Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Quality Properties of Egyptian Bio-Hard Cheese

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ABSTRACT

The quality properties of Bio-Ras cheese were investigated during ripening for 90 days at 15°C. Five different probiotic starter cultures were used (*Lactobacillus acidophilus, Lb. casei, Lb. helveticus, Lb. reuteri,* and *Lb. rhamnosus*) in Bio-Ras cheese production. The impact of probiotics on cheese quality, focusing on its physicochemical, microbial and sensory qualities besides free amino and free fatty acid profiles were also studied. Chemical properties were significantly enhanced with the use of probiotic starter culture, *Lb. helveticus* and *Lb. rhamnosus* followed by *Lb. casei* compared with control samples within 90 days of ripening. Furthermore, probiotics type and ripening period had significant effects on chemical properties. Amino acid and fatty acid profiles of fresh and 90 days-ripened Bio-Ras cheese treatments were dependent to probiotic starter culture type. Lactobacilli and Streptococci counts increased within the first 30 days of ripening in all cheese treatments followed by a gradual decrease ($p \leq 0.05$) within the ripening for 90 days. meanwhile the counts of yeast and mould were not detected during the first 30 days of ripening followed by a slight increase till the end of the ripening period. Sensory evaluation indicated that Bio-Ras cheese made with *Lactobacillus helveticus* and *Lb. rhamnosus* attained significantly high flavours, body & texture, and enhanced appearance and overall acceptability scores.

Keywords: Bio-Ras cheese, Probiotics, *Lactobacillus helveticus, Lactobacillus rhamnosus,* Ripening period.

INTRODUCTION

Conventionally Ras cheese is regarded as the most popular hard cheese in the Middle East, particularly in Egypt. It is typically made using raw cow's milk or a blend of cow and buffalo's milk in artisanal areas. The cheese is consumed after aging for 3 to 6 months, when it acquires a strong, sharp flavor similar to the Greek variety, Kefalotyri cheese (Hatteam *et al*., 2012).

All cheese types must be produced from pasteurized milk as Egyptian Organization for Standardization and Quality Control (EOS) (Hammam *et. al*., 2020). The pasteurization process has an impact on the aspects and quality properties of Ras cheese during the ripening period (Singh *et al*., 2003). LAB starter cultures must be used to meet these standards in Ras cheese manufacture. Typically, LAB starters and adjuncts are carefully selected for Ras cheese due to their significant contribution to its flavor and sensory properties. (El-Soda *et al*., 2000).

Five of most significant LAB genera that influence the cheese flavour are *Lactococcus, Streptococcus, Lactobacillus, Leuconostoc*, and *Enterococcus*. The microorganisms in hard cheese are affected by various factors, including Starter culture and Non-Starter LAB (NSLAB). To regulate NSLAB and enhance flavor properties, one way is to use adjunct culture, which is a common practice in today cheese making (Broadbent *et al*., 2003 and Johnson and Lucey, 2006).

Probiotic bacteria are commonly added to a variety of dairy foods like yoghurt, fermented milk, cheese, and pasteurized unfermented milk. When probiotics like *Lactobacillus* are added as a starter or NSLAB in cheese-making, they contain many peptidases that can break down peptides and create polypeptides, oligopeptides, and free amino acids affecting the chemical composition, flavor, texture, and organoleptic properties of Ras cheese (Santillo and Albanzia, 2008 and Soufa and Saad, 2009). Different types of Lactobacilli have been utilized in Ras cheese-making to improve their health benefits and their probiotic counts are still within the recommended range ($10^6 – 10^7$ CFU/g) during the cheese-making process and ripening period (Aumara, 2004, Abdalla *et al*., 2008, and El-Alfy *et al*., (2012).

The stability of cheese quality is influenced by the natural abilities of the probiotic strains used and the properties of the matrix. This has a major impact on the viability and shelf-life of the probiotics El-Alfy *et al*., (2012). A wide range of factors related to composition and processing can affect the viability of probiotics in cheese. These include the type and amount of inoculation, the addition of flavorings, competition from other microorganisms, the presence of bacteriocins or other antimicrobials, pH level, redox potential, incubation and storage temperatures, salt and water activity, and packaging materials.

