PRODUCTION AND PROPERTIES OF PROBIOTIC LOW-FAT FROZEN YOGHURT (ZABADY) WITH ALIVE *BIFIDOBACTERIA BIFIDUM*

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ABSTRACT: ***Milk fat was replaced in frozen yoghurt (zabady) (FY) with Simplesse 100® at the rates of 0, 25, 50, 75 and 100%, respectively. Also, traditional yoghurt starter was replaced by probiotic starter namely, Bifidobacteria bifidum DI at 50% level. Specific gravity, weight per gallon and pH values of FY mixes were increased, while titratable acidity was decreased by replacing of milk fat with Simplesse 100®. Regarding the properties of frozen product the pH values, specific gravity, weight per gallon, melting resistance, total protein, ash, carbohydrates contents, bifidobacterial and total bacterial counts were increased, while overrun, titratable acidity, fat content and calorific values were decreased. However, the changes were proportional to the rate of replacement. Substitution of milk fat in FY with Simplesse 100® up to 75% did not affect significantly ($p > 0.05$) the scores of organoleptic properties. Most of the FY properties did not change significantly during storage period, while bifidobacterial and total viable bacterial counts of all FY treatments were decreased. Generally, the organoleptic scores of all FY treatments were slightly decreased during the last two weeks of storage.***

Key Words: ***Frozen yoghurt (zabady), low fat yoghurt, probiotic yoghurt, bifidobacteria.***

INTRODUCTION

Zabady is the Arabic local name of the set-type yoghurt. It is an old fermented dairy product which is popular in Egypt as well as worldwide. Consumption of Zabady in Egypt has been increased markedly. The value of yoghurt in human diet is determined by the nutritive value of milk from which it is made, increased digestibility, prophylactic and healing effects (Rasic and Kurmann, 1978; Marshall, 1984 and Buttriss, 1997). Frozen desserts are very popular to most consumer, therefore their production has been increased steadily, in Egypt. Thus, such products could be an appropriate vehicle for delivering bifidobacteria to consumers. Thus, yoghurt ice cream (frozen yoghurt) is a hybrid of the technology and art of frozen desert and yoghurt.

Lipids play vital functional and sensory roles in food products. They carry, enhance and release the flavours of other ingredients. Lipids also interact with other ingredients to develop and mould texture, flavour

perception, flavour stability, flavour generation and the overall sensation of foods (Giese, 1996 and De Roos, 1997). Over the past decade, there has been substantial interest in the development of a new range of dairy products which are similar to the existing products but in which the fat content is substantially reduced to avoid the health problems associated with fat such as diabetes, hypertension, atherosclerosis, gallbladder disease and heart disease (Williams, 1985 and Giese, 1996). Low fat yoghurt can be achieved by lowering the fat content and / or using fat substitutes to replace the fat (Tamime *et al.,* 1994 and Giese, 1996). Using fat substitutes to replace fat in food while keeping the same functional and organoleptic properties as fat has attracted great attention in past few years. Available fat substitutes can be classified as carbohydrate-based, protein-based and fat-based fat replacers (Giese, 1996).

Bifidobacteria are well known to beneficially affect human health by improving the balance of intestinal microflora, improving mucosal defenses against pathogens, enhancing immune response, reducing serum cholesterol, reducing ammonia and free serum phenol in patients with liver disease and improving of lactose-tolerance. Additional health benefits include vitamin synthesis, anti-carcinogenic activity and anti-bacterial activity (Kebary, 1995; Badawi and El-Sonbati, 1997; Brassert and Schiffrin, 2000; Chiange *et al.,* 2000; Lourens-Hattingh and Viljoen, 2001; Ishibashi and Yamazaki, 2001; Fujwara *et al.,* 2002; Sullivan and Nord, 2002; Wright *et al.,* 2002; Gursoy *et al.,* 2005; Kebary *et al.,* 2005; Hussein *et al.,* 2006 and Kebary *et al.,* 2008a). It is estimated that more than 90 probiotic products containing bifidobacteria are produced worldwide (Shah, 2000). They include fermented milk, butter milk, sour cream, frozen dessert, cheese, baby foods, pharmaceutical preparations and livestock feed supplements (Kebary, 1996; Kebary *et al.,* 1998; Hussein and Kebary, 1999; Badran *et al.,* 2004; Boylston *et al.,* 2004; Hamed *et al.,* 2004; Kebary *et al.,* 2004; Mousa *et al.,* 2004; Kebary *et al.,* 2007; Abo Bakr, 2008 and Kebary *et al.,* 2008 b).

