Journal of Chemistry and Nutritional Biochemistry
https://jcnb.sabapub.com
E ISSN: 2709-5932

2023 Volume 4, Issue 2: 1–14
DOI: https://doi.org/10.48185/jcnb.v4i2.917

Inducing Conjugated Linoleic Acid Concentration in Tallaga-Like Cheese by Using Probiotics, Inulin and Lipolysed Milk Containing Safflower Oil as Precursor on Its Quality Characteristics

Abouelwafa, M. Y.*; Ahmed, H. A. A.; El-Safty, M. S.; Khalil, R. A. M.
Dairy Department, Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt.

Received: 20.10.2023 • Accepted: 29.11.2023 • Published: 01.12.2023 • Final Version: 01.12.2023

Abstract: Tallaga-like cheese was prepared by using some probiotic bacteria strains and lipolyzed milk with Safflower oil as a source of free linolenic acid as well as with or without inulin as prebiotic. The impact of such treatments on cheese composition and quality characteristics was studied during storage at 5°C for 28 days. Mixture of fresh buffalo`s and cow`s milk (1:1) was standardized to 3.60 % fat, pasteurized, cooled to 37°C, salted, mixed well with 0.6% (v/v) lipolyzed milk with Safflower oil, inoculated with probiotic bacteria (*Lb. plantarum*, *Lb. acidophilus*, *Lb. casei* and *B. lactis*) and supplemented with/without 1.5% inulin to induce conjugated linoleic acid (CLA) formation results showed that using inulin with 0.6% safflower oil and probiotic bacteria significantly increased the CLA concentration as compared to using probiotics with lipolysed milk only. Addition of different probiotics with inulin significantly increased the titratable acidity, lactic acid bacteria with higher sensory characteristics and gained higher total acceptability scores.

Keywords: Conjugated linoleic acid, Safflower oil, Probiotic, Tallaga-like cheese, Inulin, Quality characteristics.

1. Introduction

There has been an increasing interest in dairy products containing probiotics with potential health-improving properties [1]. This is quite important for Egyptians since cheese is the greatest part of the dairy market in Egypt. its percentage is approximately 40% of the total market value. Soft cheese grew quickly in 2012, at a rate of 26% in terms of current value and 15% in terms of volume [2]. In 2021, the percentage of the population who consumed cheese in Egypt was about 32% [3]. Cheese is an integral part of the diet consumed by Egyptians.

The most popular type of fresh soft cheese produced in Egypt is Tallaga cheese [4]. Tallaga cheese is cold stored soft cheese, easy to handle and marketing by all socioeconomic classes in Egypt due to its nutritional value, convenience and good taste, clean pleasant creamy low salty taste with a

*Corresponding author: *mohamed_abouelwafa@agr.suez.edu.eg*

spreadable mellow soft body. It is a product closely related to Domiati cheese and mainly ready for consumption within one month of cold storage [4,5,6,8].

Conjugated linoleic acids (CLAs) in their *trans–cis*, *cis–trans*, *cis–cis* and *trans–trans* configurations are conjugated isomers of linoleic acid (9-*cis*, 12-*cis* C18:2). Conjugated linoleic acid (CLA) isomers are reported to have anti-carcinogenic, -atherogenic, -diabetic and lean body mass-enhancing properties. [8, 9] A variety of animal products are good sources of CLA, but plant oils contain much less. However, plant oils are a rich source of LA, which may be isomerized to CLA by intestinal microorganisms in human. Linoleic acid is an important fatty acid in triglycerides composition of some vegetable oils which contained varied LA as 45–68% for sunflower oil or 75–80% for safflower oil [10]. Safflower (*Carthamus tinctorius L.*) oil is colorless, flavorless, and rich in the essential *n*–6 (omega-6) fatty acid; approximately 78% linoleic acid in form *c*9*c*12-linoleate [11]. One of strategies to induce CLA concentration is using vegetable oils rich in monounsaturated fatty acids, especially linoleic acid (i.e., high oleic sunflower and safflower oils) in different dairy foods [12].

Safflower oil has shown many beneficial health effects decreased fat accumulation in rats when compared to beef tallow diet [13]. The presence of conjugated linoleic acid in safflower oil has effectively shown to decrease body weight and adipose tissues as demonstrated in clinical trials [11]. Further safflower oil has been found effective in fat-induced insulin resistance [14]. Currently several applications of safflower oil in the food industry are presented owing to higher mono and poly unsaturated fatty acids. But there is no application of using safflower oil on dairy application as a precursor for inducing CLA in these products.

Inulin is widely used in processed dairy as a bulking agent, fat replacer and thickening agent [15, 16]. Also, using inulin in food can stimulate the growth of different probiotic cultures presented on gut microbiota and enhance effectively the health outcomes of human [17].

Fresh soft cheese seems to be a suitable vehicle for carrying probiotic bacteria and prebiotics as it contains high moisture and relatively low salt contents. Therefore, the major target of this research was applying some strains of probiotic bacteria and lipolyzed milk with safflower oil with/without inulin in making functional Tallaga-like cheese as well as studying the chemical, microbiological and organoleptic properties of the resultant cheese.

2. Materials & Methods

2.1. Materials:

Fresh cow`s and buffalo`s milks were obtained from the public service unit for dairy products, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Probiotic strains (*Lb. plantarum* LpU4, *Lb. acidophilus* 200711A1/ CCFM6, *Lb. casei* CCFM137 and *B. lactis* NFM7) were supplemented by China Industrial Microbiology Culture Collection Center (CICC) and Culture Collection of the Laboratory of lipids Biotechnology, Wuxi, China. Safflower oil was obtained from Agricultural Science and Technology Co., China. Inulin was obtained from Orafti, Beneo Co., England. Skim milk

powder (Nestle) was purchased from the local market. Palatase®20000 L is a microbial lipase enzyme (food grade) derived from *Rhizomucor miehei* was obtained from Novozymes Co., China. Microbial rennet powder was obtained from Hansen's Laboratories, Denmark. Refined salt (NaCl) was obtained from local market. Calcium chloride was obtained from El-Nasr Pharmaceutical Co., Alexandria, Egypt. All Solvents and all other chemicals used were of analytical grade.

