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Original Article

Improving the Properties of the Egyptian Hard Cheese (Ras Type) with Adding Some Probiotic *Lactobacillus* spp. as Adjunct Cultures

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Abstract

The objective of the present study was to improve the quality of the Egyptian hard cheese (Ras type) by adding some *Lactobacillus* spp. as probiotic adjunct cultures. Strains of *Lb. acidophilus*, *Lb. helveticus* and *Lb. casei* were used and the chemical, microbiological and sensory characteristics of the cheese during 90 days of ripening were evaluated. The obtained data indicated that pH values and moisture content were decreased, while the total nitrogen (TN), fat, fat/dry matter, titratable acidity and salt contents were increased significantly during the ripening period in all treatments. For the ripening indices, significantly higher content of soluble nitrogen (SN), SN/TN, soluble tyrosine, soluble tryptophan and total volatile fatty acids were found in the experimental cheese compared to the Control cheese. Higher *Lactobacilli* count were observed in the adjunct treated cheeses, which reflects the positive retention of *Lactobacilli* in the experimental cheeses. Also, the use of probiotic adjunct cultures reduced the growth of fungi and prevented the coliform bacteria on Ras cheese. There was higher acceptability for the experimental cheeses than for the Control. Ras cheese was considered a good carrier for *Lactobacillus* probiotic strains since they were found to survive during cheese manufacture, ripening and storage.

Keywords: *Ras cheese; Adjunct cultures; Probiotic bacteria; Cheese quality; Sensory evaluation*

Introduction

Ras cheese is one of the most famous hard cheese in Egypt. It is traditionally manufactured from raw cow's milk or a mixture of cow and buffalo milk under artisanal conditions without the use of specific starters and is consumed when it has a strong sharp flavor close to the Greek variety (Kefalotyri cheese) after 3 to 6 months (Dabiza and El-Deib, 2007; Hattem *et al*., 2012).

Fermentation in Ras cheese occurs by the natural microbes present in the raw milk and production environment. The cheese is usually stored in uncontrolled conditions of high humidity, which encourages the growth of fungi

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and yeasts that affect the final sensory properties such as flavor and texture (Awad, 2006).

The Egyptian Organization for Standardization and Quality Control emphasizes the implementation of quality standards that indicate that all types of cheese should be made from pasteurized milk. Milk pasteurization affects the characteristics and properties of cheese during maturation (Singh *et al*., 2003). Therefore, applying the standards to the Ras cheese making will require using starter cultures.

To get the best flavor and taste of cheese, careful selection of the microorganisms used in cheese making is very important. Typical lactic acid bacteria are usually selected as starters and adjuncts in Ras cheese that contribute much to the sensory properties and the rate of flavour compounds (El-Soda *et al*., 2000).

The highly autolyzed starter cultures increase the free amino acids which positively affect the flavour of cheese, while poorly autolyzed strains enhance the off-flavours. This prompts us to choose an accurate strains in cheese making (Hickey *et al*., 2007).

One of the factors affecting the microorganisms in hard cheese is the non-starter lactic acid bacteria (NSLAB), which reaches the cheese as a post-pasteurization contamination. The use of adjunct culture in this case is one way to control NSLAB and improve flavor properties (Broadbent *et al*., 2003).

Adjunct cultures are microorganisms added to cheese milk intentionally and positively affect cheese sensory properties. It is often where NSLAB has been previously isolated from mature cheese and is known to improve the quality of cheese. These cultures are common practices in cheese making today for flavour enhancement (Johnson and Lucey, 2006).

The cultures used as adjuncts should be appropriately bred to produce the desired benefit of the quality of the cheese, without causing defects such as off-flavors or gassing. The effect of adjunct cultures on flavor is known to be due to increased protein hydrolysis and the resulting production of small peptides and free amino acids (Di Cagno *et al*., 2003).

According to O'Sullivan *et al*. (2007), Lactic acid bacteria (LAB) play the main role in cheese manufacture, especially hard cheese. The most important five genera of LAB that contribute to the flavor of cheese are: *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*.

Some types of LAB are commonly used as probiotics in cheese making. These bacteria promote human health by exhibiting opposite effects toward enteropathogenic bacteria that cause intestinal diseases, reduce the risk of diarrhea, enhance immune system function, reducing cholesterol levels, relieve symptoms of lactose intolerance and synthesizing vitamins (Kebary *et al*., 2005; Hussein *et al*., 2006).

Incorporation of probiotic bacteria as a starter in cheese, especially *Lactobacilli*, possess many peptidases that can hydrolyze peptides and produce free amino acids and oligopeptides, changing the flavour, body and texture, and thus in the sensory properties of cheese (Santillo and Albanzia, 2008; Soufa and Saad, 2009). Several types of *Lactobacillus* ssp. have been used in the manufacture of Ras cheese (Abdalla *et al*., 2008) and are still within the recommended counts i.e. 10^6 – 10^7 CFU/g of probiotic in the cheese process condition to enhance their health beneficial effect (El-Alfy *et al*., 2012).

Ras cheese is the most preferred and easy food for children and adults in Egypt, especially as a school sandwich or a quick breakfast, so attention to produce a healthy Ras cheese is very important. In addition to the spread of diseases caused by the presence of mycotoxin in food even if with little amount. There are many physical, chemical, and biological ways to prevent the growth of fungi or eliminate their toxins in dairy products. However, the best way is to avoid the presence of mycotoxins by preventing mold contamination.

So, the objective of this study was to investigate the possibility to make a high-quality probiotic Ras cheese using some strains of probiotic *Lactobacilli* as adjunct cultures and to study the effect of this on the chemical, microbiological, and organoleptic properties of Ras cheese.

