

ULTRAFILTERED SOFT CHEESE AS AFFECTED BY SOY MILK, *Lactobacillus acidophilus* STARTER AND CERTAIN FLAVOUR ADDITIVES

Metry, Wedad A.

Dairy Department, Faculty of Agriculture, Cairo University, Fayoum, Egypt.

ABSTRACT

The effect of soybean milk, *Lactobacillus acidophilus* and other additives such as hot green pepper juice (HGPJ), cayenne pepper, paprika (P) and artificial green pepper flavour (AGPF) was studied. The chemical, rheological, microbiological and organoleptical properties of ultrafiltered (UF) white soft cheese during 25 days at $4 \pm 1^{\circ}\text{C}$ were investigated. Results of the study clearly indicated that, the changes in the chemical composition of cheese samples with or without soybean milk were similar and almost of the same trend during storage. Fresh and stored cheeses prepared with *Lactobacillus acidophilus* had the lowest pH values and soluble nitrogen compared to that of other treatments. The highest cholesterol content was in control followed by cheese contained starter and those contained soybean milk without starter, however the lowest cholesterol content was detected in treatment contained soybean milk and starter. Addition of starter culture led to a significant decrease ($P \leq 0.01$) in cholesterol content of fresh and during storage of UF- soft cheese. Rate of syneresis was highly significant ($P \leq 0.01$) during storage. The penetration and syneresis of cheese samples increased with adding HGPJ to retentate. The addition of starter improved the keeping quality of UF- soft cheese. The count of *Lactobacillus acidophilus* significantly increased ($P \leq 0.01$) during storage at $4 \pm 1^{\circ}\text{C}$ and reached to its maximum number after 8 days, however the viable counts at the end of storage were still enough for cheese to be successful probiotic, especially when added HGPJ. Statistically, there is significant differences ($P \leq 0.01$) between treatments containing soy milk + starter and control in total scores. The results of sensory evaluation indicated that out of 12 formulas used to manufacture UF- soft cheese, five treatments showed good organoleptic properties (total score ranged between 91 and 95.3). The best results were shown by two formulas that contained CP and HGPJ, respectively.

Keywords: Ultrafiltration process – soybean milk – *Lactobacillus acidophilus* – artificial flavour – green pepper – capsicum tincture – probiotic.

INTRODUCTION

The cheese made by ultra filtration (UF) processes has been expanded during the last decade. In Egypt more than 76000 tons of cheese was made using UF technique in the year 2000 (Hofi, 2000). With this method valuable milk components are retained with casein in the retentate, incorporated in the cheese matrix without significant losses in whey proteins which results in higher cheese yield and more nutritive product (Omar 1987, and Block *et al.*, 1996). However, UF-soft cheese has no characteristic taste and lack flavour, many trials were carried out to improve its flavour (Magda *et al.* 2000). The potential of soybean milk (SM) as a substitute for cow milk has been emphasized over the years, especially in the case of infants or children allergic to cow milk or adult with a low level of lactose in their intestine. Many

researches proved that soy milk has many advantages , the first is the high protein and nutritive value content , Osman *et al.* , (2000) mentioned that , soy cheese had higher total amino acids , particularly the essential than the Domiati cheese made from mixture of buffaloe's and cow's milk (1:1) . FAO (1965) reported a references pattern for the soybean protein which contains 3.215 g of total essential amino acids per gram of nitrogen. The second advantage is the medical effect, studies have indicated that consumption of soy protein can decrease total serum cholesterol and decrease risks for several cancers (Messina , 1997) and it also can be used as hypolipidemic (Sipos, 1988) . , The last advantage, SM can also be used as an economical protein beverage when cow milk is not available. However, soy milk and other soybean based foods are not widely accepted because of their unfavorable beany flavour , their tendency to induce flatulence and the high contents of indigestible alpha-D-galactosyl oligosaccharides such as raffinose and stachyose limit the consumption of SM as raw food material (Thananunkul *et al.*,1976) . , To overcome these limitations and to improve the acceptability and the nutritive value of SM , fermentation with various organisms

(*Lactobacilli* and *Bifidobacteria*) has been attempted (Garro *et al.*,1994 and Garro *et al.*,1999) . Shehata *et al.*, (1984 a) and Chou & Hou (2000) found that soymilk , as well as milk , is suitable for the growth of lactic acid bacteria , On the other hand , Garro *et al.* (2004) have reported that about 85% of the total amount of sugars were reduced (mainly stachyose 71% after 4 hr. of incubation at 37 ° C) in a SM prepared with the mixed culture

(*Bifidobacterium longum* CRL 849 and *Lactobacillus fermentum* CRL 251 at ratio 1:1) . Some researches used soybean milk as functional ingredient for manufacturing of nondairy and different dairy products such as fermented milk (Magdoub *et al.*, 1992 b) , Ice cream (Magdoub, *et al.* 1992 a) , Domiati cheese (Osman *et al.*,2000 ; Nermeen , 2003 and Hassanein , 2003) and Mozzarella cheese (Kumar and Jha, 1997) with good physical , chemical properties and inexpensive compared with traditional dairy products . But, under local conditions there is no available research on using soymilk for making UF-soft cheese.

Lactobacillus acidophilus (group lactic acid bacteria) are dominant *lactobacilli* in the human intestinal tract, and are expected to be useful probiotic strains (Masuda *et al.*, 2003). Consuming foods containing specific strains of probiotic bacteria has increased in most European and American countries. This due to their high nutritional benefits as well as their antibacterial and therapeutic properties (Mattila – Sandholm *et al.* , 1999) .Alleviation of symptoms of lactose intolerance and treatment of diarrhea to cancer suppression , reduction of blood cholesterol (Hoover, 1993 and Moussa *et al.*,1995) and reduction of cholesterol content in milk (Juskiwicz and Panfil – Kuncewicz , 2003) . These roles are chiefly ascribed to their metabolic activity. More than 90 products containing *bifidobacteria* or *lactobacillus acidophilus* or both are available in the market worldwide (Dave & Shah 1998). The most popular vehicles for incorporation of probiotic bacteria into the diet are fermented milk products (Kailasapathy and Rybka, 1997), karish cheese (Osman and Abbas, 1999) and Tallaga cheese (El – zayat and Osman, 2001)