In view of the aforementioned, the objectives of this study were to investigate the possibility of making a good quality low fat frozen yoghurt (FY) (Zabady) using a protein-based fat replacers, study the effect of partial replacing of normal yoghurt starter with bifidobacteria and to monitor the survival of bifidobacteria as well as quality changes of FY during storage.

MATERIALS AND METHODS

Ingredients

The following materials were used in preparing frozen yoghurt: buffaloe's milk (the herd of the Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt), non-fat dry milk (Ecoval N.V., Paris, France), sucrose (Egyptian Sugar and Distilleries Company, El-Hawamdia, Egypt), vanilla (Aromisr, Egyptian Sugar and Distilleries Company, Food Flavours and Essences

Factory, Cairo, Egypt) and emulsifier-stabilizer (Palsgaard 5936, Palsgaard Industries A/S, Juelsminde, Denmark), Simplesse®100 (the Nutra Sweet Kelco Co., Deerfield, IL., USA). Cream was obtained by separating fresh buffaloe's milk in the pilot plant of Department of Dairy Science and Technology, Faculty of Agriculture, Shibin El-Kom, Egypt.

Bacterial strains:

***Bifidobacterium bifidum* DI was provided by Diversitech Inc. (Gainesville, FL). *Streptococcus salivarius* subsp. *thermophilus* EMCC1043 and *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC1102 were obtained from Cairo Mircen (Ain Shams University, Egypt). Bifidobacteria strains were activated by two successive transfers in modified Lactobacilli MRS broth (deMan, Rogosa and Sharp), (Ventling & Mistry, 1993) followed by three successive transfers in sterile 10% reconstituted non-fat dry milk and incubated at 37°C under anaerobic condition. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were activated by three successive transfers in sterile 10% reconstituted non-fat dry milk.**

Manufacture of propiotic frozen yoghurt (FY):

Control frozen yoghurt mix containing 4% fat, 13% milk solid not fat, 15% sugar and 0.5% emulsifier-stabilizer were prepared according to Farag (1991). Simplesse 100® was used to replace the milk fat of the other four FY at the rates of 25, 50, 75 and 100%. The prepared mixes were cooled to 42°C and the starter culture was added at the rate of 1.5% *Bifidobacterium bifidum* + 1.5% normal starter (Kebary, 1996) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* at the ratio of 2 : 1, respectively). All mixes were incubated at 42°C until coagulation. Frozen yoghurt from all mixes were prepared as described by Farag (1991). The resultant FY was packaged in plastic cups and placed in deep freezer held at –18°C for hardening for 24 h., then stored at –25 ± 2°C for 5 weeks. Samples from each FY treatment were taken at zero day and every week for physical, chemical, bacteriological and sensory evaluation. The whole experiment was done in triplicate.

Physical and chemical analysis:

Each FY mix was tested before freezing for titratable acidity and pH values (Ling, 1963), freezing point (FAO, 1977), specific gravity (Winton, 1958) and weight per gallon (Burke, 1947). Fresh samples of FY products were tested when fresh and during storage period for overrun (Arbuckle, 1986), specific gravity (Winton, 1958), weight per gallon (Burke, 1947), melting resistance (Ried and Painter, 1933), titratable acidity and pH values, total solids, total protein, fat and ash according to Ling (1963). Carbohydrates were calculated by difference. Total energy was calculated based on conversion factors of 4 for

protein, 4 for carbohydrates and 9 for fat and expressed as KCal / 100 g FY product.

Bacteriological analysis:

Bifidobacterium counts were enumerated on modified MRS (Ventling and Mistry, 1993) with NPNL (Neomycine sulphate, paromycine sulphate, Nalidixicacid and Lithium chloride) solution (Samona and Robinson, 1991) under anaerobic conditions using the Baltimore Biological Laboratories (BBL) gas pak (BBL, Cockeysville, MD). Total bacterial counts were enumerated on standard plate count agar (Marth, 1978).

Sensory evaluation:

Organoleptic properties of each FY treatment were assessed by ten panelists from the staff members of Department of Dairy Science and Technology and Department of Food Science and Technology according to Kebary and Hussein (1997).

