MANUFACTURE OF FUNCTIONAL GOUDA CHEESE

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ABSTRACT: Effect of incorporating fat replacers on the quality of low fat Gouda cheese was studied. Control cheese was made from milk standardized to 3.0% fat. Another five batches of Gouda cheese were made from milk standardized to 2.0% fat. One of them was served as control, and two were made with adding Simplesse® 100 (a protein based fat replacer) at the rate of 0.5 and 1.0%, respectively the other two batches were made with adding Novagel® at the rate of 0.5 and 1.0%. All cheese treatments were salted in 20% brine solution containing 40% KCl and 60% NaCl. Reducing the milk fat caused a significant decrease in moisture content, fat content, values of ripening indices organoleptic scores and counts of total, lipolytic and proleolytic bacteria. Adding fat repalcers increased the moisture content, ripening indices values and scores of organoleptic properties. Cheese made from milk containing 2.0% fat with adding 1.0% Simplesse®100 contained the highest ripening indices values and was the most acceptable cheese beside being in significantly different from control cheese made from 3.0 fat milk. Fat, total nitrogen, acidity content, values of ripening indices and scores of organoleptic properties increased, during ripening period, while moisture content, pH values, counts of total lipolytic and proteolytic bacteria decreased. The results showed the possibility of decreasing the fat by 33.0% when Simplesse® 100 was used without significant effect on cheese quality. Key words: Functional cheese, low potassium, low fat, Simplesse® 100.

Novagel®.

INTRODUCTION

Consumption of Gouda cheese in Egypt has been increased recently, therefore it has been manufactured in some Egyptian Dairy factories (El-Sisi, et al., 2010). Salt has a crucial effect on cheese ripening and subsequently developing flavour, body and texture of cheese (Banks et al., 1993). Because sodium intake is primarily associated with hypertension (Dillon, 1987; Guinee, 2004), it has been tried to use a mixture from NaCl and KCl. It has been succeeded to use a solution brine from 60% NaCl and 40% to make a good quality low sodium Gouda cheese (El-Sisi et al., 2010).

Milk fat plays important roles in developing the distinct flavour, appearance, body and texture of cheese. Therefore, reduced fat cheeses may exhibited undesirable lack of flavour and a firm rubbery texture. Because of health problems associated with high fat intake such as obesity, diabetes,

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some types of cancer, hypertension, atherosclerosis, liver and heart disease, many efforts have been devoted to develop dairy products with a reduced fat content. Using fat replacers. The possibility of making low fat cheeses has been reviewed by Kebary *et al.* (2006).

The objectives of this study were to evaluate the possibility of making a good quality low sodium, low fat Gouda cheese, investigate the effects of adding Simplesse® (a protein based fat replacer) and Novagel® (a carbohydrate-based fat replacer) on the chemical, microbiological and organoleptic properties of low fat Gouda cheese and monitor changes during ripening period of Gouda cheese.

MATERIALS AND METHODS

1. Materials:

1.1. Milk:

Fresh whole cows' milk was obtained from the farm of Faculty of Agriculture, Cairo University, Giza, Egypt.

1.2. Starter culture:

Multiple mixed strain culture containing Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. diacetylactis was used. The culture was obtained from Chr. Hansen's Lab., Denmark.

1.3. Rennet:

Rennet powder (Hannilase L2235) was obtained from Chr. Hansen's Lab., Denmark.

1.4. Salts:

Commercial fine grade salts (NaCl, KCl and CaCl₂) was used.

1.5. Fat mimetics:

- Simplesse® 100 (Nutrasweet Kelco Co., Deerfield, IL, USA).
- Novagel® RCN 15 (FMC Biopolymer, Philadephia, PA, USA).

1.6. Annatto:

Annatto (550) was obtained from Chr. Hansen's Lab., Denmark.

1.7. Coating material:

White plastic 5% Natamycin was obtained from Chr. Hansen's Lab., Denmark.

2. Methods:

2.1. Cheese making:

Six batches of low fat Gouda cheese were made using fresh cows' milk. Control cheese was made from cow's milk standardized to 3% fat (T_1) while the other five cheese treatments were made from the same milk but was standardized to 2% fat, one of these batches was served as control (T_2), another two batches were made with adding Simplesse® 100 at the rate of 0.5 and 1.0%, respectively, while the other two treatments were made with adding Novagel® RCN15 at the rate of 0.5 and 1.0%, respectively.

