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Effect of Fat Replacer or Transglutaminase on the Quality of Low-Fat Gouda-Like Cheese

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Abstract: Eight batches of Gouda-like cheese were made to study the effect of using various concentrations of Simplesse®-100 or Transglutaminase enzyme on the quality of the resultant cheese. One of these batches was made from cow´s milk, 3% fat, (Control, C_1). The other seven batches were made from milk standardized to 1.5% fat, one of them was served as (control, C_2) and three were made with adding Simplesse® -100 at the rate of 0.2, 0.4 and 0.6 g/1 Liter of milk, whereas the other three batches were made with adding Transglutaminase (TGase) at the rate of 0.3, 0.5 and 0.8 g/1 Liter, in order. Results revealed that decreasing the fat content of cheese had an obvious effect on the chemical composition of the resultant cheese, where it increased the moisture, protein contents, pH values and decreased cheese yield, acidity, cheese ripening indices and total volatile fatty acids (TVFA) compared with full-fat cheese (C_1), when fresh and along the ripening period. Total viable, proteolytic and lipolytic bacterial counts were decreased, also, by decreasing the fat content of cheese. A reverse relationship was found between cheese texture profile and its fat content; this means that it increased as the fat content of cheese decreased. Addition of fat replacer (FR) or TGase increased the moisture content, protein, salt/DM, cheese yield, acidity, lipolysis and proteolysis were increased up to1 month of ripening then decreased thereafter with TGase whereas slight decrease in fat/DM and pH values of treated cheese than low-fat cheese free from these additives (C2) during ripening. Furthermore, data showed that all the free amino acids increased by using of FR compared with TGase during ripening. FR or TGase increased also, the counts of viable, proteolytic and lipolytic bacteria compared with cheese free from these additives (C_2), along the ripening period. Data, moreover, showed that FR or TGase improved the texture profile and organoleptic properties of low-fat cheese, when fresh and during ripening, especially its body and texture.

Key words: Cow´s milk · Simplesse®-100 · TGase · Chemical composition · Yield

INTRODUCTION

Gouda cheese is one of the most popular semi- hard cheeses consumed in Egypt. Gouda cheese is a Semi-hard cheese originated in Holland, had a wheel shaped, sized from 8 – 45 pounds, compact with firm and flexible texture, low round to oval gas holes scattered throughout the cheese, color varies from ivory white to yellow, flavour became nutty and sweet, not sour at the end of the ripening period [1].

Increased awareness of people on fitness and healthy lifestyle has led to an increased demand for low-calorie foods in particular for low and reduced fat cheese [2]. Since cheese is one of the most widely consumed food products, nutritional sciences and food industry play a prominent role in the development of healthier food ingredients. Fat content is responsible for many desirable functional, textural and sensory properties in cheese and its decrease alters physical and flavour properties and lowers cheese quality. Low and reduced fat cheeses have certain

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disadvantages stipulated by reduction in proportion of moisture in non-fat substances (NFS), level of proteolysis activity, amount of free oil and increased proportion of intact casein [3].

Various techniques used to improve the texture of low and reduced fat cheeses include process modifications, use of special starter cultures, fat replacer and enzymes e.g. Transglutaminase. In recent years, Transglutaminase (TGase) has been used by many researchers. It is naturally present in most animal tissues and body fluids and can from both inter and intra-molecular iso-peptide bonds between many proteins by cross-linking of the amino acid reduces protein bond glutamic and lysine [4]. Many food proteins are good substrates to TGase, especially casein, which is the principle protein in milk. The introduction of additional covalent cross- linking by TGase represents a promising tool to improve the functional properties for casein- based dairy products [5].

Fat replacers are of low calorie and providing some of functional properties of fat. They are divided into 2 group: fat substitutes and fat mimetics, fat substitutes are non polar, fat soluble compounds, providing sensory and functional properties of fats to food, while fat mimetics are polar, water soluble substances used to partially replace some of sensory and functional characteristics of fat. Fat mimetics bind water and thereby improving texture and yield of low-fat cheese [6]. According to the composition, fat mimetics consist mainly of micro particulate protein or carbohydrate – based materials [7]. Carbohydrate and protein – based fat replacers are widely used in food processing to improve functional properties that are adversely affected by lowering of fat levels.