Statistical analysis:

The obtained data were statistical analyzed using factorial experiment and randomized block design. Newman Keuls test was followed to make the multiple comparisons (Steel and Torrie, 1980). Significant differences were determined at $P \leq 0.05$

RESULTS AND DISCUSSION

1. Mix properties

Effect of replacing milk fat with Simplesse 100® on properties of FY mixes are presented in Table (1). These results indicate that replacing milk fat with Simplesse 100® caused significant increase in both specific gravity and weight per gallon and these increases were proportional to the replacement rate. Substitution of 25% of milk fat with Simplesse did not affect significantly ($p > 0.05$) both specific gravity and weight per gallon. FY mix (T_4) which made with substituting all milk fat with Simplesse 100® had the highest specific gravity and weight per gallon. These results are in agreement with those reported by Kebary and Hussein (1997) and Hussein and Badawi (1999).

Freezing point of FY mixes decreased by replacing milk fat with Simplesse 100®. The reduction in freezing point of zabady mixes was proportional to the amount of Simplesse 100® added (Table 1). The reduction in freezing point might be due to the presence of some soluble constituents in Simplesse 100®, which consequently lower the freezing point of FY mixes (Kebary and Hussein, 1997).

Replacement of milk fat with Simplesse 100® in FY mix caused a significant decrease in its titratable acidity (Table 1). There were negative correlation between titratable acidity of FY mix and the rate of replacing milk fat with Simplesse 100® (Table 1). These results could be attributed to the higher buffering capacity of treatments made with Simplesse 100® (Kebary and Hussein, 1999 and Badran et al., 2004). pH values of FY mixes as affected by substitution of milk fat with Simplesse 100® followed almost contrary trends to acidity (Table 1).

Table (1): Effect of Simplesse® as fat substitute on some properties of probiotic low fat frozen yoghurt mixes.

Level of milk fat substituted	Specific gravity	Weight / gl. (kg)	Freezing point °C	Titratable acidity (%)	pH value
0.0% control (C)	1.1169^{d}	4.2286^{d}	-2.05^{a}	0.83^{a}	4.60^{d}
25% (T_1)	1.1183^{d}	4.2339^{d}	-2.08^{a}	0.80^{b}	4.62^{d}
50% (T_2)	1.1205^{c}	4.2422^{c}	-2.26^{b}	0.76^{c}	4.91^{c}
75% (T_3)	1.1222^{b}	4.2486^{b}	-2.41^{c}	0.71^{d}	5.33^{b}
100% (T_4)	1.1256^{a}	4.2615^{a}	-2.58^{d}	0.66^{e}	5.86^{a}

Each value in the table is the mean of three replicates.

2. Frozen yoghurt properties:

Replacement of milk fat with Simplesse 100® up to 25% did not affect significantly ($p > 0.05$) the overrun of FY, while further increasing of the rate of replacement caused a significant ($p \leq 0.05$) reduction in overrun of the resultant FY (Tables 2, 6), and this decrease was proportional to the rate of replacement. These results could be attributed to the higher viscosity, which subsequently hindered the incorporation of air in the mix during freezing and / or lower freezing point (Khalafalla *et al.,* 1975). These results are in agreement with those reported by Badawi *et al.* (2002) and Badran *et al.* (2004).

Specific gravity and weight per gallon of FY were related to each other and inversely correlated to the overrun (Kebary and Hussein, 1997 and Badawi *et al.,* 2002) (Table 2, 6). Specific gravity and weight per gallon increased by replacing milk fat with Simplesse 100® and this increase was proportional to the rate of replacing (Kebary and Hussein, 1997; Badawi *et al.,* 2002 and Badran *et al.,* 2004). Control FY batch exhibited the lowest specific gravity and weight per gallon, while exhibited the highest overrun, conversely FY that made by complete replacement of milk fat with Simplesse® showed the highest specific gravity and weight per gallon and the lowest overrun (Tables 2, 6).

Replacement of milk fat in FY with Simplesse 100® especially above 25% decreased significantly the rate of melting after 60 min and the next 30 min, which means increasing the melting resistance of frozen product. This

increase was proportional to the rate of replacement (Tables 2, 6). The increase in melting resistance might be due to the increase in mix viscosity and / or the lower freezing point (Sommer, 1951 and Khalafalla *et al.,* 1975).

Table (2): Effect of Simplesse® as fat substitute on some properties of resultant probiotic low fat frozen yoghurt.