Gouda cheese was manufactured as described by Scott (1998) as follows: The milk was heated to 72°C for 15 sec. and immediately cooled to 31°C. Annatto and calcium chloride were added at the rate of 25 ml/100 kg milk and 0.02% in the same order. The milk was inoculated with 1.0% commercial starter culture. When acidity of milk reached to 0.19 - 0.20%, rennet powder was added at the rate of 3.0 gm/100 kg milk. The curd became firm within 25 -30 min. The curd was cut into 0.5 - 1.5 cm cubes using the American knives for 10 - 15 minutes with stirring curd to float in whey. Scalding was accomplished by replacing 30% of the whey with hot water at not more than 80°C to raise the temperature of whey to 36 - 38°C in about 30 min. with continuous stirring. The curd is lightly pressed at 2 - 4 kg/cm² for 15 - 30 min. The whey was then drained off and the curd was filled in the mould, the cheese was pressed. During pressing the cheese curd was turned and pressed to acquire the required shape. The curd wheels were then dumped into 20% salt brine (consisting from 60% NaCI + 40% KCL according to El-Sisi et al. (2010)] at 15°C for 48 hr. After salting, the green cheeses were placed for two days in ripening room for drying. The cheese wheels were then carefully coated with plastic coat. The resultant cheeses were then kept in the ripening room at 10 - 12°C and 85 - 95% relative humidity for three months. All cheese treatments were sampled when fresh and during ripening period after 15, 30, 60 and 90 days for chemical and organoleptic analysis. The whole experiment was duplicated.

2.2. Chemical analysis:

Cheese were analyzed at zero time, 15, 30, 60 and 90 days of ripening period for moisture, fat, total nitrogen (TN), soluble nitrogen (SN), pH value and titratable acidity according to Ling (1963). Total volatile fatty acids (TVFA) were determined according to Kosikowski (1966), while soluble tyrosine and soluble tryptophan contents of cheese were determined according to the method of Vakaleris and Price (1959).

2.3. Bacteriological analysis:

Total bacterial counts were enumerated on standard plat count agar according to Marth (1978). Lipolytic bacteria were determined according to

Salle (1961), while proteolytic bacteria were determined on nutrient agar medium + 10% sterile skim milk according to Sharf (1970).

2.4. Sensory evaluation:

Organoleptic properties of different cheeses were assessed according to Hammad (2008). Cheese samples were evaluated by ten panelists.

2.5. Statistical analysis:

Factorial design was used to analyze the obtained data and Duncan's test was used to calculate the multiple comparison (Steel and Torrie, 1980). Significant differences were determined at p < 0.05 level.

RESULTS AND DISCUSSION

Moisture content of low fat Gouda cheese increased significantly (p < 0.05) by replacing milk fat with fat replacers. This increase was proportional to the rate of replacement. These results are in agreement with those reported by El-Sonbaty et al. (2002) and Kebary et al. (2002) on Edam cheese; Kebary et al. (2009) on Domiati cheese. These results might be due to the higher water holding capacity of carbohydrate and protein than that of milk fat. Cheese treatment made by incorporation Novagel® contained higher moisture content than those of the corresponding cheese treatments made by adding Simplesse® 100, which means that Novagel® was more effective to increase the moisture content of cheese (Kebary et al., 2009). Moisture content decreased by decreasing the fat content of milk that used in cheese making (Tables 1, 6), which might be attributed to the ability of fat to hinder the syneresis of whey from the curd (Marshall, 1982; Van Dijk and Walstra, 1986). Moisture content of all cheese treatments decreased significantly (p < 0.05) as ripening period progressed (Tables 1, 6). These results agree with the results of Verachia (2005).

Fat content of Gouda cheese treatments decreased significantly (p \leq 0.05) by reducing the fat content of cheese milk. Similar results were reported by El-Sonbaty *et al.* (2002); Kebary *et al.* (2002) for Edam cheese, Hammam (2005); Abd Alla *et al.* (2008) for Ras cheese. There were slight differences among cheese treatments which might be due to the differences in moisture content (Table 1, 6). The results of cheese treatments made with adding the same ratio from fat repalcers (T_3 , and T_5 or T_4 and T_6) were not significantly (p > 0.05) different from each other. This means that the type of fat replacer did not affect significantly (p > 0.05) the fat content of cheese treatments. Fat content of all cheese treatments increased significantly (p \leq 0.05) throughout the ripening period (Tables 1, 6), which might be due to the loss of moisture content during ripening period. These results agree with those reported by Taha *et al.* (2007); Abd Alla *et al.* (2008) and Mehanna *et al.* (2009).