Many investigators used the artificial and natural flavouring additives in dairy products as flavours and used preservatives (antiviral , antibacterial and antifungal compounds) foods such as ethanol capsicum tincture extract (Shehata *et al.* , 1884 b) , black pepper , thyme , peppermint , clove , fennel , cardamom , (Sherine , 1999) aqueous cayenne pepper and maryoram extracts (Magda *et al.* , 2000) and toluene balsam extract in Karish cheese making (El-Nemr *et al.* , 2003) . There is currently a worldwide spurge in the use of herbal preparations and the active ingredients isolate from medicinal plants in health care . Natural flavours, very few screening programmes have been initiated on crude plant materials .fresh green vegetables such as green pepper can be added for the same effects and for its nutritive value , it is excellent source of ascorbic acid (215 mg / 100 gm) and also contain 92 % moisture , 4.5 % total carbohydrate , 1.3 % protein , 0.25 % fat , 0.45 % ash , 15 mg calcium / 100 gm , 25 mg phosphorus / 100 gm and 1.2 mg iron / 100 gm (Watt and Merrill , 1975 and Alia *et al.* , 2004) . In Egypt, some researches were carried out by adding hot green pepper (small pieces) to brine solution used in Domiati cheese pickling as natural flavouring additives (Fahmy and Hanafy, 1992 & Hassanein , 2003). The objectives of this investigation were to study .

- 1- The possibility of using soy milk in producing UF- soft cheese with good quality and low cost.
- 2- Effect of using artificial and natural additives flavour for improving UF- soft cheese flavour and to overcome the Soybean milk unpleasant taste.
- 3- To benefit from *Lactobacillus acidophilus* microbe as a probiotic bacteria.

MATERIALS AND METHODS

Materials:

Milk sources:

Fresh raw buffalo's milk and soybean milk were obtained from a private farm in Fayoum governorate and INTSOY / Food Science Soymilk Process, Food Technology Research Institute, Agriculture Research Center respectively.

Freeze dried culture *Lactobacillus acidophilus* was purchased from drug stores, manufactured by RAMEDA (the tenth of Ramadan) Co. 6th of October City. Egypt (under license of: laboratories du lacteal bu Dr. Boucard. France). Starter culture was propagated separately in a sterile skim milk by successive transfers before use. Calf powder rennet (H.A.L.A) was obtained from Chr. Hansen Laboratories (Denmark). Fresh hot green pepper, fine mild cayenne pepper and paprika (dried sweet pepper) were obtained from the local markets. Artificial green pepper flavour (produced by Kamena products Corporation, 6th of Oct. city, Giza) was obtained from local market, at Fayoum. Commercial edible grade sodium chloride was obtained from El-Nasr Company for salt, Alexandria, Egypt.

Methods:

- 1-Preparation of hot green pepper juice (HGPJ) : fresh green pepper were washed , seeds were removed , juice was obtained by using Juice – Blender , CM- 20 , Hitachi VA – 6600 GM, Japan

2-Experimental procedures of UF- soft cheese:

UF- soft cheese was made according to the method described by Renner and Abd El – Salam (1991). Ultra filtration and cheese processing were carried out at the Dairy Processing Pilot Plant, Dairy Department, Faculty of Agriculture, Cairo University. Fayoum branch. Twelve batches of UF - soft cheese were manufactured , two of them from retentate (26 % TSS as refractometer degrees) obtained from the ultra filtered fresh, pasteurized buffalo's milk and contained 3% sodium chloride & 0.02% calcium chloride without (T1) and with the addition of 1 % starter culture (T7) and the other ten patches (treatments 2,3,4,5,6,8,9,10,11and 12) from mixture of pasteurized buffalo 's retentate (36 % TSS) and pasteurized soy milk (6.5% TSS) at ratio 65 : 35 respectively which finally contained 26% TSS. Sodium chloride (3%)and calcium chloride (0.02%) were added. The mixture was divided into two parts. The first part was without starter (*Lactobacillus acidophilus*), while the second part was inoculated with 1% starter culture. Each part was subdivided into five portions. Paprika , cayenne pepper (CP) , Hot green pepper juice (HGPJ) and Artificial green pepper flavour (AGPF) were added at ratio 0.2% , 0.1% , 7% and 80 ml / 100 kg, respectively, as illustrated in Table (1) .

Table (1): Formulation of ingredients used in UF- soft cheese making:

No.	Treatments	R1 (kg)	R2 (kg)	S M (kg)	AGPF (ml/100 kg)	HGPJ (%)	PAP. (%)	CP (%)	S C (%)
T1	R1 (control)	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T2	R2+ SM	0.0	65.5	34.5	0.0	0.0	0.0	0.0	0.0
T3	R2 + SM + Pap	0.0	65.5	34.5	0.0	0.0	0.2	0.0	0.0
T4	R2+SM+ CP	0.0	65.5	34.5	0.0	0.0	0.0	0.1	0.0
T5	R2 + SM + AGPF	0.0	65.5	34.5	80	0.0	0.0	0.0	0.0
T6	R2 + SM + HGPJ	0.0	65.5	34.5	0.0	7.0	0.0	0.0	0.0
T7	R1 + SC	100	0.0	0.0	0.0	0.0	0.0	0.0	1.0
T8	R2 + SC + SM	0.0	65.5	34.5	0.0	0.0	0.0	0.0	1.0
T9	R2 + SC + SM + Pap	0.0	65.5	34.5	0.0	0.0	0.2	0.0	1.0
T10	R2 + SC + SM + CP	0.0	65.5	34.5	0.0	0.0	0.0	0.1	1.0
T11	R2 + SC + SM + AGPF	0.0	65.5	34.5	80	0.0	0.0	0.0	1.0
T12	R2 + SC +SM+ HGPJ	0.0	65.5	34.5	0.0	7.0	0.0	0.0	1.0

R1: Retentate (26% TSS)

R2: Retentate (36% TSS)

AGPF: Artificial green pepper flavour

SC: Starter culture (*Lactobacillus acidophilus*) Pap. : Paprika

* The ratio of soybean milk, hot green pepper (juice or cubes), paprika, mild cayenne pepper and artificial green pepper were used based on the preference of the panelists as established in preliminary studies.

** The amount of soy milk (6.5 % TSS) and retentate (36 % TSS) was calculated by using person square.

Rennet (5 g / 100 kg retentate) was added to every portion alone . Thereafter every treatment was transferred to small sterile plastic container (120 gm) and automatically covered by aluminum foil (sealed). After coagulation (About 30 min), the cheeses were transferred to cooling incubator at $4 \pm 1^{\circ}$ C for 25 days, and analyzed in the same day (fresh) and during storage period .

3- Methods of analysis:

Changes in chemical, rheological, microbiological and organoleptic properties were estimated and tabulated. Each value in the tables is the average of triplicates.