Materials and Methods

Materials

Milk used for the manufacture of Ras cheese was obtained from the experimental station's cow herd at Assiut University's Faculty of Agriculture. The rennet and the commercial salt were purchased from the local store in Assiut city, Egypt. Pure cultures of *Streptococcus thermophilus* 14486, *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842, *Lactobacillus casei* subsp. *casei* 393, *Lactobacillus acidophilus* 4356 and *Lactobacillus helveticus* 15009 were supplied by the American Type Culture Collection (ATCC). A fresh solution of 40% calcium chloride, purchased from El. Nasr Pharmaceutical Chemical Co., Egypt, was prepared and it was added to the heated milk to give a final concentration of 0.02% of milk. Cheese wax of commercial fine grade was bought from a local market in Assiut, Egypt.

Ras cheese manufacture

Cow's milk (100 liter) was heated momentarily to 73°C and then it was divided into 4 parts equally to make different treatments. Ras cheese was made as to the procedure of Hammam *et al*. (2020) which is explained in Fig. (1). Lactic acid starter (1%) was added at 32°C, mixed well and left for a half-hour for acid development then calcium chloride (0.02%) and rennet were added.

After coagulation within 40 minutes, the curd was cut into small cubes and the temperature was raised to 45°C in 15 minutes. The curd was held at this temperature for 50 minutes. The whey was then drained down to the level of the curd (acidity 0.14%). Then, the salt was added (2% of used milk) and mixing for

15 minutes then the curd was cooled, molded and pressed with 160 lbs for the first 2 hours. Overnight pressing was done by increasing the weight up to 1000 lbs. Fig. 2 shows the form of Ras cheese after the pressing process is completed. The cheese wheel was then dried and salted on both sides with ten grams of dry salt day after day for 12 days. Cheese then was waxed and ripened for 3 months at 13± 2°C and about 85% relative humidity.

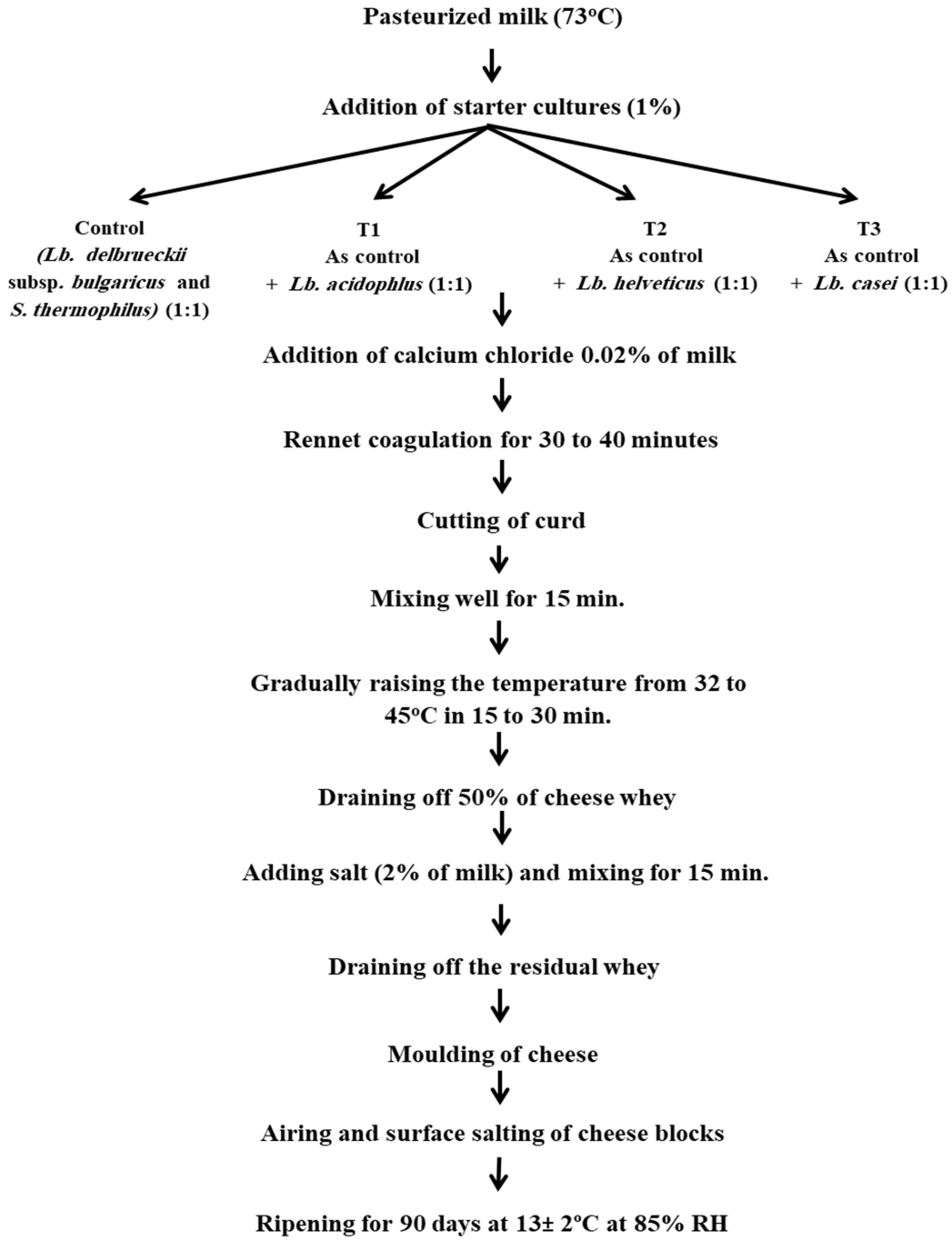

Fig. 1. Diagram showing the steps of Ras cheese making

Fig. 2. The form of Ras cheese after the pressing process

The experimental treatments varied according to the type of starters used as follow: C: (Control) (*Streptococcus thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (1:1)). T1: (*Lb. acidophilus* + (*Streptococcus thermophilus, Lb. delbrueckii* subsp. *bulgaricus*) (1:1). T2: (*Lb. helveticus* + *(Streptococcus thermophilus, Lb. delbrueckii* subsp. *bulgaricus*) (1:1). T3: (*Lb. casei* + *(Streptococcus thermophilus, Lb. delbrueckii* subsp. *bulgaricus*) (1:1).