The aim of this investigation was to study the effect of the addition of various concentration of Simplesse®-100 or Transglutaminase on the quality of low fat Gouda- like cheese.

MATERIALS AND METHODS

Materials

Chemicals: Used in this study were of analytical grade supplied by BDH, Sigma and Prolabo chemical companies.

Milk: Fresh cow's milk was obtained from the herd of Sides experimental station, Animal Production Research Institute.

Fat Replacer: Simplesse® -100, a protein- based fat replacer was obtained from CP Klco, Chicago, 11, USA (chemical composition was 40 % moisture, 7 % ash and 35.9 % carbohydrate).

Transglutaminase: A Ca-independent microbial transglutaminase (ACTIVA MP, with activity of 100 units /g powder) was obtained from Ajinomoto Europe Sales Gmbh, Hamburg, Germany .

Starter: Lactococcus lactis subsp. Lactis was obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Egypt.

Salt: From El – Naser company, Alexandria, Egypt.

Rennet: Hansen‘s powder rennet obtained from Chr- Hansen´s Laboratories, Copenhagen, Denmark.

Ready – Made Media: Tryptone Glucose Extract Agar medium, Code CM127 (TEGA); Nutrient Agar medium, were bought from Oxoid Division of Oxoid LTD, London.

Cheese Making: Eight batches of Gouda- like cheese were made as follows;

- Full-fat cheese was made from cow´s milk (3±0.1% fat) and served as Control (1).
- Low-fat cheese made from cow's milk (1.5±0.1%fat) without any addition Control (2).
- Low-fat cheese made from cow´s milk (1.5±0.1% fat) +0.2, 0.4 and 0.6 g Simplesse – 100/L milk (treatments S_1 .S_2 and S_3, respectively).
- Low-fat cheese made from cow´s milk (1.5±0.1% fat) +0.3, 0.5 and 0.8 g transglutaminase /L milk (Treatments G1, G2 and G3 in order).

Milks containing transglutaminase were incubated at 40°C/60 min. [8].

Milk of all treatments was pasteurized at 73°C/20 sec., cooled to 32°C and Cacl2.02%) was added . Gouda - milk cheese was made according to Scott [9]. Cheeses were ripened at 10 - 12°C for 3 month at relative humidity of 85%. Samples were taken periodically at 0, 1, 2 and 3 month for analysis – Three replicates of this study were done.

Analytical Methods

Chemical Analysis: pH of milk and cheese samples was measured using a combined calomel glass electrode pH-meter model HANNA instruments H 1848. Titratable acidity, moisture, Fat and total protein were determined according to Ling [10] water soluble nitrogen [11] and non protein nitrogen [12]. Phosphotungstic acid (5%) and soluble nitrogen (PTA-SN) were determined according to Jarrett *et al.* [13]. Total volatile fatty acids [14] were expressed as mL of 0.1 N Noah/100g cheese. Free amino acids were measured according to the method of Block *et al*. [15] by using Amino Acid Analyzer, A.A.A. 400.

Microbiological Analysis: Total bacterial count was determined by the plate count method [16], Proteolytic bacterial count [17] and Lipolytic bacterial count [18].

Texture Profile: Samples texture measurements were carried out with universal testing machine (Cometech., B type, Taiwan) Provided with software. Back extrusion cell with 25 mm diameter compression disc was used. Two cycles were applied, at a constant crosshead velocity of 1 mm/s, to 25% of sample depth and then returned. From the resulting force–t curve, the values for texture attributes, i.e. Firmness (N), chewiness (Nxmm), Gumminess (N), adhesiveness (N.S), cohesiveness and springiness were calculated from the TPA graphic. Bourne [19] method was applied.

Organoleptic Properties: Cheese samples were scored according to Pappas *et al*. [20] for appearance (10 points), body & texture (40 points) and flavour (50 points) by score panel of staff members of Sidis Experimental Station and Dairy Technology Department, Animal Production Research Institute, Egypt.