Table 1

Replacement of milk fat with Simplesse® 100 which is the protein-based fat replacer caused a significant ($p \le 0.05$) increase in total nitrogen content of cheese and this increase was proportional to the rate of replacement (Tables 1, 6). On the other hand, cheese treatments made by adding Novagel® were not significantly different from each other. Total nitrogen content of all Gouda cheese treatments increased as ripening period advanced (Tables 1, 6). These results could be attributed to the loss of moisture content. Similar trends were obtained by El-Sonbaty *et al.* (2002); Kebary *et al.* (2002) for Edam cheese; Badawi (1998) and Hussein (2000) for Ras cheese.

Incorporation of fat repalcers caused a significant (p \leq 0.05) increase in titratable acidity of low fat Gouda cheese (Tables 2, 6). This increase in titratable acidity was proportional to the rate of adding the fat replacers. Adding Novagel® was more effective to increase the titratable acidity of cheese. These results may be due to the higher moisture content (higher water acidity), which enhance the growth of cheese flora and subsequently developing acidity. These results are in accordance with those reported for Ras cheese by Badawi (1998); Hussein (2000). Titratable acidity of all Gouda cheese treatments increased significantly (p \leq 0.05) as ripening period proceeded (Tables 2, 6). These results are in agreement with those reported by Kebary et al. (2002) for Edam cheese, Taha et al. (2007); Abd Alla et al. (2008) for Ras cheese.

The trend of pH values of Gouda cheese treatments as affected by type and concentration of fat replacers and ripening period were opposite to that of titratable acidity (Tables 2, 6).

Ripening indices (water soluble nitrogen, soluble tyrosine, soluble tryptophan and total volatile fatty acids) of all Gouda cheese treatments followed almost similar trends (Tables 3, 6). Values of ripening indices of Gouda cheese treatments increased gradually up to the end of ripening period (Tables 3, 6). These results are in agreement with those reported for Edam cheese by Kebary et al. (2002) and for Ras cheese by Taha et al. (2007); Abd Alla et al. (2008) and Mehanna et al. (2009).

Treatments T_2 which made from 2.0% fat without adding fat repalcers contained the lowest values of ripening indices, which could be attributed to the lower moisture content which subsequently suppress the growth of proteolytic and lipolytic bacteria and might inhibit the proteases and lipases activity themselves. Similar trends were reported for Domiati cheese by Kebary *et al.* (2006). Incorporation of fat replacers increased significantly (p \leq 0.05) the values of all ripening indices of Gouda cheese treatments (Tables 3, 6). These results might be due to the higher moisture content, which enhanced the growth of lipolytic and proteolytic bacteria and subsequently increased

Table 2

Table 3

the ripening indices. Similar trends were obtained by Kebary *et al.* (2006) for Domiati cheese; Badawi (1998) for Ras cheese. Treatment T_4 that made from 2.0% fat milk with adding 1.0% Simplesse® 100 was not significantly different from control cheese (T_1) that made from 3.0% fat milk and they contained the highest values of all ripening indices. Ripening indices of all Gouda cheese increased gradually as ripening period progressed up to the end of ripening period (Tables 3, 6). These results are in agreement with those reported by Kebary *et al.* (2002) for Edam cheese and Taha *et al.* (2007); Abd Alla *et al.* (2008); Mehanna *et al.* (2009) for Ras cheese.

Scores of organoleptic properties of all cheese treatments (flavour, body & texture, appearance and total scores) followed almost similar trends (Tables 4, 6). Organoleptic scores of all cheese treatments increased significantly (p \leq 0.05) as ripening period advanced. These results are in accordance with those reported for Edam cheese by Kebary *et al.* (2002), El-Sonbaty *et al.* (2002). Reducing the milk fat caused a significant decrease in the scores of organoleptic properties (Hussein, 2000). Adding fat replacers improved the acceptability of low fat Gouda cheese. Cheese treatments made with adding Simplesse 100 gained higher scores than those of corresponding cheese treatments made with adding Novagel Cheese treatment that made from 2.0% fat milk with adding 1.0% Simplesse 100 was not significantly different from control cheese that made from 3.0% fat milk and they were the most acceptable cheeses (Tables 4, 6).