Chemical analyzes

Cheese samples of all treatments were chemically analyzed for moisture, total solids, fat content, total nitrogen and soluble nitrogen % according to the standard AOAC methods (2012). The titratable acidity, pH values and salt content were determined according to the method described by Hooi *et al*. (2004). The method of Vakaleris and Price (1959) was used to determine the soluble tyrosine and soluble tryptophan content of cheese. The Total volatile fatty acids in cheese were measured using the method of Kosikowski (1982).

Microbiological Analysis

Cheese samples were examined for total bacterial count as method recorded by Laird *et al*. (2004). *Lactobacilli* and *Streptococci* counts determined by the IDF 117B method (1997). Coliform count detected using the IDF 73A method (1985). Yeasts and Moulds counts recorded according to IDF 94A method (1985).

Sensory evaluation

Seven panelists of the staff members of the Dairy Science Department, Faculty of Agriculture, Assiut Al-Azhar University conducted the organoleptic evaluation for the sensory attributes of the cheese samples at 30, 60 and 90 days of ripening according to Hofi *et al*. (1991). The samples were presented to the panelists in random order and evaluated for the flavor, body and texture, appearance and overall acceptability with 45, 35, 20 and 100 points, respectively.

Experimental design and Statistical analyses

A factorial design with treatment and storage period as fixed factors was used. Analysis of variance was performed to examine the response variables (cheese composition) using a two-way ANOVA. For the sensory analysis, one-way ANOVA was used for every storage period separately (30, 60 and 90 days)

and the treatment of production as fixed factor. Analysis of data was performed using the GLM procedure of SPSS Statistics 22 (SPSS for windows 2013).

Results and Discussions

Gross chemical composition

Table (1) shows the effect of adding some adjunct cultures on the chemical composition of Ras cheese during ripening. It is clear from the data that the moisture content decreased gradually throughout the ripening period in all treatments. This may be due to the water evaporation during ripening or becomes bound with the protein as bound water when ripening progressed (Conner, 1980). From the present data, this trend of moisture decreased coincided with that found by Abd-Ellah (2008). Also, most of the moisture loss occurred during the first month of the ripening period which is in accordance with the results of El-Essawi *et al*. (2013). Results presented show that the T2 cheese made from adding *Lb. helveticus* as adjunct culture contained higher moisture content ($P \leq 0.05$) when fresh than other treatments, followed by those of C, T1 and T3, respectively. This result agrees with those of Kebary *et al*. (2011) who stated that the increase of cheese acidity during ripening may reduce the cheese moisture content, which consequently helps to expel the whey from the cheese curd. This is consistent with the acidity content of T2 (Table 1), which is lower ($P \leq 0.05$) than T1 and T3. In general, the mean moisture content was higher in T1 ($P \leq 0.05$) with a value of 35.55% than the other treatment.

The titratable acidity of all cheese treatments was increased gradually ($P \leq 0.05$) reaching the maximum values at the end of the ripening period (Table 1). As it is known, the total acidity of cheese is naturally caused by milk constituents in addition to acidity developing during cheese ripening. The degradation intermediates compounds of protein and amino acids, as well as fatty acids resulting from fat hydrolysis, would appreciably contribute to an increase in the acidity of cheese (Abd El-Monem, 2018). The acidity content of the cheese treatments were 0.40, 0.87, 0.67 and 0.82% for fresh C, T1, T2 and T3, respectively. However, the results were 1.50, 2.40, 1.65 and 2.25% after 90 days of storage period for the same previous treatments, respectively. The T1 cheese that made from adding *Lb. acidophilus* as an adjunct culture had significantly higher acidity ($P \leq 0.05$) than all treatments during the different stages of storage, while the Control treatment presented the lower acidity.

Similar trends were reported by Abd-Ellah (2008). The acidity differences across cheese treatments could be attributed to *Lactobacillus* strain growth rates and their ability to ferment lactose throughout the ripening stage. These findings are consistent with those of El-Zayat and Osman (2001), who found that using different *Lactobacillus* starter cultures in the production of Domiati cheese increased the acidity.

The pH values of all cheese treatments were decreased gradually ($P \leq 0.05$) and reached the minimum values at the end of the ripening process (Table 1). The cheese made from adjunct culture had significantly lowest pH values ($P \leq$

0.05) than the Control. Our study agrees with the results of Madkor *et al*. (2000) who found that the Cheddar cheese made with the *Lb. casei* strain had lower pH values than did *Lb. helveticus*-treated cheeses.

Table 1. Effect of adding probiotic adjunct cultures on chemical composition during the ripening period of Ras cheese.

