

PHYSIOCHEMICAL AND MICROBIOLOGICAL STUDIES ON YOGHURT FORTIFIED WITH VEGETABLES

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ABSTRACT

The current investigation aim to study the possibility of preparing yoghurt from buffaloe's milk mixed with different levels of potato, sweet potato or various mixtures of them. Acidity developed in all the treatments. Increasing the level of added potato or sweet potato or their mixtures resulted in an increase in the content of total volatile fatty acid (T.V.F.A) and total carbonyl (T.C). However, fat content (F), total nitrogen (T.N.), non-protein nitrogen (N.P.N), soluble nitrogen (S.N) declined with increasing the added amount of potato, sweet potato or their mixtures. All the treatments have no coliform bacteria or *Staphylococcus aureus*. However, molds and yeasts were detected after 10 days of cold storage. Spore forming bacteria were found in all treatments, as they were not affected by the heat treatments, but the control yoghurt had higher total bacterial, proteolytic and lipolytic counts compared with the potato or sweet potato based yoghurts. Lower total points of sensory evaluation were given to yoghurt containing the potato or sweet potato as compared with (control) yoghurt. Amongst the former, yoghurt containing 5% potato or 5% sweet potato or a mixture of (5+5%) gave scores more similar to those of the control. Yoghurt-mixed with 5% potato, 5% sweet potato or a mixture of them (5+5%) could be recommended. This leads to decreasing the cost of the final product due to the lower price of potato or sweet potato.

INTRODUCTION

Fermentation is the oldest and saftiest method for preserving milks. The increase in acidity consequent to fermentation results in products such as yoghurt, Quark-Labneh, Kefier and Koumiss, which are bacteriologically stable under refrigerated conditions and free from pathogens (Tamime and Robinson, 1985). Accordingly, numerous studies employed soymilk, soy protein or soy flour in the manufacture of yoghurt (Nelson *et al.*, 1971 & 1976; Farahat *et al.*, 1974). Forsumm (1975) used whey protein concentrate as a supplement to maize, rice and potato. He evaluated the chemical and biological composition using growing rats. Fermentation often improves or modifies taste, flavour and lower texture. Consequently, sufficient acid production is a main prerequisite. This would depend on the ability of the organisms to utilize the available carbohydrates in milk. Yoghurt is one of the most popular fermented milk all over the world. In Egypt, it's usually prepared from buffaloe's milk. Because of the shortage in the milk supply, trends of incorporating various milk substitutes in the manufacture of yoghurt have introduced. In addition to overcoming the insufficient milk supply, these substituents are of important economic value. However, employing them in fermented milks have associated with some technological problems. As a source of carbohydrate, required for fermentation, protein, iron found in very

limited level in milk. Vitamin A, B & C and florin, iron, potassium, phosphorus, copper, manganese, iodine potato and sweet potato might be effective milk substitute. Hegazy *et al.* (1990) reported that potato flour contains 8.60% protein, 0.40% fat, 1.38% fiber, 3.80% ash, 85.82% total carbohydrates, Ca 42.0 mg/100g, P 220.0 mg/100g, Fe 4.90 mg/100g and 381 calories. Ghazi (1996) found that sweet potato contains ash 1.80%, crude protein 4.30%, lipids 0.70%, reducing sugars 13.50% and carbohydrates 79.70% on dry weight basis. Also, he reported that sweet potato contains, P 58.0 mg/100g, Ca 30 mg/100g, Fe 3.30 mg/100g, Mg 6.20, Mn 0.21, Cu 0.50, Zn 0.40 mg/100 g on dry weight basis and caloric value = 396.30 K cal/100 g sample on dry weight basis. Youssef and Rofael (1997) reported that sweet potato slices contain starch 70.45%, total sugars 13.76%, ash 1.14% and fat 0.60% on dry weight basis.

In the present study a trail for preparing yoghurt from buffalo's milk mixed with different levels of potato and sweet potato has been conducted. Furthermore, the microbiological, chemical and organoleptic characteristics have been followed up during manufacture and storage period.

MATERIALS AND METHODS

Milk:

Fresh buffalo's milk was supplied by the herd of the Faculty of Agriculture, Mansoura University. The chemical composition of buffalo's milk, potato and sweet potato is shown in Table (1).

