

EFFECT OF FAT REPLACERS ON LOW FAT GOUDA CHEESE QUALITY

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ABSTRACT

Six batches of Gouda cheese were made to study the effect of using Simplesse[®]100 (as protein based fat replacer) and Novagel[®] (as carbohydrate based fat replacer) on the quality of low fat Gouda cheese. One of these batches was made from milk standardized to 3.0% fat (control C1). The other five batches were made from milk standardized to 2.0% fat, one of them was served as control (C2), and two were made with adding Simplesse[®] 100 at the rate of 0.5 and 1.0%, respectively, whereas the other two batches were made with adding Novagel[®] at the same mentioned previously, individually. Cheese treatments made by adding fat replacers characterized with a pronounced increase in moisture content and titratable acidity. Cheese treatments made by adding Novagel[®] (T3 and T4) were of higher moisture content and acidity than those made by adding Simplesse[®] (T1 and T2). On the other hand, cheese treatments made by adding Simplesse[®] characterized with higher ripening indices (soluble nitrogen, soluble tyrosine, soluble tryptophan and total volatile fatty acids) and were more acceptable than the corresponding cheese treatments being made by adding the addition of Novagel[®]. Fat, total nitrogen contents, titratable acidity, values of ripening indices and scores of organoleptic properties of all cheese treatments increased significantly ($P \leq 0.05$) throughout the ripening period, while moisture content decreased.

Keywords: Low fat Gouda cheese, fat replacers, Simplesse[®] 100, Novagel[®].

INTRODUCTION

Gouda is a semi-hard cheese that forms part of the continental cheese segment (McSweeney, 2004). The manufacture of cheese is a form of preservation of a highly perishable commodity. Approximately a third of milk produced worldwide is used in cheese production (Farkye, 2004). As flavour is one of the most important selling attributes of Gouda cheese it has received much attention (Ayad *et al.*, 2002). Six varieties of Gouda cheese are manufactured for three months (Vanrusselt, 1992). Consumption of this cheese in Egypt has been increased in the recent years and it has been manufactured in some Egyptian dairy factories.

Lipids play vital functional and sensory roles in food products. They carry, enhance and release the flavours of other ingredient (Huyghebaert *et al.*, 1996). 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 Huyghebaert *et al.*, 1996). 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 (Akoh, 1998). The most important problems facing the manufacture of low-fat cheese are the lack of the typical flavour and proper body and texture (Anderson and Mistry, 1994). Using fat replacers to replace fat in cheese while keeping the same functional and organoleptic properties as fat has attracted great attention in past few years. Many commercial fat replacers are available for use in foods, and they are classified as fat-based food replacer, protein-based fat replacer and carbohydrate-based fat replacer (Huyghebaert *et al.*, 1996).

The objectives of this study were to investigate the effects of two types of fat replacers, i.e. Simplese[®] 100 and Novagel[®], in different concentrations on the quality of low fat Gouda cheese and to monitor changes in cheese quality during the ripening period.

MATERIALS AND METHODS

Bacterial strain:

Multiple mixed strains culture containing *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *diacetylactis* were used. The cultures were obtained from Chr. Hansen's laboratory (Horsholom, Denmark) and used as a starter. It was activated by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Cheese making:

Six batches of low fat Gouda cheese were made using bulk fresh cow's milk obtained from the herd of Tokh Tanbasha farm, Minufiya University, Egypt. Control cheese was made from milk standardized to 3.0% fat, while other cheese treatments were made from milk standardized to 2.0% fat, one of these batches was served as control (C1), another two batches were made with adding Simplese[®] 100 (Nutrasweet Kelco Co., Deerfield, IL, USA) at the rate of 0.5 and 1.0% respectively, while the other two treatments were made with adding Novagel[®] RCN 15 (FMC Biopolymer, Philadelphia, PA, USA) at the rates 0.5 and 1.0%, respectively. Gouda cheese was manufactured as described by Scott *et al.* (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 of milk, 0.02% in the same order. The milk was inoculated with 1.0% commercial starter culture, and thoroughly mixed with the milk. When the acidity of milk reached 0.19-0.2%, rennet powder was added at the rate of 3.0 gm / 100 kg milk, the curd became firm enough almost within 25-30 min. It was then cut into 0.5 to 1.5 cm cubes using the American knives for 10-15 min. with stirring curd to float in the 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 blocks were then dumped into 20% salt brine (NaCl) at 15°C for 48 hr. After salting, the green cheeses were placed for two days in ripening room for drying. The cheese

was then carefully coated with plastic coat. The resultant cheese was 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.