Property	Treatment	Storage (days)						Mean ± SD
		Fresh	15 days	30 days	45 days	60 days	90 days	
Moisture %	C	44.37	34.74	32.54	31.5	31.00	30.33	34.08^{b}±5.3
	T1	41.41	35.65	35.11	34.43	33.97	32.72	35.55^{a}±3.0
	T2	45.75	36.47	32.01	31.45	30.89	29.90	34.41^{b}±6.0
	T3	36.71	31.92	29.90	27.50	26.90	25.74	29.78^{c}±4.1
Mean ± SD		42.06^{a} ±4.0	34.70^{b} ±2.0	32.39^{c} ±2.1	31.22^{d} ±2.8	30.69^{c} ±2.9	29.67^{e} ±2.7	
Dry matter % (DM)	C	55.63	65.26	67.46	68.50	69.00	69.67	65.92^{b}±5.3
	T1	58.58	64.34	64.88	65.57	66.02	67.27	64.44^{c}±3.0
	T2	54.25	63.52	67.99	68.55	69.10	70.10	65.59^{b}±6.0
	T3	63.29	68.07	70.09	72.49	73.10	74.25	70.22^{a}±4.1
Mean ± SD		57.94^{e} ±4.0	65.30^{d} ±2.0	67.61^{c} ±2.1	68.78^{b} ±2.8	69.31^{c} ±2.9	70.32^{a} ±2.9	
Acidity %	C	0.40	0.87	1.10	1.20	1.32	1.50	1.06^{c}±0.4
	T1	0.87	1.22	1.47	1.87	2.25	2.40	1.68^{a}±0.6
	T2	0.67	0.82	1.05	1.22	1.37	1.65	1.13^{c}±0.4
	T3	0.82	1.05	1.55	1.72	2.07	2.25	1.58^{b}±0.6
Mean ± SD		0.69^{f} ±0.2	0.99^{e} ±0.5	1.29^{d} ±0.3	1.50^{c} ±0.4	1.75^{b} ±0.5	1.95^{a} ±0.4	
pH	C	5.96	5.77	5.63	5.37	5.28	5.20	5.54^{a}±0.3
	T1	5.29	5.12	5.10	5.17	5.22	5.37	5.21^{c}±0.1
	T2	5.74	5.49	5.46	5.39	5.36	5.34	5.46^{b}±0.2
	T3	5.48	5.27	5.19	5.14	5.10	5.10	5.21^{c}±0.2
Mean ± SD		5.62^{a} ±0.3	5.41^{b} ±0.3	5.35^{c} ±0.2	5.27^{d} ±0.1	5.24^{d} ±0.1	5.25^{d} ±0.1	
Salt %	C	3.25	3.75	3.80	3.95	4.20	4.45	3.90^{c}±0.41
	T1	3.20	3.40	3.45	3.65	3.75	4.00	3.58^{d}±0.28
	T2	3.40	4.10	4.40	4.70	4.85	4.95	4.40^{b}±0.58
	T3	3.55	4.05	4.90	5.10	5.20	5.50	4.72^{a}±0.75
Mean ± SD		3.35^{f} ±0.2	3.83^{e} ±0.3	4.14^{d} ±0.6	4.35^{c} ±0.7	4.50^{b} ±0.7	4.73^{a} ±0.7	
Salt/DM %	C	5.84	5.75	5.71	5.40	6.08	6.38	5.86^{b}±0.3
	T1	5.46	5.28	5.31	5.56	5.68	5.94	5.54^{c}±0.3
	T2	6.27	6.45	6.47	6.85	7.01	7.03	6.68^{a}±0.3
	T3	5.60	5.95	7.00	7.03	7.11	7.40	6.68^{a}±0.7
Mean ± SD		5.79^{c} ±0.4	5.86^{c} ±0.5	6.12^{b} ±0.8	6.21^{b} ±0.9	6.47^{a} ±0.7	6.69^{a} ±0.7	
Fat %	C	26.07	29.30	32.50	33.50	34.00	35.00	31.73^{ab}±3.4
	T1	27.00	31.10	32.60	33.50	34.00	35.50	32.28^{a}±3.0
	T2	25.63	29.60	32.50	33.60	33.90	34.10	31.56^{b}±3.4
	T3	24.50	25.30	27.50	29.50	31.50	32.50	28.47^{c}±3.3
Mean ± SD		25.80^{d} ±1.0	28.83^{c} ±2.5	31.28^{b} ±2.5	32.53^{a} ±2.0	33.35^{a} ±1.2	34.28^{a} ±1.3	
Fat/DM %	C	46.86	44.90	48.21	48.91	49.29	50.24	48.07^{b}±1.9
	T1	46.07	48.34	50.22	51.09	51.46	52.76	49.99^{a}±2.4
	T2	47.28	46.59	47.82	49.05	49.06	48.64	41.07^{c}±1.0
	T3	38.71	37.17	39.29	40.67	43.09	43.17	40.35^{c}±2.4
Mean ± SD		44.73^{c} ±4.0	44.25^{d} ±4.9	46.39^{b} ±4.9	47.43^{a} ±4.6	48.23^{a} ±3.6	48.70^{a} ±4.1	

Means within the same columns and rows with different subscriptions are significantly different (P≤0.05).
C: (Control) (*Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus* (1:1)).
T_1: *(Lb. acidophilus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T2: (*Lb. helveticus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T_3: *(Lb. casei + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
Lb= Lactobacillus, SD= Standard Deviation

The percentages of salt and salt/dry matter values of the resultant Ras cheese are presented in Table (1). During storage, the percentage of salt % and salt/dry matter increased gradually ($P \leq 0.05$) probably due to the loss of water caused by evaporation (El-Etriby *et al*., 1998). Also, this increase might be due to more absorption of the sprinkled salt by osmosis during the salting process. Furthermore, when compared to the other treatments, the samples of T3 cheese prepared by adding *Lb. casei* as an adjunct culture had the highest salt concentration when fresh and during the ripening period ($P \leq 0.05$), followed by T2, C and T1. These results coincide with previous studies that already reported (Abd-Ellah, 2008; El-Baz *et al*., 2011).