Table (1):Chemical composition of the buffalo's milk, potato and sweet potato used in yoghurt preparation.

Items	Buffaloe's milk*	Potato**	Sweet potato**
Moisture	84.60	77.8	68.3
Total solids	15.40	22.2	31.7
Total protein	4.00	2.0	1.8
Total fat	5.85	0.1	0.7
PH	6.69	ND	ND
Acidity	0.18	ND	ND
Carbohydrate	ND	19.0	27.9

* Determined in Dairy Dept., Chem. Lab.

** According to El-Serky (1990).

ND = Not determined.

Potato and sweet potato:

It was obtained from local market of Mansoura city.

Preparation of potato and sweet potato for using in making yoghurt:

Potato [*Solanum tuberosum*, L] and sweet potato [*Ipomoea batatas* (L) Lam] were cleaned and rinsed with tap water. They were then boiled for 30-45 min. They were dehulled after discarding the boiling water. Finally, they were mixed at certain levels with buffalo's milk.

Starter cultures:

Lyophilized cultures of yoghurt starter (*Lactobacillus delbrueckii* subsp *bulgaricus* + *Streptococcus thermophilus*) were obtained from Chr. Hansen's Lab. Denmark.

Chemical analysis:

Yoghurt samples were analyzed for titratable acidity as percent of lactic acid and pH-values according to Ling (1963). Fat content (F) and total solids (T.S) according to the British Standard Institution's (B.S.I) method (1955). The total nitrogen (T.N), soluble nitrogen (S.N.) and non-protein nitrogen (N.P.N) as described by Ling (1963). The total volatile fatty acids (T.V.F.A) were determined according to Kosikowski (1978). The total carbonyl compounds were estimated as described by Bassett and Harper (1958).

Microbiological analysis:

The total bacterial count of yoghurt was determined according to the American Public Health Association (1978) by planting the proper dilution in duplicates using nutrient agar medium (Difco Manual, 1966). The spore-forming counts were determined according to Chalmer (1962). The coliform bacterial count as described in Oxoid Manual, 1982). The proteolytic bacteria count according to Chalmer (1962). The lipolytic bacterial count according to Berry (1933). The staphylococci count by Difco (1974). The molds and yeasts as described by the Oxoid Manual (1962).

Sensory evaluation:

Yoghurt made with the addition of potato, sweet potato or with their mixtures were scored for appearance out of 15 points, for body and texture out of 30 points and for flavour out of 10 points as described by Nelson and Trout, 1964).

RESULTS AND DISCUSSION

Acidity development:

It could be seen from table (2) that after pasteurization, the replacement of buffaloe's milk with various levels of potato, sweet potato or their mixture has resulted in an increase in the titratable acidity and decrease in pH-values of the treatments as compared with those of control one, which was constituted of buffaloe's milk only. Whereas, the yoghurt made from the mixtures of potato and sweet potato (5+5%) had less titratable acidity as compared with the control.

Chemical composition:

It could be seen from table (3) that increasing the level of added potato or sweet potato and their mixtures or decreasing the percent of buffaloe's milk resulted in an increase in the pH-values. On the other hand, it could be claimed that the yoghurt made from potato, sweet potato or their

Table (2): Development of acidity and pH during the making of yoghurt from buffalo milk partially substituted with potato, sweet potato or their mixtures.