2.2. Methods:

2.2.1. Preparation of lipolysed milk with safflower oil:

Lipolysed skim milk with safflower oil was prepared according to the method described by Abd [18]. Skim milk powder was reconstituted (10% total solids), mixed thoroughly with Safflower oil (10% w⁄w) and homogenized stepwise at 60°C using high speed homogenizer (Janke & Kunkel IkA® - Labortechnik, ultra-turrax T50, Germany). Lipase powder was dissolved in a minimum quantity of distilled water, added to the recombined milk to give a lipase ⁄ oil ratio of 1:100 and stirred for 1 min. at speed No. 6 by the same homogenizer. The treated milk was incubated at 40°C. The pH of lipolysed milk was adjusted to 7.0 and sterilized before use. Aliquot (6 ml) of the lipolysed recombined milk was aseptically transferred to 94 ml of milk to give 0.6% lipolysed oil in cheese milk.

2.2.2. Propagation of different strains:

Prior to the experiment, the cultures were sub-cultured at least three times in MRS broth or 10% nonfat milk (NFM) inoculated with 2% and incubated for 18 h. at 37°C. Between subcultures, the cultures were maintained at 2°C. Each tested culture was incubated until stationary phase at the corresponding temperatures employed in the fermentation of dairy products after being inoculated at a density of 10^6 CFU.ml^{-1}.

2.2.3. Tallaga-like cheese manufacture:

Tallaga-like cheese making was mainly carried out from a mixture of cow`s and buffalo`s milks (1:1) as described by [19, 20] with addition of Lipolysed milk with safflower oil, probiotic bacteria with or without inulin. The cheese milk was heated at 70°C for 10 min., cheese milk was immediately cooled to 37°C. The milk was mixed well with lipolysed Safflower oil to give 0.6% lipolysed oil in cheese. Calcium chloride and sodium chloride were added at the ratios of 0.02 and 2% (w/v), respectively. The milk was divided into 10 equal portions: **C_1**: Tallaga cheese with lipolysed milk and safflower oil was served as a Control 1 - **C_2**: Tallaga cheese with lipolysed milk with safflower oil and 1.5% Inulin was served as a Control 2. - **T_1**, **T_2**, **T_3** and **T_4**: as C_1 with adding *Lb. plantarum, Lb. acidophilus, Lb. casei* and *B. lactis* respectively. While other treatments **T_5**, **T_6**, **T_7** and **T_8** were made as C_2 using *Lb. plantarum, Lb. acidophilus, Lb. casei* and *B. lactis* in the same order. Rennet powder was added at the ratio of 0.05% (w/v) for coagulation at 37°C. The resultant curd was traditionally treated whereas all cheese samples were analyzed when fresh and after 7, 14, 21 and 28 days of cold storage.

2.2.4. Analysis of chemical composition and the changes in cheese during storage:

Moisture, fat, total nitrogen and salt were determined according to [21]. Total volatile fatty acids (TVFA) contents were estimated by the distillation method according to [22], whereas pH values were determined using pH meter (Jenway, 3505, Jenway Ltd., Felsted, Dunmow, Essex, UK).

2.2.5. Determination of conjugated dienes:

To determine the conjugated dienes in cheese samples, 10 g were vortexed with 20 ml of chloroform: methanol (2:1, v/v) and the homogenate was centrifuged at 4500 rpm for 5 min at 4°C. The separated organic phase (chloroform layer) was withdrawn and transferred to a tube by micropipettor, and then the chloroform layer passed through anhydrous sodium sulphate (Na_2SO_4) on Whatman No. 1 filter paper was rinsed with 3 mL of chloroform, and the extract (4.5 ml) was evaporated to dryness. The sample was mixed with 10 ml of hexane for further quantification. The determination of CLA was carried out by a UV spectrum analysis method described by [23]. The absorbance of the prepared extract was measured at 233 nm using a UV⁄VIS spectrophotometer (T80 UV/Vis spectrophotometer PG Instruments LTD., Felsted, Dunmow, UK), whereas a standard curve was prepared using pure conjugated linoleic acid to define the CLA concentration.

2.2.6. Microbiological analysis of cheese:

MRS agar medium [24] was used for the enumeration of lactobacillus lactic acid bacteria and Bifidobacterium after incubation at 37ºC for 3 days under aerobic conditions. Yeast and molds enumeration was determined as described by *Marshall 1992* using antibiotic standard plate count agar medium and incubated at 25ºC for 4 days under aerobic condition, whereas violet red bile agar medium was used to test presence of Coliform bacteria as recommended by *Marshall, 1992*.

2.2.7. Organoleptic properties of Tallaga-like cheese:

Sensory evaluation of Tallaga-like cheese was carried out by a panel consisting of 15 cheese experts when fresh and after 14 and 28 days of cold storage. The score card of cheese was designed in the light of the information given by [25] as follows: 50 points for flavour, 40 points for body/texture and 10 points for appearance with total perception of 100 points. The cheese was considered to be accepted at a total perception of 65points.

2.2.8. Statistical analysis:

All treatments were done in tri-replicates, and analysis of variance with two factorials (treatments and storage period) were conducted by the procedure of General Linear Model (GLM) according to [26] using Costat under windows software version 6.311 and least significant difference (LSD) test were employed to determine significant difference at $p \leq 0.01$.

3. Results and Discussion

3.1. Cheese yield:

The changes in cheese yield (%) and chemical composition of Tallaga cheese as affected by the applied treatments are presented in Table (1). Results indicated that the cheese yield for all Tallaga

cheese made with inulin (C_2, T_5, T_6, T_7 and T_8) were higher than the corresponding values of untreated Tallaga with inulin (C_1, T_1, T_2, T_3 and T_4). These increases may be due to the ability of inulin to bind more water into cheese matrix [27]. Similar finding was reported by [28] using probiotics with 2% inulin in different Tallaga cheese treatments led to a decrease in cheese yield as compared to control as a result of further acid formation by these cultures causing water expulsion causing lower dry matter. The same trend was noticed by [29, 30].

3.2. Gross chemical composition and pH values:

As seen in Table (1), results referred to the total solids, total protein, fat on dry matter basis and salt on moisture phase values of different Tallaga cheese treatments varied throughout the storage period. As storage period advanced, these values increased significantly regardless inulin addition or using different probiotic cultures as a result of loss of moisture and action by acid development which led to expel the whey from the cheese matrix. Similar trends were noticed by [31, 32] Using inulin in cheese making led to an increase in the water holding capacity due to its water binding power [33]. So, C_2 had lower total solids content throughout the storage period than that of C_1 but it had higher cheese yield as shown previously. Also, T_5, T_6, T_7 and T_8 tended to have lower total solids values than those of T_1, T_2, T_3 and T_4. Similar findings were reported by [28, 34] for Domiati and soft cheese made with inulin respectively as well as [35, 36] for Kareish cheese made with inulin. On the other hand, using probiotics increased significantly the total solids content, total protein, fat on dry matter and salt on moisture phase values of resultant cheeses as compared to control and this may be due the excess of acid formed by different cultures through fermentation of lactose. Similar findings were reported by [34, 37] for probiotic Domiati and soft cheese respectively. The highest total solids contents were in the presence *Lb. plantarum* followed by *Lb. acidophilus, Lb. casei* and *B. lactis* because of the different abilities of these cultures to form lactic acid.