Fat and Fat/DM content of Ras cheese made from different treatments presented in Table (1). The fat content of cheese is the most important component which greatly affects the palatability of the cheese as it is also partly responsible for the smoothness and richness of the body and texture of the dairy product (Abd El-Monem, 2018).

It is well established that fat is important to both the quality and yield of cheese. There is a gradual increase in fat content % of Ras cheese ($P \leq 0.05$) which is obviously due to the progressive loss in moisture accruing during ripening and also could be owing to the decrease in other non-fat constituents due to the growth and activity of microorganisms in all treatments (Kamaly, 1978 and Ezzat, 1990). Results in Table (1) show that the treatment of T1 had the highest fat and fat/DM during ripening ($P \leq 0.05$) followed by C, T2 and T3, respectively.

Proteolysis and flavour components

Table (2) showed the average TN% in Ras cheese made from different treatments during the ripening period. From the present data, it can be observed that TN values tended to increase significantly ($P \leq 0.05$) throughout ripening. Changes in TN content could be attributed to the continued loss of moisture during ripening. Also, during cheese processing, casein is physically and chemically changed by rennet enzymes. The action of these enzymes is well established for protein degradation and breakdown (Abd El-Monem, 2018). During cheese ripening, the protein undergoes several changes and more simple nitrogenous compounds are formed. The data in Table 2 show that total nitrogen content was significantly high ($P \leq 0.05$) in Ras cheese made with adjunct cultures than the Control cheese. The TN content was the highest in the treatment of T1 at the beginning of ripening followed by T3, T2 and then C. While at the end of the ripening period, the TN contents in T3 had the highest values followed by T1, T2 and then C. These results are consistent with previous studies (Abd-Ellah, 2008; El-Baz *et al*., 2011 and Ibrahim *et al*., 2011). This increase could be attributed to the acidity formed by the adjunct starter culture used in probiotic Ras cheese making. It is well known that higher acidity enhances the rennet action and the other acidic proteases enzymes (Gouda *et al*., 1992 and Dabiza and El-Deib, 2007). This is consistent with the high acidity in Ras cheese made with adjunct cultures than the Control cheese as presented in Table 1.

Table 2. Indices of ripening in Ras cheese made with adding probiotic adjunct cultures during the ripening period.

Property	Treatment	Storage (days)						Mean ± SD
		Fresh	15 days	30 days	45 days	60 days	90 days	
Total nitrogen % (TN)	C	2.40	2.56	2.82	2.89	2.65	3.15	2.75^{c}± 0.27
	T1	2.58	2.70	2.98	2.98	3.10	3.22	2.93^{ab}±0.24
	T2	2.42	2.68	2.99	2.99	3.10	3.19	2.90^{bc}±0.29
	T3	2.46	2.74	3.05	3.13	3.25	3.32	2.99^{a}±0.33
Mean ± SD		2.46^{d} ±0.08	2.67^{c} ±0.08	2.92^{b} ±0.10	2.91^{b} ±0.10	3.09^{a} ±0.26	3.21^{a} ±0.07	
Soluble nitrogen % (SN)	C	0.27	0.37	0.41	0.48	0.52	0.44	0.41^{d}±0.09
	T1	0.35	0.44	0.49	0.59	0.75	0.63	0.54^{b}±0.14
	T2	0.33	0.42	0.48	0.55	0.70	0.65	0.52^{c}±0.14
	T3	0.35	0.50	0.50	0.61	0.78	0.67	0.57^{a}±0.15
Mean ± SD		0.32^{f} ±0.04	0.42^{e} ±0.05	0.47^{d} ±0.04	0.55^{c} ±0.06	0.68^{a} ±0.12	0.59^{b} ±0.11	
SN/TN %	C	11.27	14.06	14.79	16.62	17.58	14.33	14.77^{b}±2.20
	T1	13.66	16.29	17.10	19.88	24.72	19.45	18.52^{a}±3.79
	T2	13.62	15.69	16.53	21.17	22.68	20.47	18.36^{a}±3.58
	T3	14.52	17.10	16.61	19.59	23.90	20.05	18.63^{a}±3.29
Mean ± SD		13.27^{d} ±1.39	15.78^{c} ±1.29	16.26^{c} ±1.01	19.32^{d} ±1.92	22.22^{a} ±3.20	18.58^{b} ±2.86	
TVFA [ml 0.1N NaOH/100g cheese]	C	8.00	16.00	18.50	32.50	51.00	63.50	31.58^{b}±21.76
	T1	17.00	18.00	27.00	41.00	47.00	70.00	36.66^{ab}±20.32
	T2	8.00	14.50	20.00	26.50	66.00	82.00	36.17^{ab}±30.36
	T3	12.00	16.00	25.00	37.00	58.00	77.00	37.50^{a}±25.49
Mean ± SD		11.00^{e} ±4.27	16.12^{de} ±1.44	22.54^{d} ±4.03	34.37^{c} ±6.22	55.50^{b} ±8.35	73.12^{a} ±8.09	
Tyrosine [mg/100g]	C	30.15	54.99	83.26	94.88	110.61	144.86	86.46^{c}±40.59
	T1	31.88	58.63	84.20	95.26	112.35	153.73	89.34^{b}±42.38
	T2	30.60	56.01	83.60	92.40	111.32	146.41	86.72^{c}±40.75
	T3	32.50	60.21	83.90	97.71	114.60	155.73	90.77^{a}±42.92
Mean ± SD		31.28 ^{f}±1.09	57.45^{e} ±2.39	83.74^{d} ±0.40	95.08^{c} ±2.17	112.22^{b} ±1.74	150.18^{a} ±5.35	
Tryptophan [mg/100g]	C	20.84	35.15	57.21	64.56	74.21	90.88	57.14^{b}±25.64
	T1	22.94	34.73	58.11	64.48	73.35	89.90	57.25^{b}±24.75
	T2	22.43	33.55	57.86	63.66	73.05	89.27	56.64^{b}±24.85
	T3	24.34	34.91	58.21	65.94	73.90	90.58	57.98^{a}±24.67
Mean ± SD		22.64^{f} ±1.45	34.58^{e} ±0.71	57.84^{d} ±0.45	64.65^{c} ±0.95	73.62^{b} ±0.52	90.15^{a} ±0.72	