Counts of total bacteria, lipolytic and proteolytic bacteria followed almost similar results (Table 5). Total lipolytic and proteolytic bacterial counts increased in all Gouda cheese treatments up to the 30 day of ripening period then decreased up to the end of ripening period (Table 5). Treatment T_2 which made from 2.0% fat milk exhibited the lowest counts of these bacteria, which could be attributed to the lower moisture content (lower water activity) that suppress the growth of cheese flora. Adding fat replacers increased the counts of total, lipolytic and proteolytic bacteria, which could be due to the increase of moisture content.

It could be concluded that, adding fat repalcers increased all the ripening indices, improved the acceptability of cheese and enhance the growth of lipolytic and proteolytic bacteria. It is possible to reduce the milk fat content by 33% without significant effect on Gouda cheese quality when 1.0% \times Simplesse \times 100 was used.

TABLE 4

TABLE 5

TABLE 6

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تصنيع جبن الجودا ذو الخواص الوظيفية

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الملخص العربي:

يهدف هذا البحث إلى محاولة تصنيع جبن جودا ذو خواص وظيفية منخفض الصوديوم والدهن لذلك تم تصنيع ٦ معاملات من جبن الجودا واحدة صنعت من لبن يحتوى ٣% دهن والخمس الأخرى صنعت من لبن يحتوى ٢ % دهن واحدة كنترول واثنتان صنعت بإضافة Simplesse® 100 بنسبة ٥٠٠، ١٠٠ على الترتيب ، والإثنتان الأخريتان صنعت بإضافة «Novagel بنسبة ٥٠٠، ١٠٠ على الترتيب ، ولقد تم تمليح كل المعاملات في ٢٠% محلول ملحى يحتوى على ٦٠% كلوريد صوديوم و ٤٠% كلوريد بوتاسيوم . ولقد أوضحت النتائج المتحصل عليها ما يلى :

- أدى انخفاض الدهن فى اللبن المصنع منه الجبن إلى انخفاض نسبة الرطوية والدهن والحموضة ودلائل التسوية وبرجات التحكيم وأعداد البكتريا المحللة الدهن والبروتين .
- أدى إضافة بدائل الدهون إلى زيادة الرطوية والحموضة وقيم دلائل التسوية ودرجات التحكيم وإزدادت أعداد البكتريا المحللة للبروتين والدهن والعدد الكلى للبكتريا .
- أدى إضافة 100 "Simplesse إلى زيادة نسبة النيتروجين الكلى واحتوت العينة المصنعة على ١٨ "Simplesse على أعلى القيم لدلائل التسوية ودرجات التحكيم ولم تختلف عن العينة الكنترول المصنعة من لبن يحتوى ٣% دهن .
- ازدادت نسب كل من الدهن ، البروتين ، الحموضة ، قيم دلائل التسوية ودرجات التحكم أثناء التسوية فــى كــل المعاملات ، بينما انخفضــت نسبة الرطويــة وقــيم الـ pH فى حين ازداد العدد الكلى للبكتريا وأعداد البكتريا المحللة للدهن والبروتين حتى اليوم الثلاثين ثم انخفضت تلك الأعداد بتقدم فترة التسوية .

Table (1). Effect of replacing milk fat with fat replacers on moisture, Total nitrogen and fat contents of Gouda cheese.

	Moisture content (%)					Tota	ıl nitrog	en / dry	/ matte	r (%)	Fat / dry matter (%)					
Cheese treatments	Storage period (days)						Storage	e period	d (days)		Storage period (days)					
	Zero	15	30	60	90	Zero	15	30	60	90	Zero	15	30	60	90	
T ₁	47.45	45.22	43.80	42.15	41.22	5.56	5.70	5.79	6.13	6.21	44.20	44.55	44.69	44.85	45.15	
T ₂	40.60	38.80	37.65	36.83	36.12	6.08	6.18	6.31	6.65	6.82	32.99	33.33	33.68	33.87	34.12	
T ₃	42.25	40.00	39.42	38.55	38.01	6.77	6.87	7.03	7.16	7.25	32.20	32.33	32.78	33.12	33.44	
T ₄	43.18	41.12	39.87	38.94	38.51	7.35	7.43	7.61	7.30	7.37	31.38	31.52	31.69	32.91	33.02	
T ₅	43.12	40.88	39.72	38.78	38.35	5.42	5.58	5.67	5.93	6.07	32.22	32.34	32.79	33.14	33.47	
Т ₆	44.32	42.39	41.81	40.61	39.87	4.79	4.91	5.07	5.35	5.62	31.37	31.53	31.71	32.90	33.05	

T₁: Control cheese made from milk standardized to 3.0% fat.