Treatments		Time											
		After pasteurization		After adding starter		1 h.		2 h.		3 h.		4 h.	
		A	pH	A	pH	A	pH	A	pH	A	pH	A	pH
Control	0	0.18	6.69	0.24	6.23	0.32	6.06	0.40	5.90	0.68	5.35	0.72	4.94
Potato	5	0.19	6.68	0.23	6.48	0.32	5.98	0.59	5.38	0.81	4.81	1.08	4.41
	10	0.19	6.68	0.23	6.49	0.41	5.68	0.72	5.00	0.92	4.51	1.01	4.27
	15	0.19	6.66	0.23	6.45	0.67	5.16	0.90	4.65	1.13	4.33	1.31	4.15
	25	0.19	6.60	0.23	6.31	0.63	5.18	0.81	4.65	1.08	4.33	1.22	4.16
	35	0.19	6.51	0.22	6.25	0.43	5.57	0.83	4.65	0.95	4.35	1.22	4.18
Sweet potato	5	0.19	6.62	0.24	6.38	0.35	5.98	0.62	5.28	0.93	4.73	1.13	4.45
	10	0.19	6.60	0.25	6.34	0.35	5.99	0.72	5.20	0.93	4.71	1.13	4.45
	15	0.19	6.30	0.23	6.20	0.39	5.66	0.47	4.93	0.74	4.49	0.81	4.26
	25	0.19	6.30	0.24	6.15	0.45	5.42	0.63	4.85	0.86	4.45	0.90	4.16
Sweet Potato :	5:5	0.19	6.33	0.21	6.18	0.29	5.79	0.32	5.20	0.58	4.61	0.72	4.30
Potato	25:25	0.19	6.31	0.22	6.01	0.45	5.40	0.66	4.68	0.81	4.15	0.91	4.00

A = Acidity

Table (3): Development of acidity and pH of yoghurt made from buffalo's milk partially substituted with potato, sweet potato or their mixtures as affected with storage at refrigerated temperature.

Treatments		Storage periods at refrigerator (5-10°C), (days)					
		Fresh		5 days		10 days	
		Acidity	pH	Acidity	pH	Acidity	pH
Control	0	0.72	4.94	1.12	4.08	1.13	4.27
Potato	5	1.08	4.41	1.48	3.99	1.51	3.95
	10	1.01	4.27	1.66	3.88	1.75	3.82
	15	1.31	4.15	1.71	3.85	1.80	3.82
	25	1.22	4.16	1.62	3.89	1.76	3.84
	35	1.22	4.18	1.62	3.89	1.69	3.89
Sweet potato	5	1.13	4.45	1.41	3.92	1.75	3.90
	10	1.13	4.45	1.49	3.92	1.62	3.88
	15	0.81	4.26	1.30	3.90	1.48	3.86
	25	0.90	4.16	1.35	3.89	1.57	3.68
Potato : Sweet potato	5:5	0.72	4.30	1.29	4.02	1.33	3.87
	25:25	0.91	4.00	1.35	3.97	1.37	3.84

Table (4): Contents of fat, total volatile fatty acids and total carbonyl compounds of yoghurt made from buffalo's milk partially substituted with potato, sweet potato or their mixtures as affected with cold storage.

Treatments		Time								
		Fresh			5 days			10 days		
		Fat	T.V.F.A	T.C	Fat	T.V.F.A	T.C	Fat	T.V.F.A	T.C
Control		5.85	2.00	0.015	6.10	4.00	0.067	6.30	4.60	0.315
Sweet potato	10	5.05	5.20	0.067	5.20	5.60	0.059	5.30	4.00	0.163
	15	4.45	5.20	0.192	4.65	5.20	0.052	4.80	4.00	0.785
	25	3.80	5.20	0.086	3.95	5.20	0.061	4.15	4.00	0.432
	35	3.25	5.20	0.067	3.45	4.80	0.753	3.60	4.40	0.864
Sweet potato	5	5.55	5.20	0.182	5.70	5.20	0.614	5.90	3.60	0.673
	10	5.20	5.20	0.036	5.30	5.20	0.752	5.50	3.20	0.794
	15	4.65	7.20	0.086	4.85	7.60	0.089	5.10	4.80	0.342
	25	4.05	6.00	0.447	4.25	4.00	0.149	4.50	3.20	0.369
Potato : Sweet potato	5:5	5.50	4.80	0.338	5.80	4.00	0.152	5.90	3.60	0.340
	25:25	4.00	14.4	0.851	4.25	4.00	0.797	4.50	4.40	1.149