3-1- Chemical analysis:

Fat and total nitrogen of the two kinds of milk, retentate and cheese samples were determined according to the methods described by Ling (1963), water soluble nitrogen of cheese samples was measured using Kjeldahl method according to Kuchroo and Fox (1982), moisture, ash (in soymilk, buffalo's milk, retentate, HGPJ and cheese samples) and salt (in the cheese samples) contents were determined as mentioned by Bradley et al. (1992). pH values of milks, retentate and cheese (samples prepared by homogenization 10 g / cheese with 5 ml distilled water) were measured using a digital pH meter (540 – GLP, Multical., Germany) and acidity of cheese samples was determined by titrimetric method as described in A.O.A.C (2000). Total cholesterol was colourimetrically determined as described by Paradkar and Irudayaraj (2002) and expressed as mg / 100gm sample.

3-2-Rheological properties:

Firmness of cheese:

Samples were measured at $15 \pm 1^{\circ}\text{C}$ by penetrometer (PNR 10 with Microprocessor Controls, SUR, Berlin). The standard rod weight was 50 gm. The test was performed as follows: The penetrometer cone was adjusted to touch the surface of cheese samples, then the cone was released to penetrate into the samples for 5 sec and penetration depth was recorded in units of 0.1 millimeter (mm). Penetration depth was recorded in triplicate at 3 different spots in each sample. The average of these penetration depths was taken as penetration value (firmness).

Syneresis: Dannenberg and Kessler (1988) method was used to measure the syneresis. The amount of whey drained off collected after 3 hr at 10°C (in cooling incubator) was taken as index for syneresis (g / 100 g cheese sample).

3-3-Microbiological analysis:

All microbiological tests were carried out as described in Oxoid (1990) as follows:-

Viable count of *Lactobacillus acidophilus* was estimated using Rogosa agar media, plates were incubated at 37°C for 5 – 7 days. Total bacterial count was estimated using nutrient agar medium plates were incubated at 35°C / 48 hr.

Yeasts and moulds were estimated using potato dextrose agar, plates were incubated at 30°C for 5 days. Coliform count was estimated using violet red bile (MacConky) agar media. plates were incubated at 37°C for 48 hr.

3-4- Evaluation of organoleptic properties:

Sensory tests were carried out according to the scheme of Pappas et al. (1996). Cheese samples were evaluated by a 25 panelists of the

experienced staff members of the National Research Center (Dairy department, Dokki , Cairo) , Microbiology , Food Science & Technology and Dairy Science Departments , Faculty of Agriculture , Fayoum .

3-5-Statistical analysis:

All the experiments were performed in triplicate and the results obtained were analyzed statistically (computerized) . General Linear Models (GLM) were performed using SPSS (1999) for windows, version 9.0.1 software package. Significant differences among treatments and storage periods means were determined using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition:

The average gross composition of pasteurized buffalo's milk soybean milk, retentate (26% TSS) and the mixture of retentate (36% TSS) and soybean milk (6.5 % TSS) are illustrated in Table (2). In general, buffalo's milk had higher fat, protein and ash than soybean milk. Gross composition of retentate and the mixture of retentate & soybean milk were very close. Also, the results indicated that increasing the total solids of retentate lead to decrease the pH value. This may be attributed to the higher protein content.

Table (2): Chemical composition of pasteurized buffalo's milk, soybean milk, retentate of retentate and soybean milk .

Component	Pasteurized buffalo's Milk	Soy bean milk	Retentate (26%TSS)	Retentate (36%TSS)	Mixture of retentate (36%TSS) and Soybean milk
Moisture(%)	84.06	93.39	58.33	45.52	58.08
Protein (%)	4.24	1.93	14.5	20.68	14.10
Fat (%)	6.80	1.60	19.3	26.81	19.00
Ash (%)	0.82	0.315	3.47	5.11	3.34
pH	6.61	7.02	6.57	6.50	6.68

The average results of moisture, fat, fat/dry matter, total nitrogen and ash contents of different treatments of UF- soft cheese when fresh and during storage (25 days) are presented in Table (3) . The results indicated that all cheese samples were in compliance with the Egyptian Slandered Regulation (EOSQC, 2000) , which requires that soft cheese (made from buffalo's milk) contain not more than 62% moisture , not less than 45% fat / dry matter and not less than 10% protein .

Table (3): Values of moisture, fat, fat / dry matter, total nitrogen and ash content of fresh and storied UF- soft cheese at 4 ± 1 °C.

Item	Moisture (%)		Fat (%)		Fat/dry matter (%)		Total nitrogen (%)		Ash (%)		
	Storage period (Days)	0	25	Storage period (Days)	0	25	Storage period (Days)	0	25	Storage period (Days)	0
T1	57.94 ^k	57.68 ^l	19.20 ^{cde}	19.35 ^{abc}	45.65 ^g	45.73 ^g	2.31 ^{cde}	2.35 ^{bc}	3.18 ^{eih}	3.46 ^{ab}	
T2	58.43 ^{hi}	58.00 ^k	19.00 ^{eigh}	19.20 ^{cde}	45.70 ^g	45.71 ^g	2.23 ^{ghi}	2.41 ^a	3.10 ^{ghi}	3.38 ^{bc}	
T3	58.31 ^{ij}	57.97 ^k	19.10 ^{deig}	19.35 ^{abc}	45.82 ^{gl}	46.03 ^{gle}	2.22 ^{ghi}	2.34 ^{bcd}	3.06 ^{hij}	3.38 ^{bc}	
T4	58.24 ^j	57.89 ^k	19.10 ^{deig}	19.56 ^a	45.74 ^g	46.45 ^{edc}	2.23 ^{ghi}	2.37 ^{ab}	3.12 ^{ghi}	3.35 ^{bcd}	
T5	58.96 ^g	58.55 ^h	18.80 ^{hi}	19.00 ^{eigh}	45.81 ^{gl}	45.84 ^{gl}	2.19 ^{ij}	2.27 ^{eig}	2.93 ^j	3.34 ^{bcd}	
T6	60.67 ^b	60.07 ^d	18.32 ^j	18.74 ⁱ	46.58 ^{edcb}	46.92 ^{abc}	2.09 ^k	2.12 ^k	2.98 ^{ij}	3.17 ^{cde}	
T7	58.2 ^{ij}	57.99 ^k	19.10 ^{deig}	19.35 ^a	45.77 ^{gl}	46.50 ^{edc}	2.28 ^{def}	2.29 ^{edf}	3.22 ^{deig}	3.53 ^a	
T8	59.28 ^e	59.06 ^g	18.91 ^{ghi}	19.34 ^{ab}	46.44 ^{edc}	47.47 ^a	2.19 ^{hij}	2.28 ^{def}	3.03 ^{hij}	3.38 ^{bc}	
T9	59.30 ^e	58.90 ^g	18.90 ^{ghi}	19.23 ^{bcd}	46.44 ^{edc}	46.80 ^{bcd}	2.19 ^{hij}	2.25 ^{eigh}	3.03 ^{hij}	3.18 ^{bc}	
T10	5.21 ^{ei}	58.94 ^g	18.90 ^{ghi}	19.12 ^{def}	46.35 ^{edc}	46.56 ^{edcb}	2.21 ^{hi}	2.28 ^{deig}	3.00 ^{ij}	3.30 ^{cde}	
T11	59.96 ^d	59.16 ^{ef}	18.70 ⁱ	18.88 ^{ghi}	46.70 ^{bcd}	46.23 ^{ged}	2.15 ^{jk}	2.25 ^{eigh}	3.01 ^{ij}	3.30 ^{bcd}	
T12	61.14 ^a	60.44 ^c	18.30 ^j	18.74 ⁱ	47.09 ^{ab}	47.36 ^a	2.09 ^k	2.11 ^k	2.93 ^j	3.25 ^{cdef}	