Shehata, *et. al.*, (2004 a, b,c) produced high microbiological and chemical quality of probiotic Ras cheese containing *Bifidobacterium* Sp. While El-Alfy *et al*., (2012) studied the survival of some probiotics in Ras cheese under manufacturing conditions.

Kebary *et. al.*, (2011) produced Ras cheese containing *Lb. rhamnosus* with the highest count and after the ripening period. Also, Abd El-monem *et. al*., (2022) the addition of Lactobacillus cultures to Ras cheese as an adjunct probiotic leads to an increase in acidity, SN/TN and TVFA. The number of Lactobacilli in the experimental cheeses was higher than the control cheeses throughout the ripening process. Moreover, the incorporation of Lactobacilli as

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DOI: 10.21608/jfds.2023.242823.1134

adjunct cultures to Ras cheese reduced the number of yeasts and molds compared to the control. Sensory analysis of the cheeses showed higher acceptability for experimental cheeses than for the control group. The results of the study indicate that the quality of Ras cheese in terms of its chemical, microbiological, and sensory aspects can be significantly improved by using appropriately selected Lactobacilli.

So, the main objectives of this study were to investigate the possibility of using probiotic Lactobacilli strains as an adjunct with traditional yoghurt starter culture in Ras cheese production with high viable probiotic counts. Also, to examine the Impact of this probiotic Lactobacilli strains on the chemical, microbiological and organoleptic characteristics of Bio-Ras cheese during the ripening period.

MATERIALS AND METHODS

Materials:

Whole cow's milk (Fat 3.5% and S.N.F 8.56%) was purchased from the herd of the Faculty of Agriculture at Cairo University. To standardize the milk, cow's milk was adjusted to a 0.7 casein/fat ratio. Microbial rennet powder was purchased from Valley Research Inc. and used as 3.0 g/100 Kg milk. Commercial fine grade salt (sodium-chloride, 98% NaCl) used in the study was obtained from El-Nasser Saline Company, Alexandria, Egypt. Calcium chloride ($CaCl_2$) from El Nasr Pharmaceutical Co. Was prepared as a solution and added to the milk to achieve a final concentration of 0.02% $CaCl_2$.

A plastic coating, called CESKA WL 500.0400, was obtained from CSK Food Enrichment B.V. in Holland. This plastic coating contains 0.05% Natamycin and used for coating cheese at the start of the ripening period.

Starter cultures were obtained from Microbiological Resources Center (Cairo MIRCEN), and used as probiotics in Bio-Ras cheese production. These probiotics are: *Lb. acidophilus* ATCC 20079; *Lb. casei* ATCC 2001; *Lb. helveticus* ATCC 7193; *Lb. rhamnosus* DSMZ 20245 and *Lb. reuteri* ATCC 20016. Yoghurt starter culture (*Streptococcus thermophiles*, *Lb. delbrueckii* ssp. *bulgaricus*, YOFLEX® M790) was obtained from MIFAD Co., Egypt.

Methods:

Ras cheese production:

Fresh cow's milk (72 liters) was heat treated at 73°C/15 sec. and cooled to 37 – 39 °C then it was separated into 6 equal parts to create various treatments.

Yoghurt starter culture (2%) and calcium chloride (0.02%) were added at 37°C, mixed well, and left for 20 min for acid development then reconstituted rennet (3g/100kg) was added. Ras and Bio-Ras cheese treatments were produced traditionally as in Fig. (1). Finally, after molding, and pressing the curd for two hours, it was left to press overnight. The Ras cheese wheels were salted and dried on both sides with 1% Sodium Chloride each day for two days. Next, the Ras cheese was plastic coated and aged for 90 days at 15 ± 2 °C and approximately 85% relative humidity.