T.V.F.A. = Total volatile fatty acids.ml 0.1 NaOH/100g

T.C. = Total carbonyl mg/100g

mixtures had higher total volatile fatty acids (T.V.F.A) and total carbonyl compounds. Meanwhile, the fat decreased with increasing the added level of the potato, sweet potato or their mixtures compared with the control of the fresh yoghurt. This is due to the lower content of the fat of the potato or sweet potato, *i.e.*, 0.1 & 0.7, respectively, (table 1). On the other hand, the total volatile fatty acids decreased throughout the storage period, compared with the control. Also, the fat and total carbonyl contents increased during the storage period (table 4). Meanwhile, it's clear from table (5) that increasing the added potato or sweet potato or their mixtures decreased the total nitrogen, non-protein nitrogen, and soluble nitrogen of the resultant yoghurt in the fresh control. This might be due to the lower protein content in the potato or sweet potato, *i.e.*, 3.0 & 1.8, respectively, (Table 1). On the other hand, it could also be appeared that an increase in total nitrogen, non-protein nitrogen and soluble nitrogen of the resultant yoghurt was recorded in the storage period. This could be attributed to proteolysis occurring during the storage period.

Microbiological quality:

Data presented in table (6) indicate that the higher numbers of total viable count, lipolytic and proteolytic bacteria were observed in buffalo's (control) yoghurt as compared with yoghurt made from milk containing potato, sweet potato or their mixture. This is due to the superiority of buffalo's milk for the growth of bacteria as compared to potato or sweet potato (Magdoub *et al.*, 1992). On the other hand, it's clear from the same table that no coliform

bacteria or *Staphylococcus aureus* were detected in all treatments, however, molds and yeasts were detected after 10 days of cold storage. Meanwhile, sporeforming bacteria could be detected in all the treatments as they were not affected by heat treatments.

Sensory evaluation:

Data presented in table (7) show that buffaloe's yoghurt had the highest scoring points whether when it was fresh or after cold storage for 5 or 10 days. Amongst the potato or sweet potato treatments, the yoghurt containing the lowest amount of added potato (5%) or sweet potato (5%) or their mixture (5+5%) scored the highest total points, whereas that had the highest level of potato, sweet potato or their mixture (5 + 5%) and (25 + 25%), respectively, gave the lowest score. Therefore, it could be stated that by increasing the level of added potato, sweet potato or their mixture the total score points were lower. The three elements of the total score, *i.e.*, appearance, body & texture and flavour had the same trend. It's clear, also, that by increasing the level of sweet potato, the flavour was improved and the acidity increased the total score point compared to increasing the level of potato which resulted in degrading the flavour and acidity and lowering total score. This is due to the higher content of the carbohydrates in the sweet potato. Fortifying the buffaloe's milk with (5%) potato, (5%) sweet potato or mixture of them (5 + 5%) increased the points scored for appearance, body & texture and flavour. It could be stated that increasing the level of added sweet potato improved the organoleptic properties of yoghurt.

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دراسات على الخواص الكيمو طبيعية والميكروبيولوجية لليوجورت المدعم بالخضروات

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إستهدفت الدراسة الحالية تحديد إمكانية إستخدام البطاطس أو البطاطا أو خليط منهما مخلوطاً باللبن الجاموسى فى صناعة اليوجورت ويرجع ذلك لإحتواء البطاطس والبطاطا على الحديد والفسفور والبوتاسيوم والماغنسيوم والمنجنيز والفلورين والكالسيوم وبعض الفيتامينات (B complex) ؛ وكذلك الكربوهيدرات ، ويؤدى تناوله إلى نذرة الإصابة بتسوس الأسنان ويزيد من صلابة العظام . ولقد وجد أن الحموضة قد تطورت فى كل المعاملات ، كما أن زيادة مستوى البطاطس أو البطاطا أو خليط منهما أدى إلى زيادة المحتوى من الأحماض الدهنية الطيارة والمركبات الكربونيلية فى اليوجورت الناتج ، ولكن أدى زيادة نسبة البطاطس أو البطاطا أو خليط منهما إلى خفض المحتوى من الدهن والنيتروجين الكلى والنيتروجين الغير بروتينى والنيتروجين الذائب فى اليوجورت الناتج ومن الناحية الميكروبيولوجية كانت كل المعاملات خالية من ميكروبات القولون Coliform و *Staphylococcus aureus* وأيضاً الفطر والخمائر حتى اليوم العاشر من التخزين ، ولكن عينة الكنترول كانت أعلى فى محتواها من العد الكلى للبكتيريا والبكتيريا المحللة للبروتين والمحللة للدهن مقارنة باليوجورت المحتوى على البطاطس أو البطاطا أو خليط منهما . وأيضاً فإن أعلى درجات التقييم الحسى سجلت لليوجورت الجاموسى مقارنة باليوجورت المحتوى على البطاطس أو البطاطا أو خليط منهما فإنها سجلت أقل فى درجات التقييم الحسى ، ولقد كان اليوجورت المحتوى على 5% بطاطس أو 5% بطاطا أو (5 + 5) خليط من البطاطس والبطاطا أعطت درجات تشبه اليوجورت الجاموسى (الكنترول) .