RESULTS AND DISCUSSION

Physiochemical Properties

Fat Reduction: Decreasing the fat content of cheese milk by approximately 50% resulted in increasing the moisture content, protein, salt /DM, pH values and decreasing cheese yield and acidity%, cheese ripening indices (WSN/TN, NPN/TN, PTA-SN /TN and FAA) and total volatile fatty acids (TVFA) in the resultant low-fat Gouda-like cheese, compared to full-fat cheese (C_1), when fresh and along the ripening period (Tables 1, 2 and 3). The main reason for that was the variation of fat content of full and low-fat cheeses. The same results were found by Sahan *et al*. [21]. Decreasing acidity in low-fat cheese (C_2) was confirmed by Fenelon and Guinee [22], whereas TVFA was demonstrated by Khalil [23], Shehata *et al*. [24] and Ali [25]. Moisture content, (fat, protein, salt)/DM, cheese yield, pH values, acidity, SN/TN and TVFA recorded were 41.68, 30.52, 57.42, 5.09, 8.83, 5.20, 1.80, 9.36 and 14.3% for low-fat cheese (C_2) at the end of the ripening period, in order. The corresponding values of full-fat cheese (C_1) were 38.80, 50.33, 39.17, 5.05, 11.48, 5.05, 1.86, 12.25and 19.0%, respectively.

Fat Replacer and Transglutaminase: Addition of fat replacer (FR) to cheese milk had an apparent effect on the chemical composition of the resultant cheese (S_1, S_2 and S_3 treatments), so it increased the moisture content, (protein, salt)/DM, acidity, cheese yield %, proteolysis and lipolysis and slight decreased fat/DM, pH values and lost protein in whey% of treated cheese than low-fat cheese (C_2) along the ripening period (Tables 1, 2 and 3). Mc Mahon *et al*. [26] found that low-fat cheese made with the whey-based fat replacers had 2.2 to 2.3% higher moisture contents than the control cheese. They added that Simplesse was observed as micro particles embedded with the casein matrix, which may allow greater moisture retention. The increased moisture content of low-fat cheese made by using fat replacers suggested that curd syneresis was retarded during cheese making, which can occur as a result of water being bound directly to the fat replacers or fat replacers may interfere with shrinkage of the casein matrix, thus lowering the driving force involved in expelling water from the curd particles.

Several applications supported the former results; Lacey& Gorry [27] noticed an increase in the titratable acidity when fat replacer was used. Low-fat Cheddar cheese made with protein-based fat replacer contained significantly more protein than the other low-fat Cheddar cheese [28]. Moreover, proteolysis in fat replacer Cheddar cheese [29] and Kasher cheese were higher increased the liberation of TVFA in the resultant cheese than control one and low-fat cheese. On the other side, Han and Spradin [30] found that the treatments with MTGase had enhanced the efficiency of protein retaining and improving the yield of cottage cheese with saving all sensory attributes of the product. Also, Pierro *et al.* [31]

found that the addition of TGase to cheese milk increased water content, cheese yield and the proteolysis of cross-linking cheese after 35 days of ripening markedly reduced. These results are in agreement with those found by Ya-nan *et al*. [32] in Cheddar cheese indicated that in the TG-treated samples, the degree of proteolysis was higher during the first 15 days and lower at later ripening times than the corresponding untreated samples.

Results, also, showed that a direct relationship was noticed between the level of FR used and the former parameters (chemical composition), along the ripening period and TGase behaved a similar trend to FR, during ripening, with the exception of acidity which was slightly lower when compared with FR cheeses (S1, S2 and S3) and with control full-fat cheese (C1). The results also indicated that in the TGase-treated samples, the degree of proteolysis was higher during the first month and lower at later ripening times than the corresponding TG-untreated cheeses. Compared with C2 (TG-untreated), cheeses obtained by treated TGase showed higher W/P and MNFS, which led to more proteolysis initially as discussed above. At later ripening months, TGase catalyzed additional cross-linking of proteins and TG interference with the action of the coagulant enzyme may lead to the slower degradation of the TG-treated cheeses. The availability of low molecular weight peptides needed for the growth and activity of bacteria [33] was decreased due to the raised cross-linking bonds which may explain the slow growth and activity of yoghurt starter.