T₂: Control cheese made from milk standardized to 2.0% fat.

T₃: Cheese made from milk standardized to 2.0% fat with adding 0.5% Simplesse[®] 100. T₄: Cheese made from milk standardized to 2.0% fat with adding 1.0% Simplesse[®] 100. T₅: Cheese made from milk standardized to 2.0% fat with adding 0.5% Novagel[®].

T₆: Cheese made from milk standardized to 2.0% fat with adding 1.0% Novagel[®].

Table (2). Effect of replacing milk fat with fat replacers on titrtable acidity and pH of Gouda cheese.

		Titrata	able acid	ity (%)		pH values							
Cheese treatments*		Storag	je period	(days)		Storage period (days)							
	Zero	15	30	60	90	Zero	15	30	60	90			
T ₁	1.00	1.40	1.54	1.84	2.01	6.18	5.82	5.61	5.52	5.40			
T ₂	0.93	1.27	1.43	1.71	1.89	6.20	6.01	5.83	5.70	5.59			
T ₃	0.95	1.32	1.46	1.76	1.80	6.15	5.96	5.79	5.68	5.59			
T ₄	0.98	1.37	1.52	1.83	1.84	6.11	5.94	5.73	5.62	5.53			
T ₅	0.97	1.38	1.54	1.85	2.05	6.13	5.82	5.61	5.52	5.39			
Т ₆	1.00	1.49	1.63	1.96	2.13	6.17	5.79	5.60	5.43	3.35			

^{*} see Table (1).

Table (3). Effect of replacing milk fat with fat replacers on soluble tyrosine, soluble tryptophan, soluble nitrogen (SN) and total volatile fatty acids (TVFA) of Gouda cheese.

Cheese	Soluble nitrogen (%)				n	Total volatile fatty acids (ml NaOH 0.1 N/100 g cheese)				Soluble tyrosine (mg/100 g cheese)					Soluble tryptophan (mg/100 g cheese)					
treatments*	Sto	rage	perio	d (da	ys)	Sto	Storage period (days)				S	Storage period (days)								
	Zero	15	30	60	90	Zero	15	30	60	90	Zero	15	30	60	90	Zero	15	30	60	90
T ₁	0.39	0.42	0.45	0.69	0.73	10.0	17.9	20.2	29.9	39.8	51.2	160.0	225.0	327.2	358.8	54.6	63.2	71.4	79.3	86.2
T ₂	0.37	0.41	0.43	0.65	0.70	6.8	10.1	15.3	22.4	30.7	46.6	136.7	190.0	276.0	313.2	53.0	60.3	68.6	77.0	82.9
T ₃	0.35	0.40	0.42	0.61	0.68	8.1	12.4	16.8	24.1	33.0	48.0	147.2	205.3	289.8	329.6	55.2	66.1	73.0	80.3	85.7
T ₄	0.36	0.41	0.43	0.64	0.71	9.3	15.3	18.9	26.9	37.9	50.0	154.2	219.3	325.0	355.0	55.3	68.0	74.0	82.6	89.8
T ₅	0.35	0.38	0.41	0.55	0.63	7.2	9.6	16.2	23.5	31.1	47.0	140.1	194.6	281.0	320.2	54.0	62.9	71.5	77.2	80.9
T ₆	0.36	0.39	0.42	0.58	0.65	7.9	10.9	17.3	24.8	32.5	47.2	142.3	199.6	284.2	324.2	55.2	66.2	73.4	80.2	85.5

^{*} see Table (1).

Table (4). Organoleptic evaluation of low fat, low sodium Gouda cheese.