Generally, all salt content of different Tallaga-like cheese treatments were lower than the traditional Tallaga cheeses reported by El-Kholy (2015) and Hamad and Naser-El-Deen (2021). In our study we used only 2% salt to keep the activity of probiotic cultures to induce the CLA concentrations in cheese.

However, as the storage period progressed the salt content of different cheese treatments increased. Similar findings were reported by [32, 38].

The changes in pH values of different Tallaga-like cheese treatments are illustrated in Fig. (1). pH values of different cheese treatments decreased significantly with different rates as a result of further lactic acid formed through microbial fermentation process. In cheese made with 1.5% inulin the pH significantly decreased as compared to control because of acidic nature of inulin [39] as well as it stimulates the starter cultures in Tallaga cheese leading to produce higher amounts of lactic acid (Sanders *et al*., 2019). Similar findings were reported [28, 30].

Table (1): Cheese yield and total gross chemical composition (%) of Tallaga-like cheese as affected by using probiotics and lipolysed milk containing safflower oil with or without 1.5% inulin (Average of three replicates) *.

Parameters	Storage period (days)	Treatments										Mean
		C_1	C_2	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Cheese yield %	Fresh	29.45^{e}	31.97^{a}	28.85^{h}	28.98^{g}	29.18^{f}	29.22^{f}	31.42^{d}	31.62^{c}	31.73^{b}	31.77^{b}	
Total solids	Fresh	34.59	32.14	34.86	34.91	34.74	34.76	32.68	32.74	32.55	32.57	33.65^{e}
	7	35.18	32.66	35.49	35.54	35.26	35.37	33.26	33.32	33.11	33.12	34.24^{d}
	14	36.29	33.69	36.68	36.72	36.51	36.53	34.35	35.40	34.16	34.18	35.35^{c}
	21	37.14	34.50	37.60	37.63	37.41	37.43	35.23	35.28	35.03	35.04	36.23^{b}
	28	37.68	34.97	38.19	38.21	37.97	37.99	35.78	35.83	35.57	35.58	36.78^{a}
	Mean	36.18^{c}	33.59^{f}	36.56^{a}	36.60^{a}	36.40^{b}	36.42^{b}	34.26^{d}	34.31^{d}	34.08^{e}	34.10^{e}	
Fat on dry matter basis (F/DM)	Fresh	45.88	45.25	45.89	45.83	45.82	45.80	45.15	45.19	45.19	45.16	45.52^{e}
	7	46.19	45.67	46.25	46.20	46.20	46.18	45.60	45.60	45.57	45.52	45.89^{d}
	14	46.61	46.01	46.69	46.64	46.56	46.54	45.96	45.92	45.98	45.96	46.29^{c}
	21	46.90	46.25	46.98	46.94	46.89	46.87	46.26	46.18	46.15	46.13	46.56^{b}
	28	47.10	46.47	47.14	47.11	47.08	47.15	46.49	46.40	46.39	46.37	46.77^{a}
	Mean	46.54^{a}	45.93^{b}	46.58^{a}	46.54^{a}	46.51^{a}	46.51^{a}	45.89^{b}	45.84^{b}	45.86^{b}	45.83^{b}	
Total Protein	Fresh	12.85	11.81	12.88	12.88	12.86	12.86	11.86	11.85	11.83	1.83	12.36^{f}
	7	13.08	12.02	13.13	13.13	13.11	13.11	12.09	12.08	12.05	12.05	12.59^{d}
	14	13.53	12.42	13.59	13.59	13.56	13.56	12.51	12.49	12.45	12.46	13.02^{c}
	21	13.87	12.74	13.95	13.95	13.92	13.92	12.85	12.83	12.79	12.79	13.36^{b}
	28	14.10	12.93	14.18	14.18	14.15	14.15	13.07	13.05	13.01	13.01	13.58^{a}
	Mean	13.47^{b}	12.38^{d}	13.52^{a}	13.55^{a}	13.52^{a}	13.52^{a}	12.48^{c}	12.46^{c}	12.44^{c}	12.44^{c}	
Salt on moisture phase (S/M)	Fresh	1.99	1.86	2.04	2.04	2.04	2.02	1.90	1.90	1.88	1.88	1.95^{e}
	7	2.07	1.93	2.12	2.13	2.10	2.10	1.99	1.98	1.96	1.96	2.03^{d}
	14	2.20	2.05	2.27	2.28	2.24	2.25	2.13	2.10	2.08	2.08	2.17^{c}
	21	2.31	2.15	2.40	2.41	2.35	2.38	2.25	2.25	2.20	2.19	2.29^{b}
	28	2.39	2.23	2.49	2.51	2.45	2.47	2.34	2.32	2.28	2.27	2.38^{a}
	Mean	2.19^{c}	2.04^{f}	2.26^{a}	2.27^{a}	2.23^{b}	2.24^{b}	2.12^{d}	2.11^{d}	2.08^{e}	2.08^{e}	

* a, b, ….. means with the same letter among treatments or during storage periods are not significantly different ($p \leq 0.01$).

C_1: Tallaga cheese with traditional method and lipolysed milk with safflower oil (Control 1).

C_2: Tallaga cheese with traditional method, lipolysed milk with safflower oil and 1.5% Inulin (Control 2).

T_1, T_2, T_3 and **T_4:** as C_1 with adding *Lb. plantarum, Lb. acidophilus, Lb. casei* and *B. lactis* respectively.

T_5, T_6, T_7 and **T_8:** as C_2 with adding *Lb. plantarum, Lb. acidophilus, Lb. casei* and *B. lactis* respectively.