Chemical analysis:

Cheese samples were analyzed for moisture, fat, total and soluble nitrogen contents and titratable acidity according to A.O.A.C. (1990). Soluble tyrosine and soluble tryptophan contents of cheese were determined according to the method of Vakaleris and Price (1959), while total volatile fatty acids was determined by the method described by Kosikowski (1986).

Organoleptic properties:

Cheese samples were scored for flavour, body and texture and appearance by ten panelists according to score sheet described by El-Etriby *et al.* (1998).

Statistical analysis:

Factorial design was used to analyse all the data and Newman Keuls test was followed to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. Significant differences were calculated at $p \leq 0.05$.

RESULTS AND DISCUSSION

It is clear from Table (1) that moisture content of low fat Gouda cheese treatments increased significantly ($p \leq 0.05$) by replacing milk fat with either Simplese® or Novagel®, and this increase was proportional to the rate of replacement. These results agree with the results of El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) on Edam cheese; Kebary *et al.* (2009) on Domiati cheese. This results might be attributed to the higher water holding capacity of carbohydrate and proteins than that of milk fat. Cheese treatments being made by adding Novagel® contained higher moisture content than those of corresponding cheese treatments made by adding Simplese®, which means that Novagel® was more effective to increase the moisture content than Simplese® (Tables 1, 6). These results agree with the results of Kebary *et al.* (2009) on Domiti cheese. On the other hand, moisture content of all cheese treatments decreased significantly ($p \leq 0.05$) as ripening period progressed (Tables 1, 6). Moisture content decreased rapidly during the first 30 days of ripening period, then decreased gradually as a ripening period progressed up to the end of ripening period. These results agree with the results of Verachia (2005) on Gouda cheese, Degheidi (1996); El-Shibiny *et al.* (1998); El-Sheikh *et al.* (1999); El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) on Edam cheese; Hammam (2005); Abdallah *et al.* (2006); Taha *et al.* (2007); Abd Alla *et al.* (2008) and Mehanna *et al.* (2009) on Ras cheese. These results could be due to the ability of fat to hinder the syneresis of whey from the curd (Marshall, 1982; Van Dijk and Walstra, 1986 and Mahmoud, 1995).

Fat content of low fat Gouda cheese decreased significantly ($P \leq 0.05$) as the fat content of milk reduced (Tables 1, 6). The type and the concentration of fat replacers did not affect ($p > 0.05$) significantly the fat

content of the resultant cheese. These results agree with those of Kebary *et al.* (2006) on Domiati cheese. On the other hand, fat content of all cheese treatments increased during ripening period (Tables 1, 6). Fat content of cheese treatments increased markedly during the first 30 days, then increased gradually up to the end of ripening period (Tables 2 and 7). These results are in agreement with those reported by Khader *et al.* (1995); Kebary *et al.* (1996); Abdalla *et al.* (2006); Taha *et al.* (2007); Abd Alla *et al.* (2008) and Mehanna *et al.* (2009) on Ras cheese. This increase in fat content could be attributed to the reduction of moisture content throughout the ripening period (Kebary *et al.*, 2006).

Table (1): Changes in moisture and fat contents during ripening of Gouda cheese.