Values having different superscripts within the same item (treatments & storage periods) are significantly different (P ≤ 0.01).

* Treatments: See Table (1)

In different resultant cheeses, moisture, fat, total nitrogen and ash contents showed statistically significant ($P \leq 0.001$) increase during storage at $4 \pm 1^{\circ} \text{C}$. While the differences in fat / dry matter and total nitrogen were insignificant between fresh treatments and after 25 days. The highest moisture content was found in T6 and T12 (60.67 and 61.14 % respectively). This may be due to the addition of HGPJ which lead to decrease the total solids of the resultant cheese compared to other treatments; this decrease was due to the increase in the moisture content of the HGPJ (93.04 %). Cheese made with soymilk had similar chemical composition to control. These results are confirmed with those found by Aworh *et al.* (1987).

The salt content ranged between 2.98 - 3.02 % for different fresh cheeses. Salt content in different treatments of cheeses showed a slight increase during storage. This attributed to the loss of moisture during storage, the mean values of different treatments reached 3.07 - 3.10 % at the end of storage (25 days).

Table (4) shows the rate of titratable acidity (TA), pH and water soluble nitrogen (WSN) obtained during the storage period of UF - soft cheeses. WSN and TA% tended to increase, and pH to decrease during storage at $4 \pm 1^{\circ} \text{C}$. It is clear that fresh and stored cheeses prepared with *Lactobacillus acidophilus* had the lowest pH values compared to that of other treatments. The effect of starter and storage period on pH and acidity were found to be significant ($P \leq 0.01$). WSN was taken as an index for the proteolysis of UF- soft cheese during storage period. WSN contents ranged between 0.203 % to 0.273 % in fresh cheeses, and 0.36 % and 0.613 % at the end of the storage period. From Fig (1) it can be seen that the highest cholesterol content (16.37 mg/100g) was in control (T1), followed consecutively by cheese (T7) being made with starter (15.89 mg/100g) and those contained soybean milk without starter (T3, T4, T5, T2 and T6 respectively). Also, the results indicated that the cholesterol content insignificantly increased in all treatments without starter (T1 to T6) during storage. This is probably due to the slight increase in fat content during storage. Adding starter culture to cheese retentate had a significant effect on cholesterol content during storage. The cholesterol content of fresh and stored cheeses produced from T7 to T12 were found to be lower than the control, T2, T3, T4, T5 and T6, also showed a significant decrease ($P \leq 0.01$) throughout the storage. This may be attributed to presence of soybean milk and *Lactobacillus acidophilus* which considered to be responsible for this decrease. These results are confirmed with those found by Messina (1997) and Juskiewicz & Panfil - Kuncewicz (2003).

Table (4) : Values of titratable acidity, pH and soluble nitrogen of different treatments of fresh UF- soft cheese and at 25 days of storage (4 ± 1 ° C).

Item Treat.*	Titratable acidity (%)				pH				Soluble nitrogen (%)			
	Storage period (days)				Storage period (days)				Storage period (days)			
	0	8	16	25	0	8	16	25	0	8	16	25
T1	0.20 ^l	0.337 ^{mm}	0.41 ^l	0.507 ^l	6.53 ^{abc}	6.35 ^{degh}	6.05 ^{mn}	5.86 ^{op}	0.240 ^e	0.283 ⁿ	0.443 ^{de}	0.557 ^b
T2	0.173 ^l	0.28 ^{pq}	0.37 ^{kl}	0.570 ^e	6.60 ^a	6.42 ^{bcdef}	6.09 ^{klmn}	5.68 ^{qrs}	0.210 st	0.327 ^{lm}	0.377 ^{hi}	0.507 ^c
T3	0.200 ^l	0.270 ^{pq}	0.370 ^{kl}	0.477 ^{gh}	6.57 ^{ab}	6.40 ^{cde}	6.24 ^{hijk}	6.06 ^{mn}	0.233 ^{qrs}	0.240 ^{pqrs}	0.320 ^m	0.437 ^{def}
T4	0.190 ^l	0.247 ^{qrs}	0.330 ^{lmn}	0.450 ^{hi}	6.58 ^{ab}	6.46 ^{abcde}	6.29 ^{ghij}	5.97 ^{no}	0.217 ^{rst}	0.263 ^{opqkl}	0.353 ^{kl}	0.413 ^{fg}
T5	0.190 ^l	0.323 ^{mno}	0.497 ^{fg}	0.607 ^{de}	6.50 ^a	6.37 ^{de}	6.08 ^{lmn}	5.61 ^{qrst}	0.203 ^l	0.324 ^{lm}	0.453 ^d	0.520 ^c
T6	0.210 ^{rst}	0.303 ^{nop}	0.413 ^{ij}	0.470 ^{gh}	6.51 ^{abcd}	6.35 ^{de}	6.21 ^{klmn}	5.88 ^{op}	0.247 ^{opq}	0.350 ^{klmn}	0.527 ^c	0.613 ^d
T7	0.250 ^{qr}	0.360 ^{klm}	0.503 ^{fg}	0.693 ^c	6.41 ^{cde}	6.04 ⁿ	5.57 ^{rst}	5.53 st	0.267 ^{nop}	0.320 ^{lm}	0.373 ^{ij}	0.450 ^{de}
TJ	0.217 ^{rst}	0.397 ^{hi}	0.583 ^e	0.720 ^c	6.32 ^{efgh}	6.13 ^{klmn}	5.75 ^{pq}	5.51 ^l	0.247 ^{opq}	0.330 ^{klmn}	0.347 ^{klm}	0.403 ^{gh}
T9	0.206 st	0.413 ^{ij}	0.467 ^{gh}	0.607 ^{de}	6.47 ^{abcde}	6.22 ^{hijk}	5.98 ^{no}	5.71 ^{qr}	0.240 ^{pqrs}	0.277 ^{no}	0.333 ^{klm}	0.423 ^{efg}
T10	0.283 ^{opq}	0.460 ^{gh}	0.587 ^e	0.713 ^c	6.08 ^{lmn}	5.87 ^{op}	5.64 ^{qrst}	5.48	0.240 ^{pqrs}	0.273 ^{no}	0.323 ^{lm}	0.360 ^{ik}
T11	0.273 ^{pq}	0.380 ^{jk}	0.603 ^{de}	0.763 ^b	6.25 ^{ghij}	5.97 ^{no}	5.71 ^{qr}	5.51 ^l	0.273 ^{no}	0.340 ^{klm}	0.383 ^{hi}	0.460 ^d
T12	0.297 ^{nop}	0.500 ^{fg}	0.640 ^d	0.820 ^a	6.12 ^{klmn}	5.86 ^{op}	5.59 ^{rst}	5.34	0.250 ^{opq}	0.290 ⁿ	0.323 ^{lm}	0.377 ^{hij}