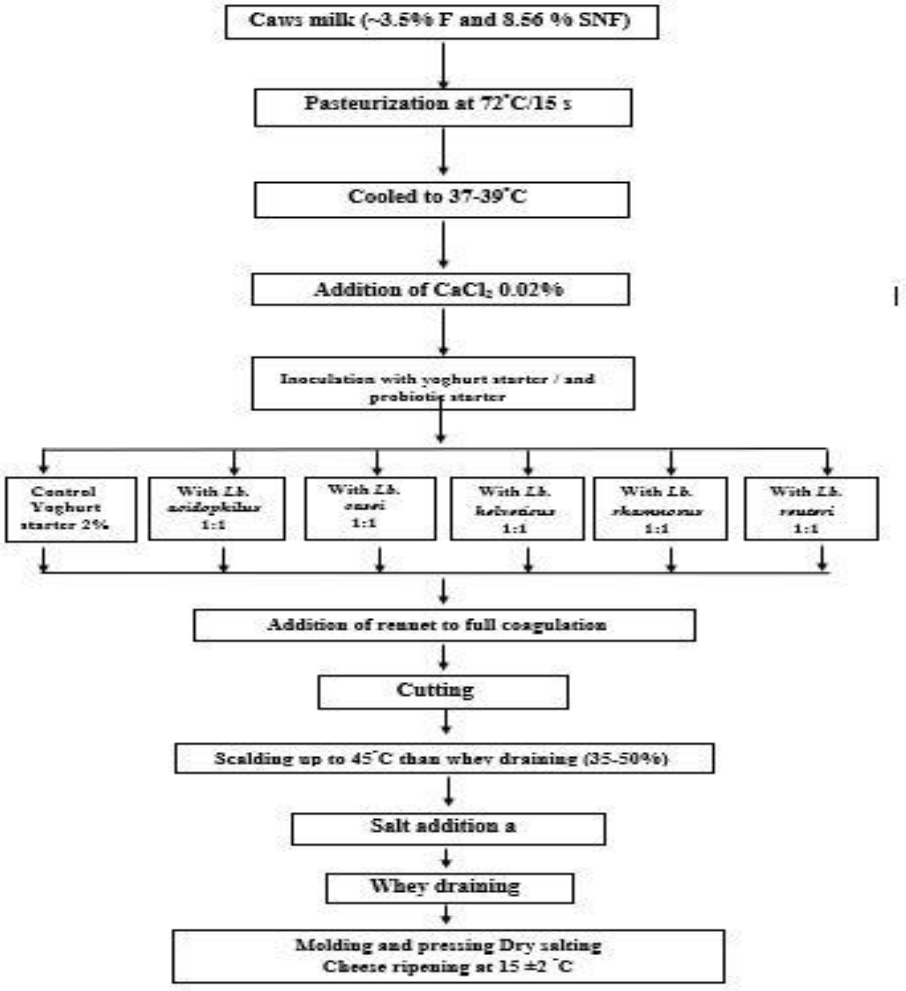

Fig. 1. Production of Ras and Bio-Ras Cheese

Ras cheese sampling:

Ras cheese samples (200 g each) were taken from fresh cheese wheels (after dry salting) and every 15 days throughout the ripening period. About one centimeter of the outer layer of cheese was removed and the leftover was divided into three parts: The first part was used for microbiological examination, and second and third parts were chemically analyzed and sensory evaluated Abd El-Tawab (1966).

Chemical analyses:

Dry matter, fat/dry matter, total nitrogen, and soluble nitrogen substances of cheese samples were analyzed in accordance with established standards of AOAC (2012). pH values were evaluated using a Beckman electric pH meter that had a glass electrode attached to it, (Model 3305). Salt in water phase content salt/moisture (%) was determined using the method of Bradley *et al.*, (1992). Ash content was established according to the methods by AOAC (2012) using a muffle furnace [Thermolyze Type 1500] at 600 °C.

Ripening indices:

Free fatty acids (FFA) profile as estimated in Ras and Bio-Ras cheese samples using a Gas chromatography system with FID detector, and free amino acids (FAA) were determined with the high-performance A A analyzer (biochrome 30) consistent with AOAC (2012).