Level of milk fat substituted	Overrun (%)	Specific gravity	Weight / gal (kg)	Melting resistance (loss % at 30°)		
				First 60 min	Next 30 min	Last 30 min
0.0% control (C)	54.6	0.651	2.4646	29	43	28
25% (T_1)	54.0	0.655	2.4798	28	42	29
50% (T_2)	52.5	0.678	2.5669	25	40	34
75% (T_3)	51.2	0.703	2.6616	21	36	42
100% (T_4)	49.1	0.719	2.7221	19	35	45

Each value is the mean of three replicates.

Gross composition of FY is presented in Table (3). Replacement of milk fat with Simplesse 100® did not affect significantly ($p > 0.05$) the total solids content of the resultant FY (Tables 3, 6). On the other hand substitution of milk fat with Simplesse 100® caused a significant increase in total protein, ash and carbohydrates contents, while caused a significant decrease in fat content of the resultant FY and this increase or decrease was proportional to the increasing the rate of replacement (Tables 3, 6). Similar results were reported by Kebary and Hussein (1997) and Hussein and Badawi (1999). Calorific value of FY decreased ($p < 0.05$) by replacing of milk fat with Simplesse. There was negative correlation between the rate of replacing milk fat and the calorific value of FY. These results are in agreement with those reported by Hussein and Badawi (1999). Gross composition and calorific value of FY did not change significantly ($p > 0.05$) throughout the storage period (Tables 3, 6).

Titratable acidity of FY (Table 4) decreased by increasing the rate of replacing milk fat with Simplesse 100®. Control FY had the highest titratable acidity, while T4 that made with complete replacement of milk fat with Simplesse showed the lowest titratable acidity which could be attributed to the higher buffering capacity, since Simplesse 100® is a protein-based fat replacers. These results are in agreement with those reported by Hussein and Badawi (1999) and Badran *et al*. (2004). Titratable acidity of all FY treatments did not change during the frozen storage period (Badran, 2004). On the other hand, pH values as affected by replacing milk fat with Simplesse 100® and storage period following contradictory trends to those of titratable acidity (Tables 4, 6).

Table (3):
Table (4):

Table (5):
Table (6):

Changes in scores of organoleptic properties during storage of FY are presented in Table (5). Replacement of milk fat with Simplesse 100® did not affect flavour and body & texture scores up to 75% replacement rate. Treatment T_3 that made with replacing 75% of milk fat with Simplesse 100® was not significantly different from control FY made with milk fat, therefore it is possible to reduce the fat content of FY to 25% by using Simplesse without significant ($p \leq 0.05$) effects on resultant FY quality. On the other hand, scores of FY organoleptic properties did not change significantly during the first two weeks of storage, while it decreased slightly during succeeding two weeks (Tables 5, 6).

Viability of bifidobacteria during storage of FY is illustrated in Table (7). Counts of bifidobacteria increased by replacing milk fat with Simplesse 100® and the increase was proportional to the replacement rate (Table 7). Control FY batch had the lowest count, while T_4 which made by complete replacement of milk fat with Simplesse 100® exhibited the highest bifidobacterial counts. These results might be attributed to the difference of acidity and / or the stimulation effect of Simplesse 100®. Bifidobacteiral counts of all FY declined as frozen storage period progressed. These results are in accordance with those reported by Kebary (1996) and Badran *et al.* (2004). Even after storage for 4 weeks all FY treatments contained higher counts of bifidobacteria than should be present to achieve their health benefits ($10^5 - 10^6$ / gm) (Hunger and Pietersen, 1992).

Table (7): Bifidobacteria counts of probiotic low fat frozen yoghurt made with Simplesse® as fat substitute throughout frozen storage.

Level of milk fat substituted	Bifidobacteria counts / gm				
	Storage period (weeks)				
	0	1	2	3	4
0.0% control (C)	89×10^7	112×10^7	96×10^7	26×10^7	78×10^6
25% (T_1)	95×10^7	125×10^7	107×10^7	32×10^7	83×10^6
50% (T_2)	91×10^7	129×10^7	112×10^7	35×10^7	95×10^6
75% (T_3)	103×10^7	141×10^7	126×10^7	41×10^7	102×10^6
100% (T_4)	121×10^7	162×10^7	141×10^7	56×10^7	111×10^6

Each value is the mean of three replicates.

Changes of total bacterial counts in FY treatments are presented in Table (8). Total bacterial counts as affected by replacing milk fat with Simplesse 100® and storage followed almost similar trends of those of bifidobacterial counts.

Table (8): Total bacterial counts of probiotic low fat frozen yoghurt made with Simplesse® as fat substitute throughout frozen storage.