As the ripening period advanced, acidity%, protein/DM, proteolysis and lipolysis were increased gradually till the end of the ripening period, whereas moisture content was decreased as a result of evaporation. These finding were demonstrated previously by several studies.

The titratable acidity of the resultant cheese from all treatments had increased with the progressive ripening period, when compared with control. These results were supported by Bonisch *et al.* [34] who detected that 88% of crosslinking had taken place in the pH range of 5.7-6.6 and the enzyme was inhibited at pH lower than 4.5. Data for pH recorded in Table (2) showed that the pH values had opposite trend to the TA. It decreased gradually in all treatments up to the end of the ripening period.

Soluble Free Amino Acids: Table (4) shows that the free amino acids contents along the ripening period for all samples were affected by using fat replacer (Simplesse-100) or TGase, this effect can be deduced to the protein degradation and partially to the decrease in the moisture content. These results are confirmed by El-Sonbaty *et al*. [35] in low fat Edam cheese. Fat replacers and TGase produced an increase in amino acids contents after ripening in comparison with the control 2 and also increased with increasing the added ratio of fat replacer (Simplesse-100) or TGase. Using Simplesse-100 gave higher contents of amino acids as compared with using TGase. These results are in agreement with the finding of El-Sissi [36] for buffaloes' Kashkaval cheese.

Microbiological Examination: Data in Table (5) indicated that the viable, proteolytic and lipolytic bacterial counts of low-fat cheese (C_2) were lower than that found in full-fat cheese (C_1), when fresh and throughout the ripening period. This trend of decreased in the bacterial counts as the fat content of cheese decreased were approved previously by Fenelon *et al*. [37].

Addition of FR or TGase to cheese milk increased the former bacterial counts compared with the corresponding ones of low-fat cheese free from these additives (C_2 treatment), during ripening. Full-fat cheese (C_1 treatment) recorded the highest counts than the rest treatments, in spit of increasing the level or concentration of FR or TGase added. A proportional relationship was noticed between the bacterial counts and the percent of FR or TGase added. Total bacterial counts of all treatments increased gradually up to the first month of ripening then decreased thereafter till the end of the ripening period. Banwart [38] referred t to the reduction of water activity because of the higher water-holding capacity and subsequently inhibiting the bacterial growth. Moreover, Ozer *et al*. [39] demonstrated that addition of TGase to yoghurt decreased the rate of growth of starter culture, owing to the cross-linking of protein molecules occurred. Fox *et al*. [40] added, furthermore, that starters provide the significant contribution to the microbial mass in young curd, typically attaining densities of $>10^8$ CFU /g within one day of manufacture. This biomass represents considerable bio catalytic potential for cheese ripening reactions. During cheese ripening many starters loose viability and release their intracellular enzymes due to autolysis.

Proteolytic & lipolytic bacterial counts were increased gradually till the end of the ripening period.

This trend of increase was found to be opposite to that happened in total viable counts. These results are in agreement with that found by Effat *et al*. [41] and Kebary *et al*. [42]. Variations noticed among the counts of all treatments were probably due to the primary environmental factors controlling growth of microorganisms in cheese include water and salt contents, pH value, presence of organic acids and nitrate, redox potential and ripening temperature [43].

G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Textural Properties Cheese Parameters: Rheology of materials e.g., cheese, may be defined simply as the study of their deformation and flow when subjected to a stress or strain. In terms, the behavior of the cheese when subject to these stresses or strains is referred to be by descriptive terms such as hardness, firmness, springiness, crumbliness or adhesiveness. Owing to the variations in the manufacturing conditions and composition, different cheese varieties exhibit a wide range of rheological behavior, ranging from the viscous behavior of soft cheese to the elastic behavior of hard cheese at low strain. The rheological of cheese is a function of its composition, microstructure, the physicochemical state of its components and the macrostructure.