Microbiological Analysis

Ras cheese samples were prepared for microbiological examinations as mentioned by Frazier and Foster (1961). The total viable bacterial counts were carried out by Laird *et al*. (2004) using plate count agar medium and the plates were incubated at 32°C for 48h. Lactobacilli was determined using MRS agar medium according to De Man *et al.* (1960) and the plates were incubated at 32°C for 48 hrs. Streptococci was determined using M17 agar, the plates were incubated at 37 °C for 48h as in IDF 117B. (1997). Yeast and Mound counts were enumerated using oxytetracycline glucose yeast extract agar medium and the plates were incubated aerobically at 25°C for 5 days according to IDF 94A (1985).

Organoleptic evaluation

The organoleptic characteristics of Ras cheese samples were evaluated at 30, 60 & 90 days of ripening based on Hofi *et al*. (1991). The panelists rated the samples on flavor, body, texture, appearance, and overall acceptability using a point system: 50 for flavor, 40 for body & texture, and 10 for appearance.

Experimental design and Statistical analyses

In this study, a factorial design was utilized with treatments of Ras and Bio-Ras cheese, and the ripening period was considered a fixed factor. To assess Ras cheese composition, we conducted a two-way ANOVA, and for sensory analysis, we performed a one-way ANOVA for each ripening period. (30, 60 and 90 days).

RESULTS AND DISCUSSION

The chemical properties of Bio-Ras cheese along the ripening period up to 90 days at 15 ±2 °C are presented in Table (1). The dry matter content gradually increased during the maturity period in all treatments. This may be attributed to water evaporation during ripening or the binding of water with protein as bound water as the ripening advanced. Conner, (1980). This trend of dry matter increases coincided with that found by Abd-Ellah (2008). Furthermore, El-Essawi *et al*. (2013) found that the moisture loss occurred within the initial month of the majority ripening process.

Fresh Bio-Ras cheese with *Lb. casei* had significantly higher dry matter content ($P \leq 0.05$) than that of other treatments (59.58%), Followed by *Lb. rhamnosus*, and *Lb. acidophilus*, (58.72 and 58.7%), respectively. DM content gradually increased as the ripening period progressed in all treatments. It has been suggested that as cheese ripens, its acidity may increase, leading to a decrease in moisture content (Kebary *et al*., 2011). This, in turn, can aid in whey removal from the cheese curd.

The Fat/DM content in Ras cheese plays a significant role in determining its taste and texture. It contributes to the smoothness and richness of the cheese product. As the cheese ripens, the Fat/DM ratio gradually increase in both Ras and Bio-Ras cheese, likely due to a loss of moisture and a decrease in non-fat constituents caused by microorganism growth and activity. This increase was statistically significant ($P \leq 0.05$) in all treatments. It is clear that the loss of moisture during the ripening process, as well as decrease in other non-fat components due to the activity of microorganisms, has caused a reduction in weight Ezzat, (1990). The obtained results from the study show that the Bio-Ras cheese with *Lb. reuteri* and *Lb. rhamnosus* had the highest Fat/DM content, followed by the control Ras cheese. These findings were statistically significant ($P \leq 0.05$).

The titratable acidity of all Ras cheese treatments gradually increased (with a significance level of $P \leq 0.05$). The maximum value is reached at the end of the ripening period. It's common knowledge that the total acidity of cheese comes from milk constituents and acidity that developed by the activity of starter culture during ripening. During this process, Compounds of protein and amino acids, as well as fatty acids resulting from fat hydrolysis, are degradation intermediates, largely contributing to the increase in cheese acidity Abd El-Monem, (2018). Acidity of fresh Ras cheese treatments ranged from 0.61% to 0.69% for Bio-Ras cheese with *Lb. reuteri* and Bio-Ras cheese with *Lb. casei*, respectively. However, titratable acidity significantly increased by increasing the ripening period to range from 0.82% to 0.91 % after 90 days of ripening period, for same treatments in same order. During storage, the Bio-Ras cheese with added *Lb. casei* had higher acidity levels ($P \leq 0.05$) compared to all other cheese treatments. Also, the Bio-Ras cheese with *Lb. reuteri* had the lowest acidity levels.