وبناء على النتائج المتحصل عليها يوصى بتحضير اليوجورت من اللبن الجاموسى المخلوط بنسبة 5% بطاطس أو 5% بطاطا أو (5 + 5) من البطاطس والبطاطا ، وكذلك تم خفض التكلفة النهائية ويرجع ذلك لإنخفاض سعر كل من البطاطس أو البطاطا .

Table (5): Nitrogenous forms of yoghurt prepared from buffalo's milk partially substituted with potatoes, sweet potatoes or their mixtures as affected with cold storage.

Treatments	Time											
	Fresh				5 days				10 days			
	T.N.	S.N.	N.P.N.	SN/TN	T.N.	S.N.	N.P.N.	SN/TN	T.N.	S.N.	N.P.N.	SN/TN
Control	0.611	0.112	0.019	0.183	0.621	0.115	0.022	0.185	0.630	0.119	0.027	0.189
Potatoes												
5	0.603	0.110	0.019	0.182	0.613	0.115	0.021	0.188	0.625	0.118	0.025	0.189
10	0.591	0.106	0.016	0.179	0.602	0.106	0.019	0.176	0.616	0.110	0.020	0.179
15	0.583	0.106	0.016	0.182	0.599	0.109	0.018	0.182	0.608	0.113	0.019	0.186
25	0.564	0.102	0.018	0.181	0.575	0.110	0.018	0.191	0.593	0.112	0.022	0.189
35	0.543	0.0998	0.019	0.184	0.561	0.108	0.019	0.193	0.570	0.110	0.023	0.193
Sweet potatoes												
5	0.603	0.109	0.018	0.181	0.614	0.115	0.022	0.187	0.621	0.119	0.022	0.192
10	0.588	0.103	0.013	0.175	0.600	0.108	0.020	0.180	0.610	0.113	0.023	0.185
15	0.575	0.103	0.013	0.179	0.592	0.105	0.019	0.177	0.600	0.112	0.024	0.187
25	0.560	0.100	0.011	0.179	0.571	0.106	0.014	0.186	0.580	0.110	0.020	0.190
Potatoes : Sweet potatoes												
5:5	0.602	0.109	0.015	0.181	0.612	0.112	0.018	0.183	0.618	0.118	0.018	0.191
25:25	0.568	0.103	0.013	0.181	0.592	0.105	0.013	0.177	0.608	0.110	0.018	0.181

T.N.: Total nitrogen

S.N.: Soluble nitrogen

N.P.N.: Non-protein nitrogen

Table (6): Microbiological analysis of yoghurt prepared from buffalo's milk partially substituted with potatoes, sweet potatoes or their mixtures.