Level of milk fat substituted	Total bacterial counts / gm				
	Storage period (weeks)				
	0	1	2	3	4
0.0% control (C)	163×10^7	125×10^7	78×10^7	21×10^7	57×10^6
25% (T_1)	196×10^7	142×10^7	91×10^7	26×10^7	62×10^6
50% (T_2)	195×10^7	145×10^7	95×10^7	30×10^7	71×10^6
75% (T_3)	213×10^7	156×10^7	113×10^7	37×10^7	78×10^6
100% (T_4)	276×10^7	180×10^7	122×10^7	42×10^7	91×10^6

Each value is the mean of three replicates.

It could be concluded that it is possible to replace up to 75% of milk fat with Simplesse in FY manufacture without significant effects on the resultant FY quality. Also replacement of 50% of normal Zabady starter with bifidobacteria did not affect the quality of resultant FY and this product could be used as a good vechile for delivering this alive probiotic bacteria to consumers.

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إنتاج وخواص اليوجورت (الزبادى) المجمد الحيوى المحتوى على بكتيريا البيفيدو الحية

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الملخص العربى

يهدف هذا البحث لدراسة تأثير استبدال دهن اللبن المستخدم فى صناعة الزبادى المجمد ببديل الدهن ذو الأصل البروتينى Simplesse على صفات الزبادى المجمد لذلك تم تصنيع ٥ معاملات من الزبادى المجمد باستبدال دهن اللبن بواسطة Simplesse بنسب صفر ، ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠% كما تم استبدال ٥٠% من بادئ الزبادى المعروف بواسطة بكتريا *Bifidobacterium bifidum* ، ولقد أوضحت النتائج المتحصل عليها بعد تحليلها إحصائياً ما يلى:

- أدى استبدال دهن اللبن بواسطة Simplesse لزيادة كلٍ من pH والوزن النوعى والوزن بالجالون بينما أدى لخفض حموضة مخاليط الزبادى المجمد وازداد التأثير بزيادة نسب الاستبدال .
- أدى استبدال دهن اللبن بواسطة Simplesse إلى خفض نسبة الريع ونسبة الحموضة بينما أدى لزيادة pH والوزن بالجالون والوزن النوعى والمقاومة للانصهار وزيادة العدد الكلى وعدد بكتريا Bifidobacteria فى الزبادى المجمد الناتج وازداد هذا التأثير بزيادة معدل الاستبدال .
- أدى استبدال دهن اللبن بواسطة Simplesse لزيادة نسب كلٍ من البروتين الكلى والرماد والكربوهيدرات بينما أدى لخفض نسب الدهن والطاقة ولم يُؤثر على الجوامد الصلبة الكلية .
- لم تتأثر درجات التحكيم الممنوحة للنكهة والقوام والتركيب أو الدرجات الكلية حتى ٧٥% استبدال بينما انخفضت الدرجات الممنوحة لخواص التحكيم بزيادة نسبة الاستبدال عن ذلك .
- أدى استبدال دهن اللبن بواسطة Simplesse لزيادة العدد الكلى للبكتريا وعدد بكتريا Bifidobacteria وكانت هناك علاقة تناسب طردية بين أعداد هذه البكتريا وبين معدل الاستبدال ولقد انخفضت أعداد هذه البكتريا فى كل المعاملات أثناء التخزين إلا أن أعداد بكتريا Bifidobacteria وحتى بعد التخزين لمدة أربع أسابيع كان أعلى مما يجب أن يكون متواجد فى المنتج الغذائى لكلى تُحقق هذه البكتريا فوائدها الصحية .

لم يتغير التركيب الكيماوى للزبادى المجمد أثناء التخزين بينما تأثرت الخواص الحسية قليلاً .

Table (3): Gross composition of probiotic low fat frozen yoghurt made with Simplesse® as fat substitute when fresh and after storage.

Level of milk fat substituted	Total solids (%)		Proteins (%)		Fat (%)		Ash (%)		Carbohydrates (%)		Caloric value (kCal / 100 g)		% Reduction of caloric value	
	Fresh	4 weeks	Fresh	4 weeks	Fresh	4 weeks	Fresh	4 weeks	Fresh	4 weeks	Fresh	4 weeks	Fresh	4 weeks
0.0% control (C)	32.15	31.93	5.22	5.18	4.01	3.98	1.15	1.11	21.77	21.62	144.05	143.02	-	-
25% (T_1)	32.03	31.91	5.91	5.86	3.05	3.00	1.23	1.20	21.84	21.85	138.45	137.84	3.99	3.62
50% (T_2)	31.98	31.90	6.34	6.30	1.95	1.93	1.29	1.26	22.40	22.41	132.51	132.21	8.01	7.56
75% (T_3)	32.05	31.95	6.91	6.88	1.00	0.95	1.30	1.28	22.84	22.84	128.00	127.43	11.14	10.90
100% (T_4)	32.10	32.05	7.32	7.27	0.15	0.15	1.35	1.32	23.28	23.11	123.75	122.87	14.09	14.09

Each value is the mean of three replicates.