Cheese Treatments	Moisture content (%)					Fat content (%)				
	Ripening period (days)					Ripening period (days)				
	0	15	30	60	90	0	15	30	60	90
C1*	43.51	41.30	40.00	39.19	38.81	28.2	28.9	29.4	30.0	30.2
C2	40.60	38.82	37.63	36.87	36.09	19.6	20.2	21.0	21.4	21.6
T1	42.12	40.04	39.13	38.43	37.98	19.5	20.1	21.1	21.3	21.6
T2	43.10	40.98	39.67	38.92	38.40	19.4	20.0	21.1	21.2	21.4
T3	43.00	40.73	39.60	38.75	38.23	19.4	20.1	21.0	21.3	21.5
T4	44.21	42.37	41.75	40.57	39.82	19.3	19.8	20.6	20.9	21.3

*C1 : Cheese made from 3% fat milk.

C2: Cheese made from 2% fat milk.

T1: Cheese made from 2% fat milk with adding 0.5% Simplese® 100

T2: Cheese made from 2% fat milk with adding 1.0% Simplese® 100

T3: Cheese made from 2% fat milk with adding 0.5% Novagel®

T4: Cheese made from 2% fat milk with adding 1.0% Novagel®

Total nitrogen content of all Gouda cheese treatments increased significantly ($P \leq 0.05$) as ripening period progressed (Tables 2 and 6). This increase might be due to the loss of moisture content. Total nitrogen content increased markedly during the first 60 days, while increased slightly at the end of ripening period. Similar trends were obtained by El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) in Edam cheese. Reduction of fat content of cheese milk resulted in a pronounced increase in the total nitrogen (Banks *et al.*, 1989). Total nitrogen content of cheese made by adding Simplese® increased significantly, this increase was proportional to the amount added from Simplese®. On the other hand, cheese treatments those made by adding Novagel were not significantly different from each other, which means that adding Novagel did not affect significantly ($P > 0.05$) the total nitrogen content of the resultant cheeses. These results are in agreement with those reported in low fat Ras cheese by Badawi (1998); Hussein (2000) and El-Sonbaty *et al.* (2002); Kebary *et al.* (2002) in Edam cheese.

Titrateable acidity of all Gouda cheese treatments increased significantly ($p \leq 0.05$) during ripening period (Tables 2, 6). It increased sharply during the first 30 days, followed by gradual increase up to the end of ripening period. These results are in agreement with those of Degheidi (1996); El-Sheikh *et al.* (1999); El-Sonbaty *et al.* (2002), Kebary *et al.* (2002) in Edam cheese and Hammam (2005), Abdalla *et al.* (2006), Taha *et al.* (2007), Abd Alla *et al.* (2008) and Mehanna *et al.* (2009) in Ras cheese. The

gradual increase in titratable acidity in all cheese treatments might be due to the consumption of lactose content by the lactic acid bacteria, and production of acid. Titratable acidity of cheese increased by increasing the concentration of the added fat replacers. Cheeses made with addition of Novagel® had higher acidity than corresponding cheeses made with Simplese® (Tables 2, 6). These results might be due to the higher moisture content (higher water activity), which enhances the growth of cheese microflora, and subsequently developing acidity. Similar results were reported in low fat Ras cheese by Khader *et al.* (1995); Badawi (1998) and Hussein (2000).

Table (2): Changes in total nitrogen content and titratable acidity (%) during ripening of Gouda cheese.

Cheese Treatments	Total nitrogen content (%)					Titratable acidity (%)				
	Ripening period (days)					Ripening period (days)				
	0	15	30	60	90	0	15	30	60	90
C1*	3.20	3.28	3.31	3.62	3.71	0.97	1.29	1.45	1.77	1.98
C2	3.73	3.81	3.94	4.14	4.23	0.86	1.15	1.37	1.69	1.87
T1	3.91	4.05	4.26	4.37	4.45	0.89	1.22	1.41	1.73	1.91
T2	4.24	4.81	4.42	4.67	4.81	0.95	1.26	1.45	1.75	1.96
T3	3.72	3.80	3.94	4.13	4.20	0.91	1.27	1.45	1.81	2.00
T4	3.71	3.78	3.93	4.11	4.20	1.01	1.36	1.53	1.83	2.05

*C1 : Cheese made from 3% fat milk.