Treatments	Time		
	Fresh	5 days	10 days

	TVC	SP	P	L	M & Y	Staph.	E. coli	TVC	SP	P	L	M & Y	Staph.	E. coli	TVC	SP	P	L	M & Y	Staph.	E. coli
Control	6.4	--	30	40	ND	ND	ND	7.2	1	32	45	ND	ND	ND	7.0	1	29	38	10	ND	ND
Potatoes																					
5	6.0	1	28	34	ND	ND	ND	6.3	1	29	38	ND	ND	ND	6.1	10	26	33	15	ND	ND
10	5.9	2	25	30	ND	ND	ND	6.2	1	27	34	ND	ND	ND	6.0	12	24	30	20	ND	ND
15	5.7	4	21	26	ND	ND	ND	6.0	3	22	29	ND	ND	ND	5.8	8	20	24	30	ND	ND
25	5.5	1	20	24	ND	ND	ND	5.8	1	22	26	ND	ND	ND	5.7	4	20	21	30	ND	ND
35	5.2	3	15	20	ND	ND	ND	5.7	--	20	23	ND	ND	ND	5.5	3	14	19	35	ND	ND
Sweet potatoes																					
5	6.1	2	25	35	ND	ND	ND	6.4	1	27	37	ND	ND	ND	6.2	3	24	33	20	ND	ND
10	5.8	4	25	31	ND	ND	ND	6.3	--	26	33	ND	ND	ND	6.0	1	23	30	20	ND	ND
15	5.6	2	20	28	ND	ND	ND	6.0	1	22	29	ND	ND	ND	5.9	--	20	26	30	ND	ND
25	5.3	1	20	26	ND	ND	ND	5.7	1	21	27	ND	ND	ND	5.2	--	18	22	35	ND	ND
Potatoes : Sweet potatoes																					
5:5	5.7	2	25	30	ND	ND	ND	6.1	1	28	32	ND	ND	ND	6.0	--	23	25	28	ND	ND
25:25	5.1	2	21	18	ND	ND	ND	5.8	1	23	20	ND	ND	ND	5.4	--	17	15	40	ND	ND

TVC = total viable count (cfu x 10⁶).
 L = lipolytic bacteria (cfu x 10²).
 P = proteolytic bacteria (cfu x 10²).
 ND = Not detected.

M&Y = molds and yeast (cfu x 10²).
 SP = sporeforming bacteria (cfu x 10²).
 Staph = *Staphylococcus aureus*

Table (7): Organoleptic properties of yoghurt prepared from buffaloe's milk partially substituted with potatoes, sweet potatoes or their mixtures as affected with cold storage.

Treatments	Time															
	Fresh					5 days					10 days					
	APP 15	B+T 30	Fla. 45	Acid 10	T.S 100	APP 15	B+T 30	Fla. 45	Acid 10	T.S 100	APP 15	B+T 30	Fla. 45	Acid 10	T.S 100	
Control	0	14.0	28.0	44.0	9.0	95.0	14.0	28.0	43.0	8.5	93.5	13.0	26.5	42.0	8.0	89.5
Potatoes	5	13.0	27.5	40.0	8.0	88.5	13.0	27.0	40.0	8.0	88.0	12.5	25.0	38.0	7.0	82.5
	10	12.5	26.5	28.5	8.0	85.5	12.5	26.0	38.0	8.0	84.5	12.0	25.0	37.0	7.0	81.0
	15	12.5	26.0	37.0	8.0	83.5	12.0	26.0	36.0	7.5	81.5	11.5	24.5	35.0	6.5	77.5
	25	12.0	25.0	36.5	7.0	80.5	12.0	25.0	36.0	7.0	80.0	11.5	24.0	34.0	6.0	75.5
	35	11.0	22.0	35.0	6.5	74.5	11.0	22.0	34.0	6.0	73.0	10.0	21.0	32.0	5.0	68.0
Sweet potatoes	5	13.0	27.0	37.5	7.0	84.5	13.0	27.0	36.0	7.0	83.0	12.0	25.0	34.0	6.0	77.0
	10	12.5	27.0	37.5	7.0	84.0	12.5	26.0	36.0	7.0	81.5	11.0	24.0	34.0	6.0	75.0
	15	12.5	26.5	38.0	7.5	84.5	12.5	26.0	37.0	7.0	82.5	11.0	25.0	35.0	6.5	77.5
	25	12.5	26.5	39.5	8.0	86.5	11.0	25.0	39.0	7.5	82.5	10.0	24.0	37.0	7.5	78.0
Potatoes : Sweet potatoes	5:5	13.0	26.5	37.5	7.5	84.5	13.0	26.0	36.5	7.5	83.0	12.0	25.0	35.0	7.0	79.0
	25:25	12.0	20.0	35.0	7.0	74.0	10.0	19.0	31.0	6.0	66.0	8.0	16.0	30.0	6.0	6.0

App.: Appearance

B + T: Body + texture

Fla.: Flavor

T.S.: Total score