Generally, treated cheese with different probiotic cultures had significantly lower pH values as compared to control treatment. This may be due to the post acidification caused by the growth of microorganism's activity [40,41]. Similar results were reported by [34, 42]. The lowest pH values were recorded with *Lb. plantarum* followed by *Lb. acidophilus, Lb. casei* and *B. lactis* because of the different abilities of these cultures to form lactic acid. [18] used different probiotics in milk media on CLA production and found that *Lb. plantarum* had lower pH value than that of *Lb. acidophilus* and *Lb. casei*. Similar findings in cheese matrix were reported by [30] found that pH values of soft cheese made with *Lb. acidophilus* were lower than that of *B. bifidum*; [29] who found that soft cheese made with *Lb. acidophilus* had higher total solids than that made with *Lb. casei*.

The differences of total CLA concentration expressed as mg/ g fat of different Tallaga-like cheese treatments are illustrated in Table (2).

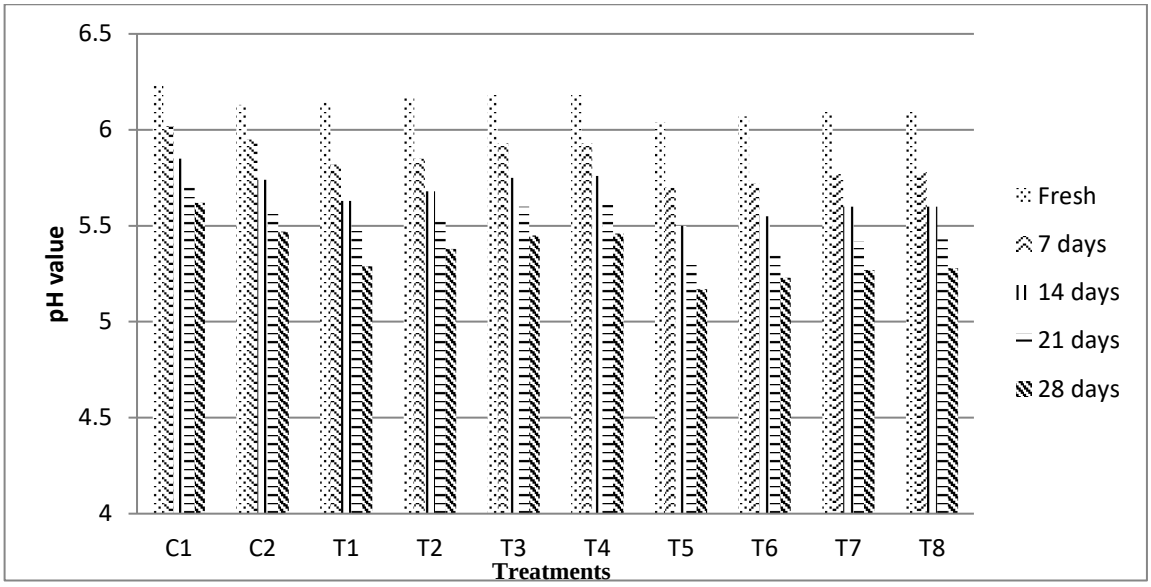

Fig. (1): Effect of using probiotics and lipolysed milk containing safflower oil with or without 1.5% inulin on the pH values of Tallaga cheese treatments during cold storage.

The obtained results showed that CLA ratio of C_1 increased gradually till 28 days of cold storage. Addition of inulin significantly increased the CLA concentration as compared to untreated-inulin cheeses because inulin can stimulate the cheese micro flora activity leading to increase the CLA [17]. A similar finding was reported by [43] who found that cream cheese made with inulin had higher CLA. So, treatments T_5, T_6, T_7 and T_8 had significantly higher CLA values than the corresponding values of T_1, T_2, T_3 and T_4 respectively. All probiotics supplemented Tallaga-like cheeses with lipolysed safflower oil increased the CLA concentration as throughout the storage period because of the ability of these cultures to induce CLA formation with using linoleic presented in safflower oil as precursor such increases varied with the power of biotransformation for these probiotics.

Table (2): Effect of using probiotics and lipolysed milk containing safflower oil with or without 1.5% inulin on the total CLA concentration expressed as mg/g fat of Tallaga like cheese treatments during storage periods (Average of three replicates) *.

Treatments**	Storage (days)					Mean
	Fresh	7	14	21	28	
C1	3.35	3.55	3.74	3.98	4.10	3.74^{j}
C2	3.92	4.23	4.41	4.84	4.98	4.48^{i}
T_1	5.32	5.65	6.98	6.34	6.63	6.18^{e}
T_2	4.97	5.14	5.45	5.88	6.21	5.53^{f}
T_3	4.56	4.75	5.11	5.23	5.56	5.04^{g}
T_4	4.42	4.68	5.04	5.18	5.44	4.95^{h}
T_5	6.28	7.20	8.45	8.98	9.64	8.11^{a}
T_6	5.97	7.01	8.17	8.60	8.98	7.75^{b}
T_7	5.53	5.82	6.58	7.22	7.76	6.58^{c}
T_8	5.40	5.69	6.45	7.01	7.67	6.44^{d}
Mean	5.40^{e}	5.69^{d}	6.45^{c}	7.01^{b}	7.67^{a}	

* a, b, ….. means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).

** See footnote of table (1) for details.

It was found that using *Lb. plantarum* supplemented cheese had the highest total CLA throughout the storage period followed by *Lb. acidophilus.* A similar trend was reported by [18] used

different probiotics in reconstituted skim milk containing 0.2% lipolysed sesame oil on CLA production and found that *Lb. plantarum* had higher CLA value than that of *Lb. acidophilus* and *Lb. casei*.

Also, [44] evaluated the availability of several *Lb. casei* and *B. bifidium* in MRS medium, MRS with skim milk with 1% hydrolysed soybean oil on the CLA concentration. It was found that the highest CLA was correlated with using skim milk with lipolysed oil with using *Lb. casei*. The majority of investigations showed that the inclusion of probiotics increased the level of CLA in cheese [43,45]. moreover [43] found that cream cheese made with *Lb. acidophilus* had higher CLA concentration through the storage period than that made with *B. animalis*; [46] found that cheese made with *Lb. paracasei* and *Lb. acidophilus* individually had higher CLA concentrations than these made *B. longum*, *B. bifidum* and *Ent. faecium* separately. From the obtained results, probiotic strain, inulin and lipolysed milk with safflower oil had a great impact on the rate of transformation of free linoleic acid to CLA.