Values having different superscripts within the same item (treatments x storage periods) are significantly different (Ps 0.01).
 * Treatments : See Table (1)

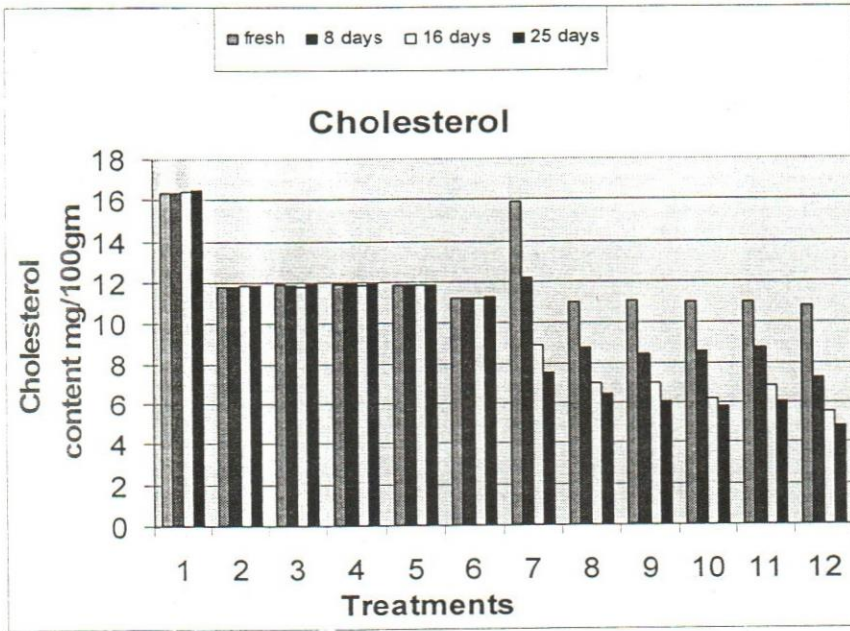


Fig (1) : Changes in Cholesterol contents of different treatments of UF – soft cheese during storage at $4 \pm 1^{\circ} \text{C}$.

Rheological characteristics:

The rate of curd syneresis of resultant cheese found in this study (table 5) significantly ($P \leq 0.01$) increased in all the treatments throughout storage at $4 \pm 1^{\circ} \text{C}$ for 25 days. This is due to changes of the physicochemical properties of cheese during cold storage , with values for fresh and 25 days ranging from 15.4 to 20.4 ml / 100 g cheese sample and from 17.8 to 24.9 ml / 100 g cheese sample , respectively . T10 exhibited low syneresis values, while T6 exhibited higher values when fresh and at the 25 day of storage at $4 \pm 1^{\circ} \text{C}$. The penetration values expressed the firmness of UF- soft cheese. Throughout the cold storage period (25 days at $4 \pm 1^{\circ} \text{C}$), an increase in penetration of cheese samples was observed .The penetration of cheese sample increased with adding HGPJ (T6 and T12) and led to increase in the rate of curd syneresis . This may be attributed to increase moisture content and acidity in these treatments. On the other hand cheeses prepared with starter culture (T7 to T12) had the high penetration compared with other treatments. This may be due to the addition of starter culture that decreased the pH values and consequently the relative cheese shrinkage which lead to increase in the firmness of the resultant cheese. These results are confirmed with those found by Azza (2004). Their data showed that, fresh cheese samples containing GDL had higher firmness than the control sample.

Table (5): Rheological properties of different treatments of fresh UF- oft cheese and days of storage at $4 \pm 1^{\circ} \text{C}$.

Item	Syneresis(g/100)		Penetration (mm)	
	Storage period (days)		Storage period (days)	
	0	25	0	25
T1	17.0 ^{ghi}	18.8 ^{def}	19.3 ^j	20.4 ⁱ
T2	17.8 ^{elg}	19.1 ^{cde}	20.6 ^{ghi}	21.6 ^{efghi}
T3	17.2 ^{igh}	19.6 ^{cd}	20.7 ^{ghi}	21.8 ^{efg}
T4	17.0 ^{ghi}	18.7 ^{def}	20.5 ^{hi}	22.2 ^{def}
T5	17.8 ^{elg}	19.07 ^{cde}	21.3 ^{ghi}	22.1 ^{def}
T6	20.4 ^{bcd}	24.9 ^a	23.1 ^{cd}	24.6 ^b
T7	15.4 ⁱ	19.8 ^{cd}	20.5 ⁱ	23.2 ^{cd}
T8	16.7 ^{ghi}	20.6 ^{bc}	21.5 ^{ghi}	23.5 ^{bc}
T9	16.0 ^{ghi}	19.4 ^{cde}	21.4 ^{ghi}	22.8 ^{cde}
T10	15.8 ^{hi}	17.8 ^{efg}	21.7 ^{efgh}	22.3 ^{cdef}
T11	16.3 ^{ghi}	19.1 ^{cde}	21.2 ^{ghi}	23.5 ^{bc}
T12	17.3 ^{igh}	21.5 ^b	23.5 ^{bc}	26.9 ^a

Values having different superscripts within the same item (treatments × storage periods) are significantly different ($P \leq 0.01$).