Means within the same columns and rows with different subscriptions are significantly different (P≤0.05).
C: (Control) (*Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus* (1:1)).
T1: *(Lb. acidophilus* + *(Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T2: (*Lb. helveticus* + *(Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T3: (*Lb. casei* + (*Streptococcus thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*) (1:1).
Lb= Lactobacillus, TVFA =Total volatile fatty acids, SD= Standard Deviation

The soluble nitrogen (SN), SN/TN, soluble tyrosine and soluble tryptophan contents in cheese is usually used as a ripening indicators. It is obvious from Table (2) that the samples of T3 cheese made from adding *Lb. casei* as adjunct culture had the highest SN ($P \leq 0.05$) during its ripening time followed by those of T1, T2 then Control. This could be due to the action of *Lb. casei*, which is a highly proteolytic *Lactobacilli* with multiple amino peptidases with caseinolytic activity, causing acceleration of cheese proteolysis (El Abboudi *et al*., 1991 and Sallami *et al*., 2004). Also it is observed that the SN content increased for all

treatments throughout the ripening period. These findings are in harmony with those of El-Batawy *et al*. (1992).

Soluble tyrosine, soluble tryptophan and volatile fatty acids were found to be a flavour contributor in some dairy products, especially hard cheese (Abd-Ellah, 2008). The increase in free amino acids (FAA) content during the ripening period was expected due to the effect of the proteolytic enzymes on proteolysis of cheese protein. These enzymes could be from rennet or the release of intracellular peptidases from dead and lysed starter cells (Folkertsma and Fox, 1992; McSweeney *et al*., 2004; Osman *et al*., 2011).

The data in Table 2 show that SN/TN was significantly high ($P \leq 0.05$) in Ras cheese made with adjunct cultures than the Control cheese. Additionally, the SN/TN increased for all the treatments during the ripening period.

According to Awad *et al*. (2001) *Lactobacilli* cell lysis released intracellular proteolytic enzymes in cheese slurry, lowering the residual percentage of αs1- and β-CN fractions within 30 days of ripening.

Data in Table (2) show that the highest soluble tyrosine and soluble tryptophan was in the treatment of T3 that made with *Lb. casei* ($P \leq 0.05$) followed by those of treatments T1, T2 then Control. Also, the soluble tyrosine and tryptophan increased for all the treatments during the ripening period. Confirmatory to these results were obtained by Abdel-Kader *et al*. (2001).

The measurement of total volatile fatty acids (TVFA) in cheese throughout ripening could be an indication of lipolysis. Volatile fatty acids are some of the micro components which have a direct effect on cheese flavour and body texture. The data which are shown in Table (2) indicated that T3 cheeses that made with *Lb. casei* containing higher TVFA ($P \leq 0.05$) than the other treatments and Control cheese. This result is in accordance with the results of Madkor *et al.* (2000), who stated that the hard cheese (Cheddar type) made with *Lb. casei* formed the highest level of free fatty acids compared with all other adjunct-treated cheeses. Also, the results indicated that the TVFA of all cheeses was increased significantly ($P \leq 0.05$) throughout the ripening period reaching the maximum values at the end of this period. These results are in harmony with those of El-Baz *et al*. (2011).

According to Khalid and Marth (1990), certain *Lactobacilli* strains release a lot of intracellular lipase when they autolyze, which could explain why the cheese ripens so quickly. Also, degradation of milk fat to short-chain fatty acids such as butyric, caproic, and capric considered among the necessary components of cheese flavor.

Microbiological properties

Table (3) showed the changes in the microbiological properties of Ras cheese for all treatments during the ripening period. Up to day 30 of ripening, the total bacterial count increased gradually in all cheese treatments, then decreased slightly up to the end of the ripening period. T2 had the highest total bacterial

counts followed by T1, T3 then Control in the 30 days from ripening. However, at the end of ripening the highest total bacterial count was in the T3 treatment with the *Lb. casei*. Which is in harmony with the results of the pH and the acidity values in Table (1). These results are in agreement with previous results (Abd-Ellah, 2008; Ibrahim *et al.*, 2011).

Table 3. Microbiological analysis (log 10 CFU/g) of Ras cheese during ripening period as affected by adding some probiotic adjunct cultures.

Microbial counts	Treatments	Ripening Period					
		Fresh	15 days	30 days	45 days	60 days	90 days
Total bacterial count	Control	8.45	8.90	9.77	9.66	9.64	8.70
	T1	8.89	9.11	9.94	9.90	9.88	8.96
	T2	9.00	9.15	10.04	9.48	9.94	9.13
	T3	8.91	8.98	9.78	9.72	9.69	9.95
Lactobacilli count	Control	8.54	8.60	9.47	9.39	9.34	8.66
	T1	8.64	8.92	9.77	9.68	9.64	8.75
	T2	8.69	8.97	9.85	9.83	9.79	8.96
	T3	8.63	8.77	9.54	9.49	9.46	8.71
Streptococcus count	Control	8.51	8.56	9.44	9.30	9.25	8.51
	T1	8.50	8.65	9.49	9.43	9.38	8.54
	T2	8.56	8.67	9.50	9.44	9.41	8.62
	T3	8.47	8.53	9.39	9.29	9.23	8.57
Yeasts and Molds	Control	1.90	1.85	1.85	1.83	1.81	1.80
	T1	1.80	1.79	1.77	1.77	1.76	1.75
	T2	1.77	1.80	1.78	1.77	1.75	1.74
	T3	1.73	1.74	1.74	1.73	1.72	1.71
Coliform count	Control	ND	ND	ND	ND	ND	ND
	T1	ND	ND	ND	ND	ND	ND
	T2	ND	ND	ND	ND	ND	ND
	T3	ND	ND	ND	ND	ND	ND