3.3 Proteolysis and lipolysis in cheese:

3.3.1 Soluble nitrogen coefficient (SN/TN) and free amino acids (FAA) values:

The differences of soluble nitrogen coefficient (SN/TN) and total free amino acids values (FAA) of different fresh and stored Tallaga-like cheese treatments are illustrated in Table (3). Results indicated that both SN/TN and FAA values of all treated cheeses tended to increase significantly as storage period progressed. This may be due to the protein breakdown that occurred through the growth of micro flora and/or the activity of resistance proteolytic enzymes. Using 1.5% inulin caused higher SN coefficient and FAA values as compared to C_1 that may be due to the differences in total protein contents of both treatments as seen in Table (1). Similar result was noticed by [30]. On the other hand, using different probiotic cultures increased the SN/TN and FAA as compared to the control throughout the storage period. This could be due to the distinct contribution of the released enzymes into cheese matrix through storage period. These results coincided with those obtained by [47] who found that the SN/TN contents and free amino acids values of soft cheeses made with *Lb. casei*, *Lb. rhamnosus* and *Lb. delbrueckii* ssp. *bulgaricus* show an increase at the end of storage period. In between treatments, the highest proteolysis rate was noticed by using inulin as prebiotic and probiotic cultures than using probiotic cultures only. So, the highest values of SN/TN and FAA were found for T_5 and T_6 among treatments.

3.3.2 Total volatile fatty acids (TVFA):

The differences in TVFA, expressed as expressed as ml NaOH 0.1 N per 10 grams of cheese, as affected by the applied treatments are illustrated in Table (3). It was noticeable that the TVFA values in all treatments tended to increase significantly as the storage period progressed. The rate of increase varied considerably among the treatments. This increase in TVFA during storage period could be attributed to the residual activity of heat resistance lipase which may cause fat hydrolysis.

Table (3): Proteolysis and lipolysis of Tallaga-like cheese as affected by using probiotics and lipolysed milk containing safflower oil with or without 1.5% inulin during cold storage (Average of three replicates) *.

Parameters	Storage period (days)	Treatments**										Mean
		C_1	C_2	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Soluble nitrogen coefficient %	Fresh	7.20	7.56	7.83	7.58	7.39	7.34	8.23	8.08	7.98	7.87	7.71^{e}
	7	9.02	9.66	9.91	9.77	9.59	9.54	11.24	11.04	10.85	10.64	10.13^{d}
	14	10.66	11.30	11.83	11.60	11.39	11.20	13.46	13.13	12.81	12.60	12.00^{c}
	21	13.25	14.02	14.59	14.42	14.21	14.07	16.53	16.16	15.51	15.23	14.80^{b}
	28	14.93	16.43	17.73	17.32	16.95	16.64	19.43	18.87	18.14	17.65	14.80^{b}
	Mean	11.01^{i}	11.79^{j}	12.38^{e}	12.14^{f}	11.91^{g}	11.76^{h}	13.78^{a}	13.46^{b}	13.06^{c}	12.80^{d}	
Total Free amino acids values (FAA)***	Fresh	0.012	0.011	0.014	0.014	0.013	0.013	0.012	0.012	0.011	0.011	0.013^{e}
	7	0.088	0.101	0.128	0.122	0.115	0.111	0.135	0.133	0.126	0.121	0.122^{d}
	14	0.126	0.146	0.186	0.183	0.174	0.167	0.206	0.194	0.181	0.175	0.170^{c}
	21	0.185	0.213	0.260	0.252	0.242	0.240	0.269	0.265	0.249	0.248	0.241^{b}
	28	0.220	0.260	0.354	0.333	0.310	0.310	0.379	0.361	0.338	0.324	0.321^{a}
	Mean	0.126^{h}	0.146^{g}	0.188^{c}	0.181^{d}	0.170^{f}	0.168^{f}	0.200^{a}	0.193^{b}	0.181^{d}	0.176^{e}	
Total Volatile Fatty Acids (TVFA)****	Fresh	1.33	1.46	1.58	1.55	1.53	1.51	1.68	1.64	1.58	1.57	1.54^{e}
	7	1.62	1.75	1.86	1.83	1.78	1.75	1.95	1.92	1.85	1.82	1.81^{d}
	14	2.21	2.34	2.64	2.55	2.48	2.46	2.72	2.66	2.58	2.56	2.52^{c}
	21	2.86	3.06	3.58	3.46	3.30	3.26	3.74	3.60	3.48	3.44	3.41^{b}
	28	3.44	3.68	4.14	4.03	3.86	3.81	4.38	4.24	4.07	4.01	3.97^{a}
	Mean	2.29^{j}	2.46^{i}	2.76^{c}	2.68^{e}	2.59^{g}	2.55^{h}	2.89^{a}	2.81^{b}	2.70^{d}	2.64^{f}	

* a, b, ….. means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).
** See footnote of table (1) for details.
*** (FAA) Expressed as absorbance at 507 nm /100 µl.
**** (TVFA) expressed as ml NaOH 0.1 N per 10 grams of cheese.

Tallaga-like cheese with inulin contained more TVFA values than the control throughout the storage period as a result of higher moisture which induced the fat hydrolysis. Similar findings were found by [30]. Using different probiotics in Tallaga-like cheese led to higher TVFA values as compared to the control that maybe due to the lipolytic activity of these cultures in cheese matrix. Similar findings were reported by [34]. The highest TVFA values were found for treated probiotic cheese with inulin (4.38 mls 0.1NaoH/10 g cheese) for *Lb. plantarum* treated cheese followed by *Lb. acidophilus* among other treatments throughout the storage period. A similar finding was noticed by [48].

3.4. The microbiological quality of Tallaga- like cheese:

3.4.1. The enumeration of different probiotic cultures:

The changes in counts of different probiotic cultures of Tallaga- like cheese as affected by using different probiotic with lipolysed milk with safflower oil with or without inulin are illustrated in Table (4). The viable bacterial counts were different between treatments and increased during the storage period up to 14 days of storage, followed by a decreasing trend up to 28 days of storage. At the end of the storage period, the highest CFU was found in T_6 while the lowest CFU was found in C_1 and C_2. This result can be explained by the decrease in the viability of the species which are sensitive to the increase of acidity. All the total viable counts of treated cheeses were higher than those given by [49]. This may be correlated to the lower salt content of our treated cheeses.

Table (4): Counts of lactic acid bacteria (log CFU. g^{-1}) of Tallaga-like cheese as affected by using probiotics and lipolysed milk containing safflower oil with or without 1.5% inulin during cold storage (Average of three replicates) *.