	Flavour (60)				Boo	Body and texture (30)				Appearance (10)				Total scores (100)						
Cheese treatments*	Sto	Storage period (days)				Sto	Storage period (days)				Stor	Storage period (days)				Storage period (days)				
	Zero	15	30	60	90	Zero	15	30	60	90	Zero	15	30	60	90	Zero	15	30	60	90
T ₁	-	28	39	52	56	-	21	23	26	27	-	6	7	8	9	_	55	69	86	92
T ₂	-	25	34	46	49	_	18	20	23	25	-	5	6	7	7	_	48	60	76	81
T ₃	-	26	37	50	53	_	19	21	24	26	-	5	6	8	8	_	50	64	82	87
T ₄	-	27	39	51	55	_	21	23	25	27	-	6	7	8	9	_	54	69	84	91
T ₅	_	25	35	48	51	_	19	21	23	25	-	5	6	6	7	_	49	62	77	83
T ₆	_	26	38	49	52	_	20	22	24	26	_	6	7	7	8	_	52	67	80	86

^{*} see Table (1).

Table (5). Effect of replacing milk fat with fat replacers on micropiological analysis of Gouda cheese.

Cheese	Total bacterial count (cfu / gm × 10 ⁶)					Pro	•	bacte / gm ×	•	ints	Lipolytic bacterial counts (cfu / gm × 10 ⁶)					
treatments*	;	Storage	e period	l (days))		e period	d (days))	Storage period (days)						
	Zero	15	30	60	90	Zero 15 30 60 90 Z				Zero	15	30	60	90		
T ₁	65	81	86	66	46	3.9	6.0	7.4	2.5	1.9	3.1	5.8	8.3	4.5	3.0	
T ₂	60	74	81	56	41	3.4	5.0	7.0	2.2	1.6	2.5	4.5	7.4	3.6	2.7	
T ₃	61	76	82	58	42	3.5	5.2	7.1	2.3	1.7	2.5	4.3	7.5	3.8	2.8	
T ₄	62	79	84	60	44	3.8	5.6	7.4	2.4	1.8	2.6	4.4	7.6	4.0	2.9	
T ₅	60	74	80	55	40	3.5	5.5	7.1	2.1	1.6	2.4	4.2	7.3	3.7	2.6	
Т ₆	61	78	82	57	41	3.6	5.4	7.2	2.2	1.7	2.3	4.3	7.5	3.9	2.7	

^{*} see Table (1).

Table (6). Statistical analysis of low fat and low sodium Gouda chees	ese properties.
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			Effec	ct of ti	eatm	ents"			Effect of storage (days)						
Gouda cheese properties	Means square		Mult	iple c	ompa	rison		Means square	Multiple comparison						
	oquaio	T ₁ •	T ₂	T ₃	T ₄	T ₅	T ₆		0	15	30	60	90		
Moisture (%)	4.34*	Α	Е	D	С	D	В	61.82*	Α	В	С	D	Е		
Fat (%)	0.45*	Α	В	С	D	С	D	13.50*	Ε	D	С	В	Α		
Total nitrogen (%)	9.18*	D	С	вс	Α	D	D	0.70*	Ε	D	С	В	Α		
Acidity (%)	0.06*	Α	С	вс	В	AB	Α	1.71*	Ε	D	С	В	Α		
pH values	0.07*	В	Α	Α	AB	В	В	0.90*	Α	В	С	D	Е		
Soluble nitrogen (%)	0.03*	Α	D	В	Α	С	В	0.27*	E	D	С	В	Α		
Soluble tyrosine (mg/100g)	746.18*	Α	D	В	Α	С	В	2312.819*	Ε	D	С	В	Α		
Soluble treptophan (mg/100g)	15.015*	Α	С	В	Α	С	В	2078.532*	E	D	С	В	Α		
TVFA (ml 0.1 N NaOH/100 g)	43.12*	Α	D	В	Α	С	В	1260.78*	Ε	D	С	В	Α		
Organoleptic properties:															
Flavour (60)	71.35*	Α	С	В	Α	В	С	962.26*	_	D	С	В	Α		
Body & texture (30)	31.90*	Α	С	В	Α	С	В	165.22*	-	D	С	В	Α		
Appearance (10)	5.15*	Α	С	В	Α	С	В	14.78*	_	D	С	В	Α		
Total score (100)	270.28*	Α	С	В	Α	С	В	2226.22*	<u> </u>	D	С	В	Α		

^{*} see Table (1).

* Significant at 0.05 level.

For each different letters (the same row) means the multiple comparison are different from each others letter A is the highest followed by B, C... etc.