Results in Table (6) indicated that the decrease of fat percent in low-fat cheese affected greatly the texture profile of the resultant cheese. A reverse relationship was found between firmness, cohesiveness, springiness, gumminess & chewiness and the percent of fat in cheese, when fresh and along the ripening period. This means that these parameters were increased as the fat content decreased. Kahyaoglu *et al*. [44] stated that reducing fat content from 50.4% w/w, (full fat), to 13.5% (Low-fat) in the manufacture of Gaziantep cheese (pasta philata cheese) increased textural parameters (hardness, gumminess, cohesiveness and springiness) in the resultant cheese. Texture parameters were correlated with each other except cohesiveness. Fat in dry matter correlated only with hardness and springiness. These results were supported by Fox *et al*. [45] and Madadlou *et al*. [46]

Addition of FR improved the texture profile of the resultant cheese compared with low-fat cheese (C_2), along the ripening period. In general, reduction of cheese fat caused a harder texture in cheese while use of fat replacer caused a decrease in the hardness as in various types of cheese such as Kashar cheese, Cheddar cheese. A linear relationship was found between the concentration of FR used and the texture profile of the cheese.

TGase improved, also, the texture profile of cheese but its effect was slightly lower than FR (Simplesse-100). Bonish *et al*. [47] said that the cross-linking of protein occurred by TGase led to decrease the firmness in the resultant cheese. Li- Chan [48] added, moreover, that the use of TGase in the production of low-fat cheese increased the water-holding capacity, emulsification, foaming, viscosity and solubility in the product. Fredirick & Duley [49] and Diskinson [50] confirmed the former results.

Organoleptic Properties: Full-fat cheese (C_1) was found the best treatment "gained 91 score points at the end of the ripening period" compared to the rest treatments either contained or free from additives "FR or TGase", along the ripening period. It was characterized by nutty and sweet flavour, being firm & compact and with springy texture and low numbers of gas holes (Table 7).

On the other hand, low-fat cheese (C_2) "which had the lowest score points (72)" was characterized by flat flavour, more hard texture and had slighter brittle body and crumbling under compression forces.

Addition of FR or TGase improved the organoleptic properties of the resultants cheese "especially the body and texture" as a result of increasing its water-holding capacity and the cross-linking bonds between protein molecules. Ozrenk [5] stated that the introduction of additional covalent cross-linking by TGase represents a promoting tool to improve the functional properties for casein-based dairy products. Gunasekaran and Ak [51] added that presence of additional moisture content by FR can improve functionality of low-fat cheese and the highest percent of moisture in fat replacer treated cheese was the main reason for that.

CONCLUSION

From the foregoing, it can be concluded that the reduction of cheese fat had a reverse effect on the texture properties and the addation of Fr or TGase improved the chemical composition, texture profile and organolyptic properties of low-fat Gouda like cheese when fresh and during ripening, especially its body and texture.

Table 1: Effect of use of Simplesse®-100 or transglutaminase on some physiochemical properties and yield of low-fat Gouda like cheese during 3 months of ripening