Storage period (days)	Treatments**										Mean
	C_1	C_2	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Fresh	2.11	2.66	9.49	9.51	9.45	9.42	9.69	9.74	9.64	9.62	8.13^{d}
7	2.39	2.86	9.80	9.85	9.76	9.73	9.80	9.84	9.71	9.71	8.35^{b}
14	2.52	2.95	9.95	9.99	9.88	9.85	9.91	9.94	9.82	9.81	8.46^{a}
21	2.44	2.75	9.44	9.49	9.42	9.38	9.82	9.86	9.74	9.73	8.21^{c}
28	2.31	2.64	9.18	9.21	9.15	9.13	9.74	9.78	9.65	9.64	8.04^{e}
Mean	9.70^{c}	9.71^{c}	9.83^{a}	9.79^{b}	9.50^{g}	9.53^{f}	9.61^{d}	9.57^{e}	2.77^{h}	2.35^{i}	

* a, b, ….. means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).

** See footnote of table (1) for details.

Generally, all treated cheeses with probiotic had higher viable counts than untreated Tallaga-like cheese because of the excess of lactic acid bacteria in cheese matrix. Similar results were observed by [49, 50]. [51] noticed a gradual increase in probiotic bacteria (above 10^6 CFU.g^{-1}) during cold storage of probiotic white cheese. Using inulin with prebiotic cultures in Tallaga-like cheese increased the bacterial counts because of its prebiotic action which induced the activity of these cultures in cheese matrix. So, all probiotics treated Tallaga-like cheese with inulin had higher bacterial counts than that made with probiotics only. Similar trend was reported by [43] for cream cheese. The number of living cells for the Tallaga-like cheese made using *Lb. plantarum* and *Lb. acidophilus* had higher bacterial count (above 9 log CFU.g^{-1}) after 28 days of cold storage. This demonstrated that such cheese was considered as probiotic cheese.

Generally, control cheeses had little viable counts than other treated probiotic cheeses as a result of the presence of NSLAB (nonstarter lactic acid bacteria). NSLAB was also found in all cheese samples, although at noticeably higher counts in treated cheeses with inulin during cold storage.

Generally, it was found that inducing CLA production in the study had no significant impact on growth and activity of different probiotics used in the experiments, as apparent from the normal acid development in treated Tallaga-like cheeses during the cold storage. A similar finding was reported by [43] who studied the impact of using inulin and *Lb. acidophilus* and *B. animalis* on the quality characteristics of cream cheese and found a decrease trend for pH value during storage.

3.4.2. Yeast and molds and Coliform bacteria count:

All probiotic Tallaga cheese treatments made with or without inulin had no counts for yeasts and molds or Coliform bacteria throughout the storage period. The resultant phenomena may be due to the good manufacturing conditions used to make different Tallaga-like treatments.

3.5. Sensory evaluation:

The impact of using different probiotic cultures, lipolysed milk with safflower oil with or without inulin on the sensory evaluation of Tallaga-like cheeses are shown in Fig. (2). Generally, using

1.5% inulin (C_2) improved the sensory properties, especially body & texture than C_1 because of its ability to increase water holding capacity with forming gel with smooth soft body & texture leading to get higher total acceptability scores. This may be attributed to both the higher ratio of moisture to protein and to the increase in filler volume that results in a decrease in the amount of protein matrix [15]. Similar findings were reported by [34, 35] for Karish and Tallaga cheeses made with inulin. While, using probiotics in Tallaga-like cheese increased the flavour scores as compared to its control. This may be correlated to its higher proteolysis and lipolysis rates. The highest total scores were recorded for Tallaga-like cheese treated with *Lb. plantarum* followed by *Lb. acidophilus*, *Lb. casei* and *B. lactis.* The mixed culture of both *Lac. lactis* subsp. *cremoris* and *Lb. casei* subsp. *casei* at a ratio of 1:1 have been recommended for the manufacture of Tallaga cheese [48].

Similar findings were reported by [49] who made Tallaga cheese with *B. longum* and *B. lactis* which gained higher total acceptability scores than untreated cheese. [37] mentioned that probiotic white soft cheese with *B. bifidum* gained higher flavour scores and total acceptability scores than the control. Also, it was found that inoculation of probiotic led to enhancement for body & texture as well as flavour scores of the resultant cheeses. So, T_5 and T_6 gained higher body & texture, flavour and total acceptability scores than T_1 and T_2. The same trend was noticed by [34] who found that Domiati cheese made with *Lb. rhamnosus* and inulin had higher total acceptability scores than that made with culture only.

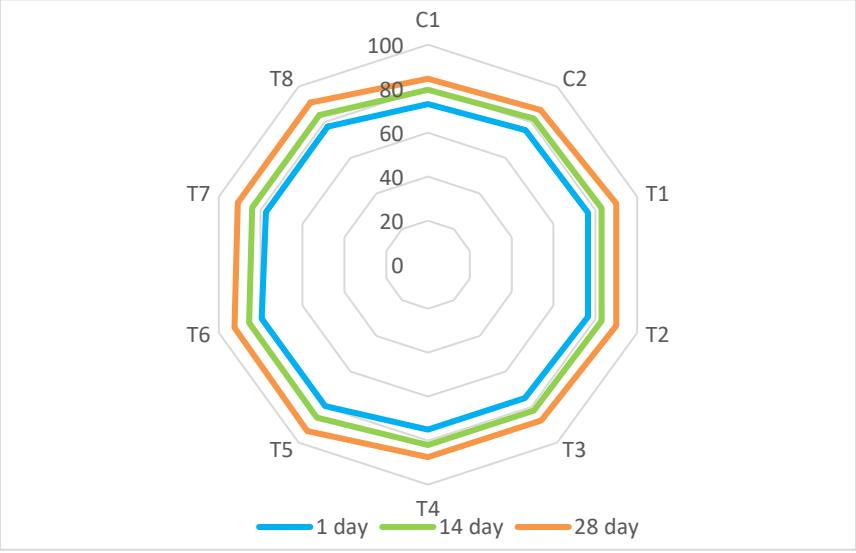

Fig. (2): Effect of using probiotics and lipolysed milk with safflower oil with or without inulin on the sensory evaluation of Tallaga-like cheese treatments as total acceptability during cold storage periods.

4. Conclusion

Functional Tallaga-like cheese with more than double CLA, presented in control cheese, can be made using *Lb. plantarum* and 1.5% inulin in the presence of lipolysed milk with safflower oil as a source of free linolenic acid. The resultant treated cheese gained higher body & texture, flavour and total acceptability scores. The attained results can be recorded but with lower scores by using *Lb. acidophilus* while keeping all the circumstances constant.