C1: Cheese made from 2% fat milk.

T1: Cheese made from 2% fat milk with adding 0.5% Simplese® 100

T2: Cheese made from 2% fat milk with adding 1.0% Simplese® 100

T3: Cheese made from 2% fat milk with adding 0.5% Novagel®

T4: Cheese made from 2% fat milk with adding 1.0% Novagel®

Degradation of milk proteins plays an important role in the formation of the proper flavour, body and texture of cheese. Proteolysis indices (water soluble nitrogen, soluble tyrosine and soluble tryptophan) of low fat Gouda cheese followed almost similar trends (Tables 3, 4 and 6). Values of soluble nitrogen, soluble tyrosine and soluble tryptophan of all cheese treatments increased gradually ($p \leq 0.05$) throughout the ripening period.

Table (3): Changes in soluble nitrogen (SN) % and soluble tyrosine during ripening of Gouda cheese.

Cheese Treatments	S.N (%)					Soluble tyrosine (mg / 100 g cheese)				
	Ripening period (days)					Ripening period (days)				
	0	15	30	60	90	0	15	30	60	90
C1*	0.38	0.46	0.51	0.70	0.79	46.4	145.2	211.0	296.3	338.1
C2	0.36	0.45	0.49	0.68	0.71	44.5	123.1	188.2	272.4	312.0
T1	0.35	0.43	0.47	0.66	0.70	45.1	138.1	196.3	280.2	329.3
T2	0.36	0.45	0.49	0.67	0.71	46.2	144.2	207.5	291.3	335.6
T3	0.34	0.42	0.46	0.64	0.68	44.6	135.0	193.1	277.0	325.4
T4	0.35	0.43	0.47	0.65	0.69	45.4	140.1	205.4	284.4	331.2

*C1 : Cheese made from 3% fat milk.

C1: Cheese made from 2% fat milk.

T1: Cheese made from 2% fat milk with adding 0.5% Simplese® 100

T2: Cheese made from 2% fat milk with adding 1.0% Simplese® 100

T3: Cheese made from 2% fat milk with adding 0.5% Novagel®

T4: Cheese made from 2% fat milk with adding 1.0% Novagel®

Table (4): Changes in soluble tryptophan and total volatile fatty acids (TVFA) during ripening of Gouda cheese.

Cheese Treatments	Soluble tryptophan (mg / 100 g cheese)					TVFA (ml 0.1N NaOH / 100 g cheese)				
	Ripening period (days)					Ripening period (days)				
	0	15	30	60	90	0	15	30	60	90
C*	53.2	61.7	67.6	77.0	82.1	9.4	14.8	16.7	26.1	33.6
C1	52.8	59.4	65.3	75.2	79.3	5.9	10.0	13.1	20.8	25.4
T1	53.7	62.5	70.2	75.6	80.2	6.8	11.6	14.7	23.7	26.3
T2	53.9	63.0	69.7	77.2	81.0	7.5	11.8	15.2	24.9	29.1
T3	53.1	61.2	67.6	74.3	78.1	6.0	10.0	13.4	21.0	25.5
T4	53.2	62.9	69.4	75.5	80.0	6.2	10.3	13.6	21.3	25.8

*C1 : Cheese made from 3% fat milk.

C1: Cheese made from 2% fat milk.

T1: Cheese made from 2% fat milk with adding 0.5% Simplesse® 100

T2: Cheese made from 2% fat milk with adding 1.0% Simplesse® 100

T3: Cheese made from 2% fat milk with adding 0.5% Novagel®

T4: Cheese made from 2% fat milk with adding 1.0% Novagel®

Table (5): Organoleptic properties (scores out of 100) of Gouda cheese during ripening period.

Cheese treatments	Ripening period (days)			
	15	30	60	90
C1*	58	70	89	92
C2	56	61	77	83
T1	58	72	89	91
T2	59	77	90	92
T3	56	62	79	85
T4	57	65	82	87

*C1 : Cheese made from 3% fat milk.

C1: Cheese made from 2% fat milk.