* Treatments: See Table (1)

Microbiological characteristics:

The survival rates of total count and *Lactobacillus acidophilus* in different treatments during storage at $4 \pm 1^{\circ} \text{C}$ are given in table (6). It was clearly noticed that the count of *Lactobacillus acidophilus* significantly increased ($P \leq 0.01$) during storage and reached the maximum number after 8 days , and decreased with extending the storage period. The main factors in loss of the viability of *Lactobacillus acidophilus* cultures have been attributed to the increase in the acidity of medium and accumulation of organic acids as a result of growth and fermentation. Similarly, Kailasapathy and Rybka (1997) found that, acidity of dairy products is one of the principal factors contributing to poor viability of probiotic culture. But the counts at the end of storage in the present study were still enough for cheese to be successful probiotic in T12 (3.6×10^7 cfu/g). Soy milk enhanced the growth of *lactobacillus acidophilus*. These results are in accordance with those reported by Shimakawa *et al.* (2003). In contrast El-Sayed and El- Sayed (1988) reported that the higher concentration of soymilk (20-30%) decreased the *Lactobacillus acidophilus* counts. Also, addition of HGPJ enhanced the viability of *lactobacillus acidophilus*. The analysis of variance indicated that the total count was significantly affected ($P \leq 0.01$) by storage period, different treatments and starter culture (table 7). Statistically , the interaction between the storage period and different treatments with or without *lactobacillus acidophilus* was significant ($P \leq 0.01$) for tctal count. The same table indicated that the total count was significantly increased ($P \leq 0.01$) during storage period . The number ranged between 3.3×10^5 to 2.7×10^6 , 1.07×10^6 to 8.47×10^8 , 3.2×10^7 to 8.13×10^9 and 1.1×10^8 to 2.26×10^{10} cfu/g cheese at 0 , 8 , 16 and 25 days of storage, respectively at $4 \pm 1^{\circ} \text{C}$. T10 had lowest total count . Generally, the addition of starter culture significantly decreased ($P \leq 0.01$) the total counts. These results are confirmed with those

reported by Charteris *et al.* (1998), who found that lactobacilli have to produce a number of different bacteriocins (the inhibitory activity may be restricted to gram positive bacteria since inhibition of gram negative bacteria has not been demonstrated) and bacteriocin like substances (act on both gram positive and gram negative bacteria) . Coliform were not detected in all treatments. Yeast and mould were detected in some stored treatments (T1 , T2 and T5) . This may be due to recontamination during the manufacturing processes. The numbers were 28 , 10 and 63 cfu/g cheese at 16th day and 57 , 88 and 119 cfu/ g at the end of storage in T2 , T1 and T5 respectively . Few visible colonies of yeast and bacteria started to appear on the surfaces of the cheese in the most treatments except T10 and T12 after 28 – 30 days of storage, therefore the analysis stopped after 25 days .

Sensory evaluation of UF- soft cheese:

List of the parameters used to evaluate the different cheese samples are shown in fig. (2). The scoring of cheese was carried out at 4 different stages namely fresh, 8, 16, 25 days. Sensory evaluation illustrated that, the cheese produced from T12 and T10 were superior in organoleptic properties as compared with control or the other treatments. This may be due to the addition of HGPJ or cayenne pepper with starter culture lead to improve the flavour of cheese. Addition of HGPJ (T6 and T12) resulted in good flavour, smooth texture and softer body compared with the other treatments. This might be attributed to the increase of its moisture content compared to other treatments .

While, the lowest value of total score was found in cheese samples containing soybean milk without any other additives (T2). This may be due to beany flavour and slight yellowish color. However the cheese was accepted and was not improve during storage These results have the same direction with the findings of Metwally (2000) . The addition of AGPE, CP and HGPJ resulted in significant improved ($P \leq 0.01$) in taste of the resultant cheese sample containing SM (T2) . These additives masked the beany flavour and off – flavour. But, paprika had no effect on the flavour of the finished cheese (T3 and T9), compared with control (T1) . On the other hand, the use of *Lactobacillus acidophilus* lead to better results, (T7) compared with control (T1) . These results are in accordance with those reported by Bayomi (1991) and Okda (2001). The scores of body and texture of UF- soft cheese manufactured from mixture of SM and buffaloe's milk were very close to that of control (T1) . Total sensory scores of all treatments were decreased at the end of storage (25 days) .Total organoleptic scores in all cheeses significantly ($P \leq 0.01$) decreased during storage from 88.7 , 76.3 , 86.5 , 91.0 , 88.0 , 93.7 , 91.3 , 84.0 , 85.7 , 95.0 , 85.7 and 95.3 to 76.3 , 63.1 , 74.0 , 78.0 , 74.0 , 72.0 , 75.3 , 68.8 , 76.0 , 58.7 and 74.0 after 25 days of storage in T1 to T12 respectively .

UF – soft cheese remained acceptable for 25 days at $4 \pm 1^{\circ}$ C without any apparent changes in sensory quality. Results of statistical analysis indicated that , there are significant differences ($P \leq 0.01$) between treatments containing SM and control in total scores .

Table (6) : Total count and *Lactobacillus acidophilus* of different treatments of fresh UF- soft cheese and 25 days of storage at 4 ± 1 °C.

Item Treat.*	Total count (cfu / ml)								<i>Lactobacillus acidophilus</i> (cfu / ml)				
	0	8	16	25	0	8	16	25	0	8	16	25	
T1	2.70E ^{6rs}	8.47E ^{6klm}	1.07E ^{9kl}	2.26E ^{10q}	—	—	—	—	—	—	—	—	
T2	1.27E ^{6rs}	3.77E ^{6nopqr}	8.13E ^{9l}	2.05E ^{10b}	—	—	—	—	—	—	—	—	
T3	2.17E ^{6rs}	6.80E ^{6klmno}	1.10E ^{9k}	1.40E ^{10e}	—	—	—	—	—	—	—	—	
T4	1.03E ^{6rs}	3.30E ^{6nopqr}	4.97E ^{6mnopq}	7.70E ^{9h}	—	—	—	—	—	—	—	—	
T5	1.83E ^{6rs}	4.50E ^{6mnopq}	7.07E ^{6klmn}	1.52E ^{10d}	—	—	—	—	—	—	—	—	
T6	2.50E ^{6rs}	6.33E ^{6klmnop}	1.08E ^{9kl}	1.58E ^{10c}	—	—	—	—	—	—	—	—	
T7	1.10E ^{6rs}	2.80E ^{6r}	7.30E ^{6r}	5.00E ^{6mnopq}	3.20E ^{5l}	2.03E ^{9c}	7.40E ^{9gh}	5.27E ^{9kl}	—	—	—	—	
T8	1.03E ^{6rs}	3.70E ^{6r}	8.93E ^{6r}	5.83E ^{6mnopq}	1.20E ^{5l}	2.47E ^{9d}	9.90E ^{9g}	9.63E ^{9kl}	—	—	—	—	
T9	9.67E ^{5s}	2.73E ^{6rs}	6.53E ^{6r}	3.30E ^{6nopqr}	2.20E ^{5l}	2.50E ^{9d}	11.30E ^{9g}	1.63E ^{6jk}	—	—	—	—	
T10	3.33E ^{5s}	1.07E ^{6rs}	3.20E ^{6r}	1.10E ^{6qr}	1.63E ^{5l}	1.87E ^{9el}	2.73E ^{6jk}	1.23E ^{9kl}	—	—	—	—	
T11	1.03E ^{6rs}	2.37E ^{6r}	6.97E ^{6r}	4.50E ^{6mnopq}	4.60E ^{5l}	2.10E ^{9e}	5.20E ^{9h}	8.03E ^{9kl}	—	—	—	—	
T12	9.33E ^{5s}	2.03E ^{6r}	5.30E ^{6r}	1.60E ^{6pqr}	4.80E ^{5l}	5.57E ^{9a}	4.13E ^{9b}	3.60E ^{6jk}	—	—	—	—	