The variation in acidity levels among different cheese treatments could be attributed to the growth of Lactobacillus strains, and this capacity to ferment lactose during the ripening process, the utilization of various *Lactobacillus* strains in the manufacture of Domiati cheese led to an increase in its acidity levels, as reported by El-Zayat and Osman (2001).

During the ripening process, the percentage of salt/moisture % significantly increased ($P \leq 0.05$). This is due to the water loss caused by evaporation El-Etriby *et al.*, (1998). It is possible that the increase in salt/moisture % content is due to the salt getting absorbed through osmosis during the salting process. The Bio-Ras cheese samples prepared with *Lb. casei* followed by *Lb. helveticus* starter had

the highest salt/moisture (%) content when they were fresh and throughout the ripening period compared to the other treatments ($P \leq 0.05$). These findings are consistent with previous studies conducted by (Abd-Ellah, 2008), and (El-Baz *et al.*, 2011).

Ash content in all Ras cheese treatments slightly increased by extending the ripening period and with a high concentration of Bio-Ras cheese with *Lb. casei* due to a decrease in the moisture content in Ras cheese during the ripening period. Bio-Ras cheese with *Lb. casei* followed by with *Lb. helveticus* and with *Lb. rhamnosus* had the highest and significant ash content within the ripening period.

Table 1. Chemical properties of Bio-Ras cheese among ripening for 90 days at 15 ±2 °C.