T1: Cheese made from 2% fat milk with adding 0.5% Simplesse® 100

T2: Cheese made from 2% fat milk with adding 1.0% Simplesse® 100

T3: Cheese made from 2% fat milk with adding 0.5% Novagel®

T4: Cheese made from 2% fat milk with adding 1.0% Novagel®

These results are in accordance with those of El-Shibiny *et al.* (1998), El-Sheikh *et al.* (1999), El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) in Edam cheese; El-Batawy *et al.* (1987); Kebary *et al.* (1996); Hussein (2000); Hammam (2005); Abdalla *et al.* (2006); Taha *et al.* (2007); Abd Alla *et al.* (2008) and Mehanna *et al.* (2009) in Ras cheese.

Total volatile fatty acids of all cheese treatments had similar trends being noticed for soluble nitrogen, soluble tyrosine and soluble tryptophan contents. This means that factors either inhibiting or stimulating proteolysis, had the same effect on lipolysis (El-Sonbaty *et al.*, 2002 and Kebary *et al.*, 2002). Total volatile fatty acids of all cheese treatments increased significantly ($p \leq 0.05$) as ripening period proceeded (Tables 4, 6).

These results are in agreement with those reported by El-Shibiny *et al.* (1998); El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) in Edam cheese and Abdalla *et al.* (2006); Taha *et al.* (2007); Abd Alla *et al.* (2008) and Mehanna *et al.* (2009) in Ras cheese; Kebary *et al.* (2006) and Kebary *et al.* (2009) in Domiati cheese. Cheese treatments made from 2% fat milk contained lower total volatile fatty acid contents than those of cheese treatments made from 3% fat milk (control C) (Tables 4, 6), which could be attributed to the lower moisture content (lower water activity) in cheese made from 2% fat milk, which suppress the growth of cheese microflora, especially lipolytic bacteria and lipases activity. Similar results were reported in Ras cheese (Khader *et al.*, 1995 and Hussein, 2000); Kebary *et al.* (2009) in Domiati cheese. Cheeses treatments those made by adding Simplese contained higher TVFA than corresponding cheese treatments made by adding Novagel (Tables 4, 6).

Organoleptic scores of all cheese treatments increased significantly as ripening period progressed (Tables 5, 6). These results are in agreement with the findings of Degheidi (1996); El-Shibiny *et al.* (1998); El-Sheikh *et al.* (1999); El-Sonbaty *et al.* (2002) and Kebary *et al.* (2002) in Edam cheese. Lowering the fat content of cheese milk caused a significant decrease in the scores of organoleptic quality. Control cheese C1 gained higher score than control cheese C2 which made without adding fat replacers. Similar results were reported by Khader *et al.* (1995); Badawi (1998) and Hussein (2000). Adding fat replacers improved significantly ($P \leq 0.05$) the acceptability of cheese. Cheese treatments made from 2% fat milk with 1.0% Simplese[®] and cheese treatment control (C1) gained higher scores than those of cheese treatments made from 2% fat milk with adding Novagel[®] (fat replacer) (Tables 5, 6). These results might be due to the higher water activity of these cheese treatments, which enhances the growth of proteolytic and lipolytic bacteria, proteases and lipases activities and subsequently formation of the proper flavour, body and texture. These results are in accordance with those reported for Ras cheese by Khader *et al.* (1995) and Hussein (2000).

It could be concluded that adding fat replacers to low fat Gouda cheese increased the values of ripening indices (soluble nitrogen, soluble tyrosine, soluble tryptophan and total volatile fatty acids contents) and scores of organoleptic properties. It is possible to make a good quality low fat Gouda cheese from milk containing 2% fat with adding 1.0% Simplese[®].