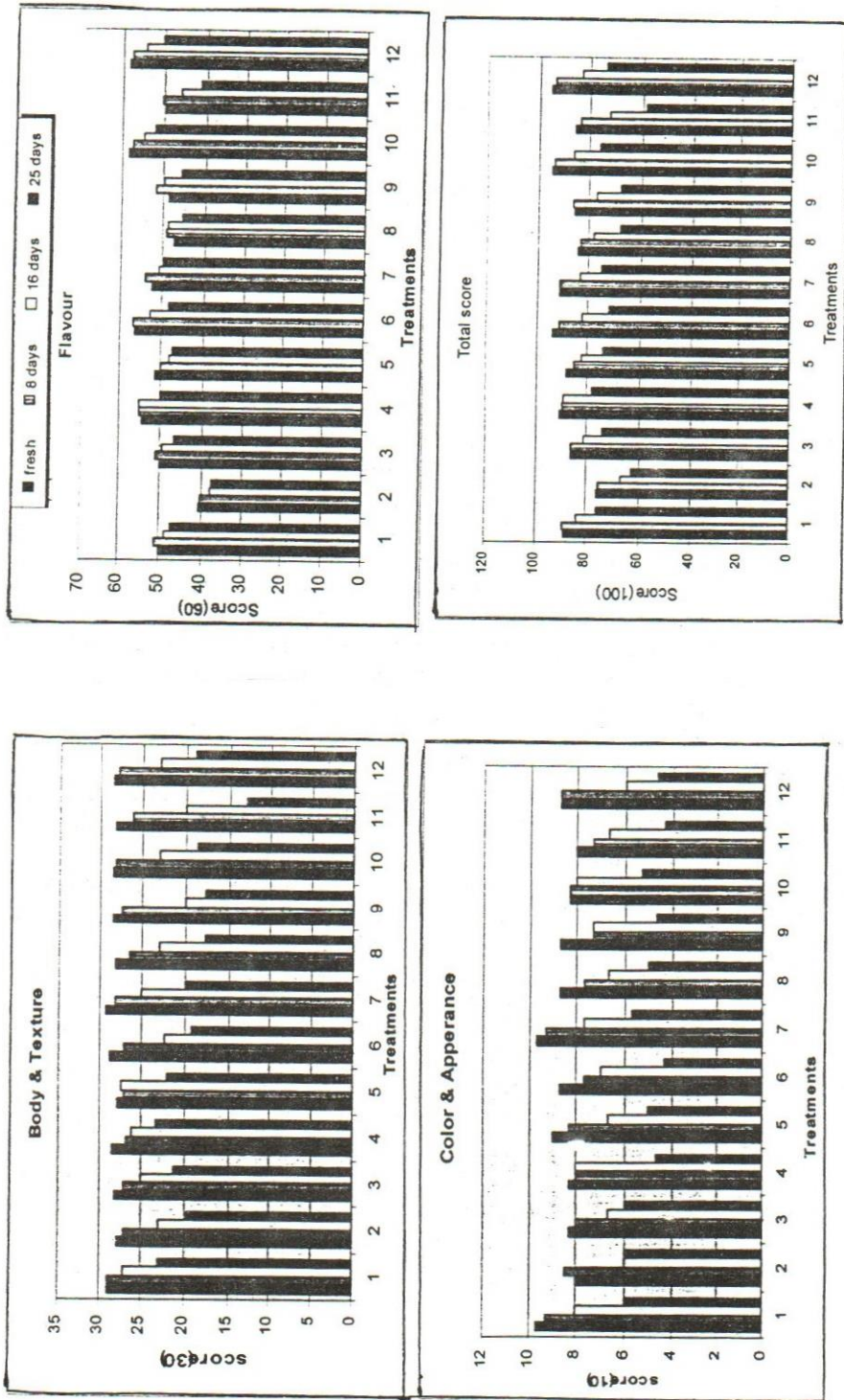
Values having different superscripts within the same item (treatments × storage periods) are significantly different (PS 0.01) .

* Treatments: See Table (1)

Table (7) : Effects of starter , storage period and treatments on total count of UF- soft cheese .

Main effect	Total count (cfu / g)											
	Without starter						With starter (Lactobacillus acidophilus)					
Starter	4.35 E ^{9A}						1.17 E ^{9B}					
	0						16					
Storage period	1.11 E ^{6B}						1.72 E ^{9B}					
	8						9.73 E ^{8B}					
Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
	0.13 E ^{9A}	5.4 E ^{9B}	3.95 E ^{9D}	2.13 E ^{9F}	4.10 E ^{9D}	4.40 E ^{9C}	1.03E ^{9GH}	9.70 E ^{8H}	1.14 E ^{9G}	5.7 E ^{8I}	8.6 E ^{8H}	2.4 E ^{9E}

Means having different capital super scripts within each main effect are significantly different (PS 0.01) .



Fig(2) : Changes in organoleptic properties of different treatments of UF — soft cheese during storage at 4 ± 1 °C .

CONCLUSION

It can be concluded that acceptable UF-soft cheese with high organoleptic properties, reasonable viable count of *Lactobacillus acidophilus* and lower cost can be manufactured using mixture of soybean milk and buffalo's milk (35 : 65), 1% *Lactobacillus acidophilus* culture, HGPJ (7%) or CP (0.1%) and stored about 25 days at $4 \pm 1^{\circ} \text{C}$.