	Treatments							
			Simplesse-100 %			Transglutaminase %		
Ripening period (months)	C1	C2	S1	S2	S3	G1	G2	G3
Moisture %								
Fresh	42.75	46.82	47.80	48.52	49.66	46.72	48.16	48.46
1	41.40	44.93	45.37	46.89	47.15	45.19	46.77	46.88
2	40.50	43.77	44.70	45.74	46.58	43.55	45.56	45.92
3	39.80	41.68	42.07	43.56	44.34	41.22	43.18	43.46
	F/DM %	F/DM						
Fresh	50.13	29.90	29.88	29.91	30.19	29.09	29.71	29.10
1	50.24	29.24	29.10	29.56	29.42	28.64	29.12	28.61
2	50.25	29.52	30.31	29.49	29.76	28.87	29.02	28.42
3	50.33	30.52	30.55	30.47	30.18	29.43	29.74	28.48
	P/DM %							
Fresh	38.86	56.83	59.27	60.45	62.26	59.35	61.44	62.98
1	38.91	57.38	59.93	62.12	62.59	60.14	62.28	63.87
2	39.03	57.57	59.34	62.22	63.53	60.44	63.30	64.73
3	39.17	57.42	58.90	60.88	62.32	60.48	63.04	61.91
	S/DM %							
Fresh	3.76	3.91	4.29	4.41	4.55	4.17	4.24	4.23
1	4.33	4.43	4.87	5.12	5.30	4.72	4.83	4.80
2	4.86	4.98	5.36	5.75	5.89	5.26	5.27	5.20
3	5.05	5.09	5.37	5.67	5.77	5.26	5.31	5.27
	Protein in whey (%) and Cheese Yield%							
Protein in whey	0.67	0.71	0.65	0.58	0.49	0.40	0.31	0.26
Yield %	11.48	8.83	9.24	9.55	9.85	9.57	9.82	9.98

C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk)Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Table 2: Effect of use of Simplesse®-100 or Transglutaminase on Titratable acidity (%) and pH values of low-fat Gouda-like cheese during 3 months of ripening

	Treatments							
			Simplesse-100 %			Transglutaminase %		
Ripening period (months)	C1	C2	S1	S2	S3	G1	G2	G3
	Titratable acidity (%)							
Fresh	0.80	0.77	0.83	0.93	0.98	0.71	0.74	0.79
1	0.97	0.95	1.13	1.19	1.24	0.82	0.92	0.96
2	1.29	1.26	1.40	1.49	1.56	1.10	1.15	1.16
3	1.86	1.80	1.92	1.98	2.05	1.47	1.53	1.56
	pH values							
Fresh	5.63	5.80	5.57	5.55	5.54	5.90	5.80	5.63
1	5.56	5.57	5.44	5.42	5.39	5.62	5.60	5.44
2	5.50	5.54	5.45	5.41	5.37	5.51	5.43	5.40
3	5.06	5.10	5.13	5.10	5.04	5.29	5.20	5.24

C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk)Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Table 3: Effect of use of Simplesse®-100 or Transglutaminase on Proteolysis and Lipolysis in low-fat Gouda-like cheese during 3 months of ripening

	Treatments							
			Simplesse-100 %			Transqlutaminase %		
Ripening period (months)	C1	C2	S1	S2	S3	G1	G2	G3
	WSN /TN							
Fresh	8.14	6.42	8.76	9.14	9.27	7.15	7.35	8.26
1	9.73	7.85	9.98	10.22	10.66	8.01	8.22	8.59
2	11.21	8.77	11.85	12.07	12.33	8.23	8.42	8.72
3	12.25	9.36	12.78	13.16	13.85	8.75	8.85	9.10
	NPN / TN							
Fresh	3.57	2.88	4.12	4.36	4.77	3.45	3.45	3.83
1	4.32	3.94	4.88	5.13	5.84	4.11	3.96	4.14
2	5.12	4.17	5.76	5.99	6.18	4.13	4.06	4.16
3	5.69	4.52	6.33	6.54	6.86	4.25	4.33	4.40
	PTA-SN /TN							
Fresh	1.10	0.86	1.28	1.37	1.42	1.23	1.26	1.38
1	1.66	1.43	1.77	1.80	1.93	1.41	1.48	1.62
2	2.74	2.32	2.89	2.96	2.99	2.03	2.09	2.29
3	3.24	2.47	3.44	3.57	3.72	2.23	2.28	2.34
	TVFFA							
Fresh	7.6	6.6	7.5	7.7	7.9	7.3	7.8	7.9
1	12.0	10.9	12.5	12.8	12.9	12.3	12.6	13.7
2	16.2	12.2	17.6	17.9	18.2	15.7	16.5	17.8
3	19.0	14.3	20.3	20.7	21.1	18.5	19.6	20.3

TVFFA: (milliliters of 0.1 N NaoH/ 100g cheese)
C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk)Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Table 4: Effect of use of Simplesse®-100 or Transglutaminase on the Amino Acids contents (mg /100 g cheese) of low-fat Gouda like cheese, after 3 months of ripening