	Ripening period (days)					
	Green	**15**	**30**	**45**	**60**	**90**
			Dry matter content (%)			
Control	58.39^{Db}	58.81^{Db}	59.28^{Cc}	59.99^{Bab}	60.59^{Ab}	60.76^{Ab}
Lb. acidophilus	58.70^{Db}	59.19^{Dab}	59.71^{Cb}	60.06^{Bab}	60.40^{Bb}	60.81^{Ab}
Lb. casei	59.58^{Da}	59.93^{Da}	60.27^{Ca}	60.84^{Ca}	61.30^{Ba}	61.81^{Aa}
Lb. helveticus	57.28^{Fc}	58.12^{Ec}	58.47^{De}	60.04^{Cab}	60.73^{Bb}	61.42^{Aa}
Lb. reuteri	57.22^{Ec}	57.71^{Dc}	58.30^{Cd}	58.95^{Bb}	59.52^{Ac}	59.93^{Ac}
Lb. rhamnosus	58.72^{Fb}	59.08^{Eb}	59.59^{Db}	60.17^{Cab}	60.63^{Bb}	60.89^{Ac}
			Fat/DM content (%)			
Control	51.14^{Ca}	52.28^{Cb}	53.32^{Ba}	53.8^{Bb}	54.68^{Ab}	55.13^{Aa}
Lb. acidophilus	50.24^{Cb}	51.45^{Cc}	52.06^{Bb}	52.61^{Bc}	53.11^{Bb}	54.03^{Ab}
Lb. casei	49.86^{Cc}	50.74^{Cc}	51.28^{Bc}	51.73^{Bc}	52.32^{Ac}	53.06^{Ac}
Lb. helveticus	50.41^{Cb}	51.85^{Bc}	52.50^{Bb}	53.20^{Ab}	53.71^{Ab}	54.43^{Ab}
Lb. reuteri	51.26^{Ca}	52.91^{Ba}	53.71^{Ba}	54.51^{Ba}	55.33^{Aa}	56.10^{Aa}
Lb. rhamnosus	51.04^{Da}	52.72^{Ca}	53.64^{Ba}	54.11^{Ba}	55.13^{Aa}	55.52^{Aa}
			Titratable acidity%			
Control	0.63^{Ea}	0.67^{Db}	0.71^{Cb}	0.75^{Bc}	0.79^{Bc}	0.85^{Ab}
Lb. acidophilus	0.66^{Da}	0.70^{Ca}	0.73^{BCb}	0.77^{ABb}	0.81^{Bb}	0.87^{Aab}
Lb. casei	0.69^{Da}	0.73^{Ca}	0.78^{Ba}	0.81^{Aa}	0.85^{Ba}	0.91^{Aa}
Lb. helveticus	0.65^{Ca}	0.71^{Ba}	0.76^{Ba}	0.80^{Aa}	0.84^{Ba}	0.90^{Aa}
Lb. reuteri	0.61^{Bb}	0.65^{BC}	0.69^{Bc}	0.72^{Ac}	0.76^{Bc}	0.82^{Ab}
Lb. rhamnosus	0.64^{Ca}	0.69^{Cb}	0.73^{Bb}	0.78^{Bb}	0.82^{Ab}	0.89^{Aab}
			Salt/moisture (%)			
Control	6.12^{Cc}	6.54^{Cc}	7.17^{Bc}	7.51^{Bd}	8.03^{Ac}	8.51^{Ac}
Lb. acidophilus	6.20^{Dc}	6.33^{Cd}	7.16^{Bc}	7.74^{Bc}	8.22^{Ab}	8.75^{Ab}
Lb. casei	6.21^{Cc}	6.53^{Cc}	7.55^{Ba}	8.15^{Aa}	8.77^{Aa}	9.10^{Aa}
Lb. helveticus	6.38^{Cb}	6.91^{Cb}	7.59^{Ba}	8.08^{Bb}	8.56^{Aa}	8.89^{Aa}
Lb. reuteri	6.23^{Cc}	6.80^{Cb}	7.31^{Bb}	7.73^{Bc}	8.11^{Ab}	8.40^{Ac}
Lb. rhamnosus	6.52^{Ea}	7.01^{Da}	7.65^{Ca}	8.17^{Ba}	8.57^{Aa}	8.79^{Ab}
			Ash content (%)			
Control	7.33^{Cb}	7.47^{Cb}	7.67^{Bb}	7.89^{Ab}	7.98^{Ac}	7.95^{Ac}
Lb. acidophilus	7.21^{Cc}	7.48^{Bb}	7.68^{Bb}	7.87^{Bb}	8.01^{Ac}	8.15^{Ac}
Lb. casei	7.50^{Ca}	7.69^{Ca}	7.87^{Ba}	8.07^{Ba}	8.39^{Aa}	8.70^{Aa}
Lb. helveticus	7.30^{Cb}	7.47^{Cb}	7.80^{Ba}	8.03^{Ba}	8.24^{Ab}	8.41^{Ab}
Lb. reuteri	7.12^{Cd}	7.33^{Cc}	7.48^{Bc}	7.59^{Bc}	7.67^{Ad}	7.75^{Ad}
Lb. rhamnosus	7.51^{Ca}	7.53^{Ca}	7.90^{Ba}	8.03^{Ba}	8.26^{Ab}	8.39^{Ab}

Means with the same superscript capital letter in the same row along the ripening period is not significantly different ($P \leq 0.5$). The total Means with the same superscript small letter in the same column among treatments is not significantly different ($P \leq 0.5$).

Ripening Indices:

The soluble nitrogen/Total nitrogen (SN/TN) and Total Volatile Fatty Acids (TVFA) contents of Ras and Bio-Ras cheese are often tested as ripening indices. Regarding the data presented in Figure 2, it is clear that Bio-Ras cheese samples containing *Lb. casei* had the maximum SN/TN ($P \leq 0.05$) during the ripening process, followed by *Lb. rhamnosus* and *Lb. helveticus*. This may be attributed to the proteolytic activity of *Lb. casei*, which has various amino peptidases with caseinolytic properties. As a result, it accelerates the proteolysis of Ras cheese (El Abboudi *et al*., 1991) and (Sallami et al., 2004).