Table (4): Changes in titratabe acidity (%) and pH value during storage of probiotic low fat frozen yoghurt made with Simplesse® as fat substitute.

Level of milk fat substituted	Titratable acidity (%)					pH value				
	Storage period (weeks)					Storage period (weeks)				
	0	1	2	3	4	0	1	2	3	4
0.0% control (C)	0.82	0.82	0.84	0.85	0.85	4.65	4.62	4.62	4.62	4.60
25% (T_1)	0.81	0.81	0.82	0.82	0.82	4.70	4.70	4.68	4.68	4.65
50% (T_2)	0.76	0.76	0.78	0.78	0.78	4.92	4.90	4.92	4.90	4.90
75% (T_3)	0.73	0.73	0.73	0.75	0.75	5.06	5.05	5.05	5.02	5.02
100% (T_4)	0.70	0.70	0.72	0.72	0.72	5.13	5.10	5.10	5.10	5.10

Each value is the mean of three replicates.

Table (5): Sensory evaluation during storage of probiotic low fat frozen yoghurt made with Simplesse® as fat substitute.

Level of milk fat substituted	Flavour (50)					Body and texture (40)					Melting quality (10)					Total scores (100)				
	Storage period (weeks)					Storage period (weeks)					Storage period (weeks)					Storage period (weeks)				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
0.0% control (C)	45	44	44	44	44	35	34	34	33	33	9	9	8	8	8	89	87	86	85	85
25% (T_1)	45	45	45	44	43	35	35	34	34	34	9	9	8	8	8	89	89	87	86	85
50% (T_2)	45	46	46	44	44	35	35	35	33	34	8	8	9	8	8	88	89	89	85	86
75% (T_3)	45	46	45	44	44	34	35	35	34	33	8	8	8	8	8	87	89	88	86	85
100% (T_4)	42	42	40	40	40	34	34	32	32	30	8	8	8	8	8	84	84	80	80	78

Each value is the mean of three replicates.

Table (6): Statistical analysis of probiotic low fat frozen yoghurt properties made with Simplesse® as fat substitute.

Properties of frozen zabady	Effect of treatments						Effect of storage period (weeks)					
	Mean squares	Multiple comparisons•					Mean squares	Multiple comparisons•				
		C♦	T_1	T_2	T_3	T_4		0	1	2	3	4
Overrun (%)	9.368*	A	A	B	C	D						
Specific gravity	15.513*	D	D	C	B	A						
Weight / gal (kg)	2.672*	D	D	C	B	A						
Melting resistance:												
First 60 min	52.351*	D	D	C	B	A						
Next 30 min	41.236*	D	D	BC	B	A						
Last 30 min	26.513*	A	B	C	D	E						
Titratable acidity (%)	0.815*	A	A	B	C	D	0.0931	A	A	A	A	A
pH value	1.251*	D	D	C	B	A	0.1256	A	A	A	A	A
Composition:												
Total solids (%)	24.231	A	A	A	A	A	26.03	A				A
Protein (%)	6.825*	E	D	C	B	A	12.16	A				A
Fat (%)	43.026*	A	B	C	D	E	23.58	A				A
Ash (%)	11.581*	E	D	C	B	A	9.18	A				A
Carbohydrate (%)	23.105*	E	D	C	B	A	40.31	A				A
Calorific value (Kcal/100 gm)	38.638*	A	B	C	D	E	55.63	A				A
Organoleptic properties:												
Flavour	145.361*	A	A	A	A	B	5.832*	A	AB	AB	B	B
Body and texture	97.235*	A	A	A	A	B	16.516*	A	AB	AB	B	B
Melting quality	23.091*	A	A	B	B	B	3.781*	A	A	A	AB	AB
Total scores	305.253*	A	A	AB	AB	B	25.316*	A	AB	AB	B	B

♦ See Table (1). * Significant at 0.05 level.

• For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, … etc.