5. Competing Interests

The authors declare that they have no competing interests in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

References

[1] Ong, L. and Shah, P. N. (2009). Probiotic Cheddar cheese: influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. LWT Food Sci. Tech., 42:1260–1268. DOI:10.1016/j.lwt.2009.01.011

[2] Euromonitor International, 2012. Cheese in Egypt. http://www.euromonitor.com/cheese-in-egypt/report.

[3] Statista (2021). https://www.statista.com/statistics/1305838/share-of-people-who-recently-consumed-cheese-in-africa-by-country/

[4] Tamime, A. Y. (2006). Brined cheese. 1st Edition. Blackwell Publishing. UK. DOI:10.1002/9780470995860

[5] Mehanna, A. S. and Rashed, M. A. (1990). At attempt to improve the keeping quality of Tallaga cheese by using milk treated with carbon dioxide. Egyptian J. Dairy Sci., 18: 377-388.

[6] Shehata, A. E.; Gaafer, A. M. and Hussein, G. A. (1995). Fate of enterotoxigenic *S. aureus* in Tallaga cheese. Proceedings of the 6th Egyptian Conf. Dairy Sci. and Techn., November 4-6, 1995, Cairo, Egypt., pp: 169-182.

[7] Saleh, A. E.; Abd El-Malek, A. F. and Moussa, M. A.M. (2020). Extended shelf life of Tallaga cheese by natural preservatives. J. Product. & Dev., 25(1): 25- 37. DOI: 10.21608/JPD.2020.81013

[8] Yang, B.; Chen, H.; Stanton, C.; Ross, R.P.; Zhang, H.; Chen, Y.Q. and Chen, W. (2015). Review of the roles of conjugated linoleic acid in health and disease. J. of Functional Foods, 15: 314–325. https://doi.org/10.1016/j.jff.2015.03.050.

[9] Basak, S. and Duttaroy, A. (2020). Conjugated linoleic acid and its beneficial effects in obesity, cardiovascular disease, and cancer. nutrients, 12(7):1913. https://doi.org/10.3390/nu12071913.

[10] Chorfa, N.; Hamoudi, S.; Belkacemi, K. (2010). Conjugated linoleic acid formation via heterogeneous hydrogenation/isomerization of safflower oil over mesostructured catalysts. App. Catalysis A: General 387: 75–86. DOI:10.1016/j.apcata.2010.08.006

[11] Norris, L. E.; Collene, A. L.; Asp, M. L.; Hsu, J. C.; Liu, L. F.; Richardson, J. R.; Li, D.; Bell, D.; Osei, K.; Jackson, R. D. and Belury, M. A. (2009). Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. Am. J. Clin. Nutr.;90: 468–476. DOI: 10.3945/ajcn.2008.27371

[12] Mozzon, M.; Foligni, R. and Tylewicz, U. (2018). Chemical characteristics and nutritional properties of hybrid palm oils. In book, Palm oil book, by Waisundara, 5th Edition., Intechopen. DOI: 10.5772/intechopen.75421.

[13] Shimomura, Y.; Tamara, T. and Suzuki, M. (1990). Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. J. Nutriti., 120: 1291-1296. DOI: 10.1093/jn/120.11.1291

[14] Neschen, S.; Moore, I.; Regittnig, W.; Yu, C. L.; Wang, Y.; Pypaert, M., *et al*. (2002). Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Amer. J. of Physiol.-Endocrinology and Metabo., 282(2): 395-401. DOI: 10.1152/ajpendo.00414.2001

[15] Meyer, D.; Bayarri, S.; Tárrega, A. and Costell, E. (2011). Inulin as texture modifier in dairy products. Food Hydroc., 25: 1881-1890. DOI:10.1016/j.foodhyd.2011.04.012

[16] Karimi, R.; Azizi, M. H.; Ghasemlou, M. and Vaziri, M. (2015). Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review. Carbohydrate polymers, 119: 85-100. DOI: 10.1016/j.carbpol.2014.11.029

[17] Sanders, M. E.; Merenstein, D. J.; Reid, G.; Gibson, G. R. and Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nature Reviews Gastroenterology & Hepatology, 16 (10): 605–616. DOI: 10.1038/s41575-019-0173-3

[18] Abd El-Salam, M. H. A., El-Shafei, K., Sharaf, O. M., Effat, B. A., Asem, F. M. and El-Aasar, M. (2010). Screening of some potentially probiotic lactic acid bacteria for their ability to synthesis conjugated linoleic acid. Inter. J. of Dairy Techn., 63(1), 62–69. DOI:10.1111/j.1471-0307.2009.00541.x

[19] Abou-Donia S. A. (1991) Manufacture of Egyptian, soft and pickled cheeses. In book, Robinson, R.K. and Tamime, A.Y. Feta and Related Cheeses. 2nd Edition. Woodhead Publishing Ltd., England. p.160-208.

[20] Abd-El Salam, M. and Benkerroum, N. (2006) North African Brined Cheeses. In book, Tamime, A. Y. Brined cheese. 1st Edition. Blackwell Publishing.UK. P.139- 187.