C: (Control) (*Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus* (1:1)).
T1: *(Lb. acidophilus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T2: (*Lb. helveticus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T3: (*Lb. casei* + (*Streptococcus thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*) (1:1).
Lb= Lactobacillus

Results of the *Lactobacilli* and Streptococci counts were in the same direction as the total bacterial count (Table 3). The counts gradually increased until the fourth week of the ripening period, then gradually decreased slightly until the end of the ripening period. The higher *Lactobacilli* counts in adjunct treated cheeses than Control cheese reflects the positive retention of *Lactobacilli* in the experimental cheeses (Madkor *et al*., 2000). The *Lactobacilli* count in T2 was the highest followed by T1, T3 then Control in the 30 days from ripening. On the other hand, at the end of ripening the highest *Lactobacilli* count was in the T2 treatment with the *Lb. helveticus* which is in agreement with the results of Madkor *et al.* (2000).

Counts of yeasts and molds in the same Table show that the control treatment had the highest number of yeasts and molds followed by T1, T2 then T3 in the fresh treatments. The number of yeasts and molds of all treatments decreased slightly up to the end of the ripening period. These results suggest that *Lactobacilli* bacteria incorporated in the manufacture of Ras cheese prevented and reduced the fungal growth in cheese. The inhibition of growth and

sporulation of moulds by *Lactobacilli* bacteria could be due to the facultative anaerobic conditions created by these bacteria in the cheese (Batish *et al*., 1997), or due to their lactic and organic acids production, which decreasing the pH of the growth environment (Caplice and Fitzgerald,1999). Also the results of Zabouri *et al*. (2021) proved that LAB could produce a large number of natural compounds with antifungal properties which makes it a good bio-preservatives against phytopathogenic and food-spoilage fungi.

Results in Table (3) show that the coliform count was not detected in all treatments of Ras cheese from fresh up to the end of the ripening period. This result is in agreement with the results of Abd Alla *et al*. (2008), who study the influence of two *Lactobacillus* strains on the total coliform count in Ras cheese. The production and synergistic activity of organic acids and hydrogen peroxide is the main factor for the antimicrobial activity of *Lactobacilli*, whereas the antagonistic activity of *Lactobacilli* against gram-negative bacteria depends on the fermentation group of *Lactobacilli* (Annuk *et al*., 2003).

Organoleptic properties

Several researches support the importance of adding adjunct *Lactobacilli* to enhance the Organoleptic properties of hard cheese (El-Abboudi *et al*., 1991; Muir *et al*., 1996; Madkor *et al*., 2000). The sensory scores of Ras cheeses at 30, 60 and 90 days of ripening are summarized in Fig. 3. The results showed that the acceptability of cheese in terms of flavour, body and texture, appearance, and overall acceptability could be related to the addition of the probiotic *Lactobacillus* sp. as adjunct cultures. It can be seen that the treatment of T3 which made with the addition of *Lb. casei* gain the highest score at 30 days of ripening ($P \leq 0.05$), however, the T1 treatment which made with the addition of *Lb. acidophilus* obtained the highest score ($P \leq 0.05$) at the end of the storage period for all the attributes of flavour, body and texture, appearance, and overall acceptability.

The significant increase result in Ras cheese flavor made from adjunct *Lactobacilli* in the present study could be associated with its effect through increasing the aminopeptidolytic activity as reported by Madkor *et al*. (2000). This result is proved by the significant increase of soluble tyrosine and soluble tryptophan concentrations in the treated cheese as shown in Table 2. Also, high TVFA liberation in adjunct-treated cheese (Table 2) seems to promote a positive effect on the flavor of Ras cheese.

The scores for body and texture in Fig. 3 indicated that Ras cheese made with adjunct *Lactobacilli* promoted some improvement in T1, T2 and T3 compared to the Control cheese at the beginning of ripening (30 days), however, T1 present the highest score at the end of ripening. This could be because *Lactobacilli* with high intracellular peptidase activity caused more casein hydrolysis in cheese, resulting in smaller peptide fragments and free amino acids, which could affect the conformation of secondary structure like the αs-helix (Creamer and Olson, 1982). This may contribute to changes in the curd firmness,

resulting in an enhancement and improvement in body and texture quality of adjunct-treated cheese (Muir *et al*., 1996).