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تأثير استخدام لبن فول الصويا و باديئ *Lactobacillus acidophilus* و

بعض مواد النكهة على خواص الجبن الطري المصنع بالترشيح الفوقي

وداد عزب متري

قسم الألبان – كلية الزراعة – جامعة القاهرة – فرع الفيوم – جمهورية مصر العربية

تهدف هذه الدراسة إلى إنتاج جبن طري منخفض التكاليف و ذو قيمة صحية عالية و لذلك تم في هذا البحث تصنيع ١٢ معاملة من الجبن الأبيض المصنع بطريقة الترشيح الفوقي مع استبدال جزء من اللبن الجاموسى (المستخدم في التصنيع) بلبن فول الصويا (نسبة استبدال ٣٥ %) و كذلك إضافة بعض مواد النكهة الصناعية مثل أسانس الفلفل الأخضر بنسبة ٨٠ مل / ١٠٠ كجم مترکز و كذلك استخدمت مصادر نكهة طبيعية مثل البابريكا ، الفلفل الأحمر الحريف المجفف ، عصير الفلفل الأخضر الحار بنسبة ٠,٢ ،

- ٠,١ ، ٧ % على التوالي لتحسين نكهة الجبن و جودتها و ذلك في وجود و غياب بادئ يحتوى على ميكروب *lactobacillus acidophilus* بنسبة ١ % و قد تم إضافة كلوريد الكالسيوم (٠,٠٢ %) و كلوريد صوديوم (٣ %) إلى المترکز ، ثم أضيفت المنفحة إلى كل جزء على حدة . خزنت الجبن الناتجة على 4 ± 1 م لمدة ٢٥ يوم تم خلالها دراسة التغيرات الكيميائية ، الريولوجية و الميكروبيولوجية و الحسية للجبن الناتجة من المعاملات المختلفة بالمقارنة بالعينة الكنترول (بدون إضافات) . و كانت أهم النتائج المتحصل عليها هي :
- وجد ان التركيب الكيميائي للجبن الناتجة من المعاملات المختلفة كان متقارب و كان له نفس الاتجاه اثناء التخزين ، كما لوحظ زيادة معنوية في نسبة النيتروجين الذائب في الماء بالعينات الغير محتوية على بادئ مقارنة بتلك المحتوية على البادئ بينما وجد عكس ذلك بالنسبة للحموضة المعيارية .
 - ارتفاع المحتوى من الكوليسترول في العينة الكنترول عن العينات الأخرى ، إضافة لبن الصويا ادت الى حدوث انخفاض معنوي في محتوى عينات الجبن من الكوليسترول و التي زاد بمعدل طفيف جدا اثناء التخزين و من ناحية أخرى فان إضافة بادئ محتوي على *lactobacillus acidophilus* أدى الى خفض محتوى العينات من الكوليسترول بالمقارنة بالعينات الغير محتوية على بادئ ، و قد زاد هذا الانخفاض بشكل معنوي اثناء فترة التخزين .
 - حدث زيادة في معدل اختراق الخثره penetration وكذلك معدل انفصال الشرش اثناء فترة التخزين و قد أدى إضافة عصير الفلفل الى زيادة كمية الشرش المنفصلة وكذلك زيادة مسافة اختراق الخثره بالمقارنة بالعينات الأخرى .
 - تراوحت اعداد الخلايا الحية من مزرعة *L.acidophilus* في المعاملات الطازجة من $1,2 \times 10^8$ الى $4,8 \times 10^8$ خلية / مللي بينما تراوح هذا العدد ما بين $1,23 \times 10^6$ الى $9,63 \times 10^6$ خلية / مللي بعد مرور ٢٥ يوم تخزين و قد سجلت العينات المحتوية على عصير الفلفل الأخضر اعلى حيوية للميكروب سواء للعينات الطازجة ($4,8 \times 10^8$ خلية / مللي) او عند تخزين على درجة حرارة 4 ± 1 م ($3,6 \times 10^7$ خلية / مللي) و من ناحية أخرى فان العدد الكلي للميكروب قد ازداد معنويا اثناء فترة التخزين و كان معدل الزيادة اقل معنويا في حالة عينات الجبن المصنع بإضافة بادئ مقارنة بتلك الغير محتوية على بادئ و كانت العينات المحتوية على الفلفل الأحمر الحار المجفف الاقل معنويا في العدد الكلي للميكروبات سواء طازجة او اثناء التخزين و قد ظلت فترة أطول من المعاملات الأخرى بدون فساد كما قد دلت النتائج على خلو العينات كلها من مجموعة ميكروبات القولون او الخمائر و الفطريات فقد ظهرت في بعض العينات دون الأخرى اثناء فترة التخزين حيث سجل عدد ٢٨ ، ١٠ ، ٦٣ خلية / جرام في اليوم الـ ١٦ من التخزين و وصل في نهاية التخزين وهي ٢٥ يوم الى ٥٧ ، ٨٨ ، ١١٩ خلية / جرام جبن ناتجة من تلك المعاملات المحتوية على لبن صويا فقط (معاملة ٢) ، العينة الكونترول (معاملة ١) و عينة الجبن المحتوية على إضافة الفلفل الأخضر بدون بادئ على التوالي و قد بدء ظهور عدد من المستعمرات المرئية من الخمائر والبكتريا على سطح الجبن الناتج من معظم المعاملات بعد مرور ٢٨ - ٣٠ يوم من التخزين ولذلك تم إيقاف التحليلات المختلفة عند ٢٥ يوم .
 - دلت نتائج التقييم الحسي لعينات الجبن الناتجة من المعاملات المختلفة على حدوث انخفاض معنوي في نكهة الجبن الناتج من كل المعاملات حصلت الجبن المحتوية على لبن صويا فقط (معاملة ٢) على اقل درجات التحكيم بالمقارنة بالكونترول و باقي المعاملات و إضافة مواد النكهة المختلفة الطبيعية او الصناعية أدت الى ظهور تحسن ملحوظ في نكهة الجبن و كانت أعلى المعاملات في الدرجات هي تلك المحتوية على عصير الفلفل الأخضر الحار .
 - إضافة البادئ الى المترکز المستخدم في التصنيع أدى الى زيادة درجات التحكم عن العينة الكونترول وكذلك عينات الجبن المصنعة من ملحوظ لبن صويا مع لبن جاموسى مع إضافة البادئ و مواد النكهة الطبيعية قد حصلت على اعلى درجات التحكيم وخاصة تلك العينات المحتوية على عصير الفلفل الأخضر الحار ومسحوق الفلفل الأحمر الحار فكانت هي المفضلة من معظم المحكمين طوال فترة التخزين .