	Treatments							
			Simplesse-100 %			Transqlutaminase %		
Amino Acids %	C1	C2	S1	S2	S3	G1	G2	G3
Thr	0.76	0.58	0.61	0.64	0.69	0.52	0.54	0.55
Ser	0.78	0.61	0.64	0.67	0.70	0.55	0.57	0.58
Glu	5.90	4.77	4.88	4.91	4.97	4.69	4.71	4.72
Pro	5.77	4.93	5.01	5.16	5.23	4.85	4.88	4.90
Tyr	0.83	0.74	0.78	0.80	0.81	0.69	0.71	0.73
His	1.51	1.18	1.22	1.26	1.32	1.13	1.14	1.16
Lys	3.64	3.19	3.23	3.25	3.31	3.12	3.14	3.16
Arg	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Phe	2.24	1.97	2.00	2.16	2.18	1.89	1.92	1.94
Val	1.57	1.34	1.38	1.41	1.46	1.28	1.30	1.32
Ala	0.68	0.57	0.61	0.63	0.65	0.52	0.53	0.55
Asp	0.76	0.55	0.59	0.63	0.68	0.49	0.50	0.52
Met	1.82	1.59	1.64	1.68	1.72	1.53	1.54	1.56
Lle	1.69	1.32	1.37	1.41	1.45	1.26	1.28	1.30
Leu	3.87	3.24	3.28	3.32	3.37	3.17	3.20	3.22
Gly	2.32	2.07	2.15	2.18	2.23	2.00	2.02	2.03
Total	34.14	28.65	29.39	30.11	30.77	27.69	27.98	2.284

C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk)Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Table 5: Effect of Simplesse®-100 or Transglutaminase on the total bacterial, proteolytic and lipolytic bacterial counts of low-fat Gouda like cheese during 3 months of ripening

	Treatments							
			Simplesse-100 %			Transqlutaminase %		
Ripening period (months)	C1	C2	S1	S2	S3	G1	G2	G3
	Total bacterial count (cfu $\times 10^7$/g)							
Fresh	7.4	5.6	6.8	7.1	7.3	6.7	6.9	7.1
1	9.1	7.3	8.6	8.7	8.8	8.4	8.6	8.7
2	7.9	6.2	7.6	7.8	8.0	7.5	7.6	7.8
3	6.0	4.4	5.2	5.5	5.6	5.0	5.2	5.3
	Proteolytic bacterial count (cfu $\times 10^3$/g)							
Fresh	39	31	30	31	34	35	37	38
1	77	52	59	63	65	56	60	64
2	95	63	70	73	73	69	71	72
3	116	84	98	101	107	86	90	93
	Lipolytic bacterial count (cfu $\times 10^3$/g)							
Fresh	24	11.5	16.5	18	20.5	17.5	19.5	21
1	45	22.5	36	38.5	40	39	41	42
2	54	30.5	39	44	43	42.5	43	44
3	61	38	43	47	50	43.5	45	48

C1: (control): Full-fat untreated cheese
C2:(control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with (0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk) Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase

Table 6: Effect of Simplesse®-100 or Transglutaminase on the Textural Properties of low-fat Gouda like cheese, during ripening