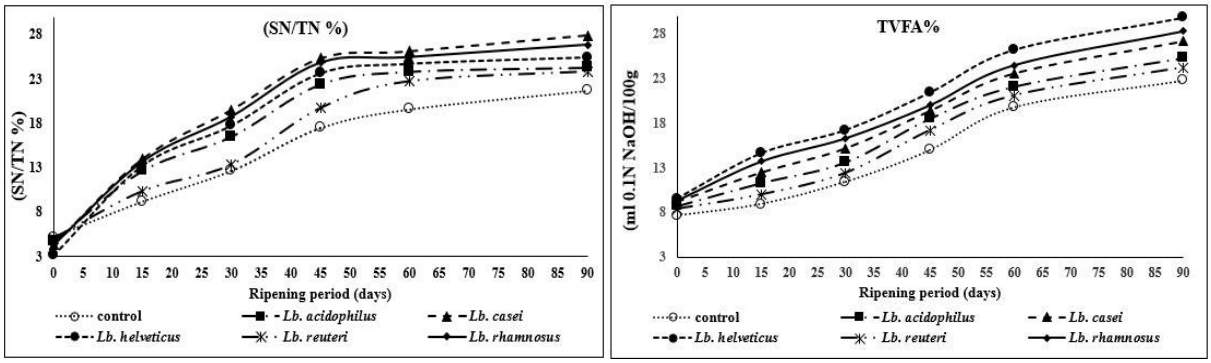

Fig. 2. Soluble nitrogen in total nitrogen (SN/TN %) and total Volatile Fatty Acids (TVFA%) of Ras and Bio-Ras cheese during ripening for 90 days at 15 ±2 °C.

Cheese flavor and texture are affected by micro components called volatile fatty acids. The amount of TVFA in cheese changes during ripening and can indicate lipolysis. Fig. (2) shows that Bio-Ras cheese produced with *Lb.*

rhamnosus had the highest TVFA ($P \leq 0.05$) than other all treatments and control Ras cheese. Throughout ripening, the TVFA significantly increased ($P \leq 0.05$) for all Ras and Bio-Ras cheese, reaching maximum values at 90 days of the ripening period. These results were similar to those of Madkor *et al.* (2000). Certain Lactobacilli strains release intracellular lipases during autolysis, which may explain why the cheese ripens quickly. Additionally, milk fat degradation to short-chain fatty acids like butyric, caproic, and capric is essential for cheese flavor.

Free amino acids:

Some dairy products, particularly hard cheese, contain soluble tyrosine and tryptophan which contribute to their flavor Abd-Ellah, 2008). As the cheese matures, the content of free amino acids (FAA) increases. This is because proteolytic enzymes break down the cheese protein. These enzymes can come from dead or lysed starter cells that release intracellular peptidases (Folkertsma and Fox, 1992); (McSweeney *et al.*, 2004) and (Osman *et al.*, 2011). Awad *et al.* (2001) found that when Lactobacilli cells break open in cheese slurry, they release proteolytic enzymes that reduce the number of αs_1- and β-CN fractions during the first 30 days of ripening.

The amount of total free amino acids (FAA profile) in fresh and 90-day ripened Ras cheese depends on many factors. During the ripening process of cheese, proteolytic enzymes and microorganisms are involved in proteolysis which is vital for texture and flavor development. The FAA contents of Ras and Bio-Ras cheese in table (2) were very low in fresh Ras and Bio-Ras cheese, and then a significant increase was observed in all cheese treatments at 90 days of the ripening period. Generally, Bio-Ras cheese with *Lb. helveticus*, followed by *Lb. casei*, then *Lb. rhamnosus* had higher free amino acids compared with control Ras cheese. While the least free amino acids contents were observed in Bio-Ras cheese with *Lb. acidophilus* and *Lb. reuteri*.

The FAA of Glutamic, Histidine, Alanine, Proline, Methionine, Leucine, Phenylalanine then Lysine contents were high in all Ras and Bio-Ras cheese samples. While the least free amino acids were Cysteine and Arginine. At 90 days of the ripening, the free amino acid content of Ras cheese significantly increased, and the higher content of FAA was in Bio-Ras cheese with *Lb. helveticus*, followed by *Lb. casei*, then with *Lb. rhamnosus*.

