاج كبديل للدهن في تصنيع زبادي منخفض في الدهن مع فوائد غذائية إضافية دون التأثير على الخصائص الفيزيائية الكيميائية للزبادي الناتج.