	Treatments							
			Simplesse-100 %			Transqlutaminase %		
Ripening period (months)	C1	C2	S1	S2	S3	G1	G2	G3
	Hardness (kg)							
Fresh	8.72	10.94	8.76	9.14	9.27	7.35	8.12	8.26
1	8.98	11.68	9.98	10.22	10.32	8.22	8.44	8.59
2	9.67	12.91	11.85	12.07	12.07	8.63	8.67	8.72
3	9.68	13.43	12.78	13.16	13.16	9.05	9.17	9.23
	Cohesiveness							
Fresh	0.51	0.62	0.57	0.55	0.53	0.55	0.58	0.59
1	0.55	0.73	0.59	0.57	0.56	0.64	0.63	0.61
2	0.62	0.81	0.68	0.66	0.64	0.69	0.68	0.67
3	0.64	0.82	0.73	0.71	0.68	0.75	0.74	0.73
	Springiness							
Fresh	6.7	7.2	6.5	6.7	6.9	6.6	6.8	7.0
1	11.1	11.9	11.5	11.8	12.9	11.7	11.9	13.2
2	15.4	15.5	15.6	16.9	18.2	15.0	16.4	17.8
3	18.8	18.3	18.3	19.1	19.4	18.5	19.6	20.3
	Gumminess (kg)							
Fresh	2.18	3.04	2.61	2.57	2.41	2.47	2.32	2.20
1	2.50	3.31	2.75	2.70	2.60	2.69	2.58	2.47
2	2.82	3.80	2.99	2.87	2.74	2.86	2.73	2.62
3	3.16	4.52	3.67	3.50	3.37	3.41	3.34	3.23
	Chewiness (kg × mm)							
Fresh	17.32	31.03	28.01	26.74	25.12	24.16	22.98	22.53
1	23.65	35.29	30.58	28.23	26.80	28.09	26.75	25.36
2	28.95	43.31	34.63	33.54	28.89	30.21	29.44	27.15
3	34.04	48.27	37.97	35.28	34.95	34.89	32.47	31.19

C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk)Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

Table 7: Effect of Simplesse®-100 or Transglutaminase on the Organoleptic properties of low-fat Gouda- like cheese, during ripening

Treatments	Ripening period (months)	Appearance (10)	Body & texture (40)	Flavour (50)	Total Scores (100)	Remarks
C1(control)	Fresh	8.0	27	34	69.0	Best Nutty and sweet flavour.
	1	8.0	31	37	76.0	Firm,
	2	9.0	34	42	85.0	Compact and Springy body.
	3	9.0	37	46	92.0	
C2(control)	Fresh	7.0	18	28	53.0	Flat flavour. Slightly hard, brittle
	1	7.0	22	30	59.0	and crumble body. Poor texture.
	2	8.0	25	33	66.0	
	3	8.0	27	35	70.0	
S1	Fresh	8.0	22.0	30	60.0	Improved body and texture.
	1	8.0	25.0	33	63.0	Enhanced flavour than low-fat
	2	8.0	28.0	37	73.0	cheese (C_2)
	3	9.0	32.0	39	80.0	
S2	Fresh	8.0	24.0	31	63.0	Slightly better than S1 and control.
	1	8.0	27.0	35	70.0	
	2	9.0	30.0	38	77.0	
	3	9.0	34.0	41	84.0	
S3	Fresh	7.0	22.0	30	59.0	Slightly less than FR cheese (S_1)
	1	8.0	26.0	33	67.0	
	2	8.0	27.0	36	71.0	
	3	9.0	31.0	39	79.0	
G1	Fresh	7.0	22.0	30	58.0	Slightly less than FR cheese (S_1)
	1	8.0	24.0	31	63.0	
	2	8.0	26.0	35	69.0	
	3	9.0	31.0	37	77.0	
G2	Fresh	8.0	23.0	30	61.0	Slightly better than (G_1)
	1	8.0	25.0	33	66.0	
	2	9.0	28.0	36	73.0	
	3	9.0	33.0	38	80.0	
G3	Fresh	8.0	25.0	32	65.0	Better than G1 and G2 and near
	1	8.0	27.0	35	70.0	from S3
	2	9.0	30.0	37	76.0	
	3	9.0	34.0	40	83.0	

C1: (control): Full-fat untreated cheese
C2: (control): Low-fat untreated cheese
S1: Low-fat cheese with (0.2 g /1kg milk) Simplesse-100
S2: Low-fat cheese with(0.4 g /1kg milk) Simplesse-100
S3: Low-fat cheese with (0.6 g /1kg milk) Simplesse-100
G1: Low-fat cheese with (0.3 g /1kg milk) transglutaminase
G2: Low-fat cheese with (0.5 g /1kg milk) transglutaminase
G3: Low-fat cheese with (0.8 g /1kg milk) transglutaminase

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