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تأثير بدائل الدهون على خواص جبن الجودة المنخفض في نسبة الدهون
على حسن السنباطي^١ و أحمد صابر السيسى^٢
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تم تصنيع ٦ معاملات من جبن الجودة المنخفض في نسبة الدهون وذلك باستخدام بدائل الدهون حيث صنعت المعاملة الأولى من لبن يحتوى على ٣% دهن ككترول (C1) بدون اضافة بدائل الدهون، وصنعت خمس معاملات أخرى من لبن يحتوى على ٢% دهن إحداهما إستخدمت ككترول (C2) بدون اضافة بدائل الدهون، وصنعت معاملتان باستخدام Simplese[®] وهو بديل دهن ذو أصل بروتيني (T1) بنسبة ٠.٥%، (T2) بنسبة ١.٠% ، كما تم تصنيع معاملتان باستخدام Novagel[®] وهو بديل دهن ذو أصل كربوهيدراتي (T3) ، (T4) بنفس النسب السابقة على التوالي، ولقد استخدمت هذه البدائل بمفردها ، وتم دراسة تأثير اضافة هذه البدائل على صفات الجبن الناتج، ولقد أوضحت النتائج المتحصل عليها بعد تحليلها إحصائياً على مايلي:

١- أدى إستخدام بدائل الدهون إلى زيادة نسبة الرطوبة والحموضة في الجبن الناتج وكانت هذه الزيادة أعلى في المعاملات التي إستخدم فيها بديل الدهن Novagel[®] عن مثيلاتها تلك المستخدم فيها Simplese[®].

٢- احتوت العينات التي صنعت بواسطة Simplese[®] على نسب أعلى من النيتروجين الكلى وقيم دلائل التسوية (النيتروجين الذائب في الماء، التيروسين والتربتوفان الذائبان والأحماض الدهنية الطيارة) وكذلك درجات التحكيم عن تلك المصنعة بواسطة Novagel[®].

٣-ازدادت نسب الدهن والنيتروجين الكلى والحموضة وقيم دلائل التسوية (النيتروجين الذائب، التيروسين والتربتوفان الذائبان، الأحماض الدهنية الكلية الطيارة) وكذلك درجات التحكيم أثناء فترة التسوية بينما انخفضت نسبة الرطوبة لكل معاملات الجبن.

٤- كانت أكثر الجبن قبولاً هي تلك المصنعة من لبن يحتوى على ٣% دهن (C1) وكذلك المصنعة من لبن ٢% والمضاف إليها ١.٠% Simplese[®] (T3) بدرجة أفضل من تلك المضاف لها Novagel[®] أو الكترول ٢% دهن (C2).

الخلاصة: يمكن تصنيع جبن جودة منخفض الدهن ذو صفات عالية وذلك من لبن ٢% دهن والمضاف إليه ١.٠% Simplese[®]

قام بتحكيم البحث

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Table (6): Statistical analysis of low fat Gouda cheese properties.

Cheese properties	Effect of cheese treatments							Effect of ripening period (days)					
	Mean squares	Multiple comparisons \diamond						Mean squares	Multiple comparisons \diamond				
		C1*	C2	T1	T2	T3	T4		0	15	30	60	90
Moisture (%)	19.616*	AB	D	C	B	C	A	118.728*	A	B	C	D	E
Fat (%)	63.514*	A	B	B	B	B	B	7.688*	E	D	C	B	A
Total nitrogen (%)	5.980*	D	C	B	A	C	C	5.002*	E	D	C	B	A
Titrateable acidity (%)	0.047*	F	E	D	C	B	A	2.985*	E	D	C	B	A
Soluble nitrogen (%)	0.007*	A	B	BC	BC	C	C	0.067*	E	D	C	B	A
Soluble tyrosine (mg/100 g)	746.186*	A	F	D	B	E	C	2309.819*	E	D	C	B	A
Soluble tryptophan (mg/100 g)	15.015*	A	C	B	B	C	D	2078.539*	E	D	C	B	A
Total volatile fatty acids (ml. 0.1N NaOH/100g cheese).	37.903*	A	E	C	B	E	D	1534.065*	E	D	C	B	A
Organoleptic properties (Total score)	208.400*	A	E	B	A	D	C	3735.500*		D	C	B	A

• See Table (1).

* Significant at 0.05 level.

\diamond 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, Cetc.