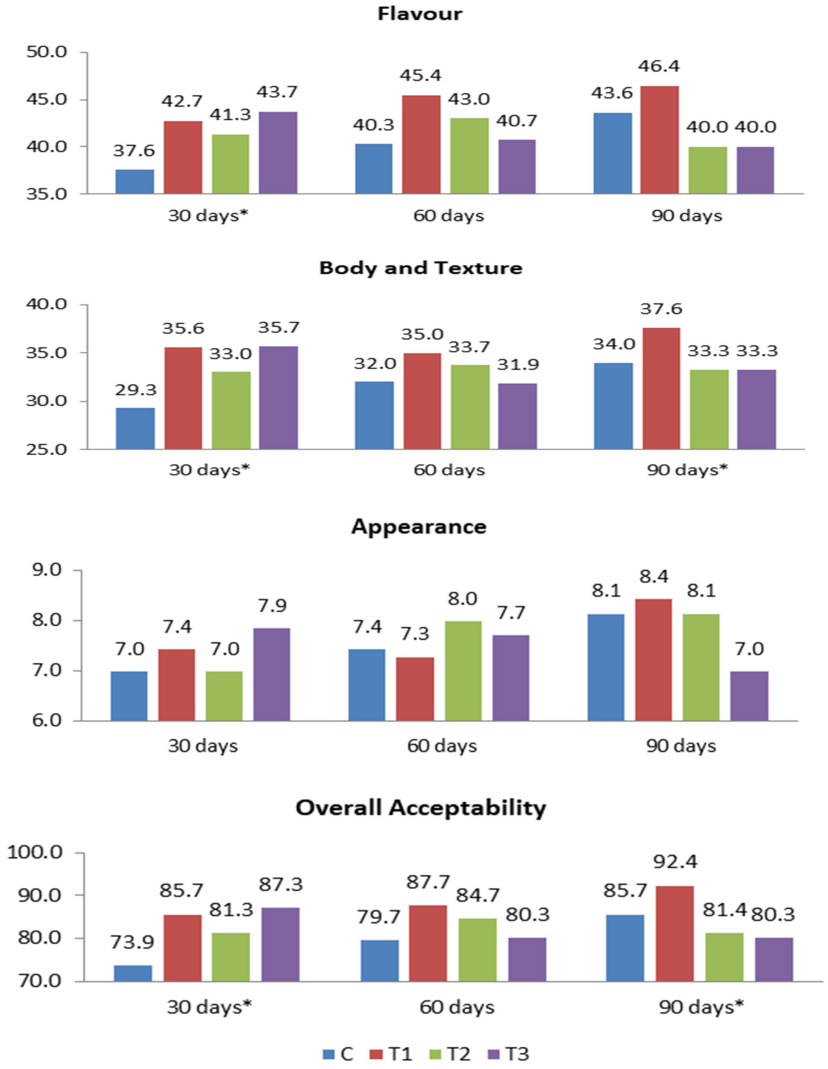

Fig. 3. Sensory evaluation of Ras cheese during ripening period as affected by using some of the adjunct cultures. *Means within the storage period are significantly different (P≤0.05).
C: (Control) (*Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus* (1:1)).
T1: *(Lb. acidophilus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1).
T2: (*Lb. helveticus + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1)
T3: *(Lb. casei + (Streptococcus thermophilus, Lb. delbrueckii subsp. bulgaricus)* (1:1)
Lb= Lactobacillus

Sensory results regarding the appearance and overall acceptability demonstrate that *Lactobacilli* cultures did not cause defects in sensory scores and could even improve the organoleptic characteristics of Ras cheese at the end of ripening, in particular, the *Lb. acidophilus* cheese (T2). Similar results were reported in several types of cheese made with adjunct *Lactobacillus* cultures (Minervini *et al*., 2012; Cuffia *et al*., 2018 and Tomar, 2018).

Conclusion

The addition of adjunct probiotic *Lactobacillus* cultures for Ras cheese leads to an increase in acidity, TN, SN, SN/TN, soluble tyrosine, soluble tryptophan and TVFA. The numbers of *Lactobacilli* in the experimental cheeses were higher than the control cheeses until the end of ripening. Also, adding *Lactobacilli* as adjunct cultures to Ras cheese reduced the number of yeasts and moulds compared to control. Sensory analysis of the cheeses showed higher acceptability for experimental cheeses than for the control. The results of the current study indicated that the chemical, microbiological and sensory quality of Ras cheese can be improved by using the appropriately selected *Lactobacilli*.

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تحسين خصائص الجبن الراس المصري بإضافة بعض سلالات *Lactobacillus* الداعمة للحيوية

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[٣] قسم النبات والميكروبيولوجي – كلية العلوم – جامعة اسيوط

الملخص

الهدف من هذه الدراسة هو تحسين جودة الجبن الراس المصري بإضافة بعض أنواع *Lactobacillus* spp. كبادئات حيوية مساعدة. تم استخدام سلالات من *Lb. acidophilus* و *Lb. helveticus* و *Lb. casei* وتم تقييم الخصائص الكيميائية والميكروبيولوجية والحسية للجبن خلال ٩٠ يومًا من التسوية. انخفضت قيم ال pH ومحتوى الرطوبة، بينما زادت نسبة النيتروجين الكلي (TN) والدهن و الدهن / المادة الجافة والحموضة و نسبة الملح بشكل معنوي خلال فترة التسوية في جميع المعاملات. بالنسبة لمؤشرات التسوية، تم العثور على محتوى أعلى بكثير من النيتروجين القابل للذوبان (SN) و SN / TN، التيروسين القابل للذوبان، التربتوفان القابل للذوبان والأحماض الدهنية الكلية المتطايرة في الجبن المعامل مقارنة بجبن الكنترول. كما لوحظ ارتفاع عدد ال *Lactobacillus* في الجبن المعامل، مما يعكس الاحتفاظ الإيجابي بهذه البكتريا في الجبن الناتج. كما أن استخدام البكتريا الداعمة الحيوية كبادئات مساعدة يقلل من نمو الفطريات ويمنع نمو بكتريا القولون في الجبن الراس. كان هناك قبول أعلى من المستهلكين للجبن المعامل مقارنة بالجبن الكنترول. يعتبر جبن الراس ناقلاً جيدًا لسلالات بكتريا *Lactobacillus* حيث وجد أنها تعيش أثناء تصنيع الجبن ونضجه وتخزينه.