[21] AOAC (2007). Official Methods of Analysis, 19th Edition., Association of Official Analysis Chemists Inc., USA.
[22] Koiskowski, F.V. (1982). Cheese and Fermented Milk Foods. 2nd Edition. Brooktonale, New York.
[23] Rosson, R.A. and Grund, A.D. (2001). Linoleate isomerase. World Patent 100846, 30.
[24] Marshall; R. T. (1992). Standard Methods for the Examination of Dairy Products. 16th Edition. American Public Health Association (APHA); Washington. USA. https://doi.org/10.2105/9780875530024
[25] Clark, S.; Costello, M.; Drake, M. and Bodyfelt, F. (2009). The Sensory Evaluation of Dairy Products. 2nd Edition, Springer Science Business Media, LLC. New York, USA. DOI:10.1007/978-0-387-77408-4
[26] Snedecor, G.W. and Cochran, W.G. (1969) Statistical Methods. 6th Edition. Iowa State University press, Iowa, USA.
[27] Yahyavi, F. and Kalajahi, S. E. M. (2014). A study of the possibility of low- fat feta cheese production using dietary fiber. Advance Environment. Biol., 8, 1245- 1249.
[28] Ghazal, M. M.; Al-Hilfy, N. A. and Ali, Hadeir, A. (2021). Production of Functional Soft Cheese and Studying its Chemical and Sensory Evaluation Properties. Basrah J. Agric. Sci., 34(1): 67-83. DOI:10.37077/25200860.2021.34.1.07
[29] Hafez, Y.M.; Sobeih, Azza M. and Mansour, Nehal M. (2019). Pivotal role of Lactobacillus strains in improvement of soft cheese quality and inhibiting the growth of harmful and dangerous bacterial pathogens. Slov. Vet. Res., 56 (22): 657–63. DOI: https://doi.org/10.26873/SVR-804-2019
[30] El-Baz, A. M. (2013). The use of inulin as a dietary fiber in the production of symbiotic UF- soft cheese. J. Food and Dairy Sci., Mansoura Univ., 4 (12): 663 – 677. DOI: 10.21608/JFDS.2013.72121
[31] El-Kholy, A. M. (2015). The use of artichoke (*Cynara Scolymus, L*) extracts for the production of Tallaga cheese. Intr. J. Food and Nutr. Sci., 4(1): 34-41.
[32] Abbas, Halima F.; Tammam, A. A.; Gomah, Nanis H.; Elderwy, Y. M. A. and Moneeb, Asmaa H. M. (2020). Making Domiati cheese from mixtures of cow's milk and milk powder. J. of Food and Dairy Sci., Mansoura Univ., 11 (3):83 – 89. DOI: 10.21608/JFDS.2020.87014
[33] Krishna, A.; Krishna, K.A. and Patel, S.S. (2020). Inulin-Benefits and Scope of Use in Dairy Products. Inter. J. of Curr. Micro. and Appl. Sci., 9(8): 1911-1921. DOI: https://doi.org/10.20546/ijcmas.2020.908.219
[34] Abd-Rabou, Hagar S.; El-Ziney, M. G.; Awad, Samah M.; AlSohaimy, S. A. and Dabour, Nassra, A. (2016). Impact of probiotic and synbiotic supplementation on the physiochemical, texture and sensory characteristics of wheyless Domiati-like cheese. MOJ Food Process Technol., 3 (3):317-325. DOI: 10.15406/mojfpt.2016.03.00074
[35] Alnemr, T. M.; Abd El-RazeK, Amal M.; Hasan, Hoda M. A. and Massoud, Mona I. (2013). Improving of Karish cheese by using enhanced technological texturizing inulin. Alex. J. Agric. Res., 58 (2):173-181.
[36] Mohammed, Ragia, O.Y. (2016). Improve microstructure and rheological properties of Kareish cheese. J. Food and Dairy Sci., Mansoura Univ., 7 (1): 1 – 9. DOI: 10.21608/JFDS.2016.42767
[37] Soliman, A. H. Y.; Abdalla, M.I. and El Zubeir, I. E. M. (2018). Effect of level of milk fat on the compositional quality of Sudanese white cheese during storage, Sky J. of Food Sci., 2:1- 9. DOI:10.24966/DRT-9315/100008
[38] Hamad, M. N. F. and Naser El-Deen, Mervat O. M. H. (2021). Attempting to produce Egyptian Tallaga cheese with international specifications. Open Access Res. J. of Sci. and Techno., 01(01): 008–023. DOI:10.30574/oarjst.2021.1.1.0020
[39] Rodrıguez_Garcıa, J.; Puig, A.; Salvador, A. and Hernando, I. (2012). Optimization of a sponge cake formulation with inulin as fat replacer: Structure, physicochemical, and sensory properties. J. of Food Sci., 77 (2): 189 – 97. DOI: 10.1111/j.1750-3841.2011.02546.x
[40] Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. J. Dairy Sci., 83: 894-907. https://doi.org/10.3168/jds.S0022-0302(00)74953-8
[41] Elshaghabee, F. M. F. (2023). Probiotics in Dairy Foods: Advantages and Disadvantages. Egypt. J. Agric. Sci., 74 (1): 1-18. DOI: 0.21608/EJARC.2023.304665
[42] El-Zayat, A. I. and Osman, M. M. (2001). The use of probiotics in Tallaga cheese. Egyptian J. Dairy Sci., 29: 99-106.
[43] Alves, Larissa D.; Richards, N. S. P.; Mariutti, Lilian R. B.; Nogueira, G. C. and Bragagnolo, Neura (2011). Inulin and probiotic concentration effects on fatty and linoleic conjugated acids in cream cheeses. Eur. Food Res. Technol, 233: 667–675. DOI:10.1007/s00217-011-1558-x
[44] Al-Hindi, R. R. and Abd El Ghani, S. (2015). Production of free conjugated linoleic acid by fermentation performed using *Lactobacillus casei* and *Bifidobacterium bifidum*. Global Veterinaria 14 (5): 720-728.
[45] Van Nieuwenhove, C. P.; Oliszewski, R.; Gonzalez, S.N. and Chaia, A. B. P. (2007) Influence of bacteria used as adjunct culture and sunflower oil addition on conjugated linoleic acid content in buffalo cheese. Food Res. Int., 40:559–564. DOI:10.1016/j.foodres.2006.08.003

[46] Gursoy, O.; Seckin, A. K.; Kinik, O. and Karaman, Aysed D. (2012). The effect of using different probiotic cultures on conjugated linoleic acid (CLA) concentration and fatty acid composition of white pickle cheese. Inter. J. of Food Sci. and Nutr., 63(5): 610–615. DOI: 10.3109/09637486.2011.643295

[47] Shehata, A. E.; EL- Nawawy, M. A.; E L- Kenany, Y. M. and Aumara, I. E. (2001). Production of soft cheese with health benefits. In Proc. 8th Egyptian conf. Dairy Sci. & Techn., p. 635 – 651.

[48] El-Abd, M. M., Abd El-Fattah, A. M., Osman, S. G. & Abd El-Kader, R. S. (2003) Effect of some lactic acid bacteria on the properties of low salt Domiati cheese. Egyptian J. of Dairy Sci., 31: 125–138.

[49] Hamad, M. N. F.; Gomaa, M. S.; El-Kadi, S. M. and Al-Esawy, Shymaa A. I. (2017). The effect of starter type on the quality of Tallaga soft white cheese. Egypt. J. of Appl. Sci., 32 (7): 146-165.

[50] Amer, A. (2011). Probiotics as a tool to improve microbial quality of soft cheese. Assiut Vet. Med. J., 57 (129) 1- 14.

[51] Yerlikaya, O. and Ozer, E. (2014). Production of probiotic fresh white cheese using co-culture with *Streptococcus thermophilus*. Food Sci. and Technol., 34, 471–477. https://doi.org/10.1590/1678-457X.6365.