The essential amino acid content is "threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine". Higher remarkable contents of threonine (17.11mg /100g), isoleucine (52.02 mg /100g), phenylalanine (26.15 mg/100g), and lysine (45.1mg/100g) were noticed in Bio-Ras cheese with *Lb. helveticus* followed by all other treatments. However, higher aspartic (20.6 mg/100g), serine (17.01mg/ 100g), glycine (5.99mg/100g), histidine (16.1mg/100g), arginine (4.02mg/100g), alanine (23.77mg/100g), proline (27.82mg/ 100g), valine (35.04 mg/100g), methionine (40.22mg/100g) and Cysteine (1mg/100g) acids were found also, in Bio-Ras cheese treatment with *Lb. helveticus*. Also, glutamic and leucine acids were higher in Bio-Ras cheese with *Lb. helveticus* (66.87mg/100g) and (58.98 mg/100g) than all other treatments.

(Awad, *et.al.*, 2001) found that the concentration of free amino acids was higher in pseudo curd and cheese slurries containing lactobacilli compared to those containing *Lactococci* or *Enterococci***.** Cheeses made with mesophilic starters also showed similar results. Among the adjunct single cultures used, *Lactobacillus paracasei* ssp. *paracasei* produced the highest level of free amino acids.

Table 2. Free amino acid (mg/100 g) content of fresh and 90 days ripened Ras and Bio-Ras cheese.

Free amino acid	Control	*Lb. acidophilus*	*Lb. casei*	*Lb. helveticus*	*Lb. reuteri*	*Lb. rhamnosus*
			Fresh			
Aspartic	1.92	1.02	1.71	2.01	0.52	1.52
Glutamic	14.02	3.34	4.55	13.96	3.29	4.51
Serine	0.62	0.33	0.48	0.96	0.29	0.46
Glycine	0.42	0.29	0.30	0.40	0.29	0.29
Histidine	8.45	1.20	1.82	8.51	1.20	1.30
Arginine	0.44	0.05	0.10	0.53	0.05	0.08
Threonine	0.93	0.53	0.73	1.21	0.47	0.66
Alanine	4.96	0.77	1.26	5.12	0.40	1.11
Proline	9.86	6.73	8.55	10.52	6.66	8.30
Valine	7.78	1.08	1.53	8.12	1.06	1.49
Methionine	6.82	2.65	3.01	7.21	2.47	2.87
Tyrosine	0.28	1.30	1.22	0.35	1.02	0.29
Isoleucine	1.78	0.39	0.51	1.82	0.37	0.42
Leucine	8.51	3.87	4.17	7.87	3.77	4.09
Phenylalanine	6.39	3.02	3.22	6.52	2.96	3.06
Lysine	6.88	4.11	5.01	7.00	3.81	4.46
Cysteine	0.22	0.05	0.10	0.30	0.05	0.07
			90 days of ripening			
Aspartic	16.20	14.76	18.01	20.60	18.40	17.76
Glutamic	55.20	31.79	56.66	66.87	62.15	35.44
Serine	14.90	4.73	16.22	17.01	16.30	5.98
Glycine	6.28	3.35	5.41	5.99	5.34	3.39
Histidine	15.45	7.83	15.20	16.10	18.00	8.01
Arginine	3.76	0.38	4.90	4.02	4.31	0.59
Threonine	13.72	7.66	16.10	17.11	16.30	8.93
Alanine	23.24	14.55	22.00	23.77	21.80	16.27
Proline	27.61	15.92	25.40	27.82	24.80	18.74
Valine	23.60	13.73	36.70	35.04	32.40	16.23
Methionine	33.60	12.77	38.50	40.22	36.80	14.82
Tyrosine	3.02	1.60	1.58	1.368	1.75	2.36
Isoleucine	41.40	20.11	50.45	52.02	51.30	23.23
Leucine	50.80	38.51	52.90	58.98	56.90	40.93
Phenylalanine	25.09	18.21	24.50	26.15	25.10	19.77
Lysine	34.20	22.32	40.60	45.10	40.80	26.43
Cysteine	0.90	0.10	0.86	1.00	0.92	0.11

Free fatty acids:

To measure the extent of lipolysis in Ras cheese during ripening, the total FFA was calculated and expressed as mg/100g of cheese fat. The flavors in cheese are mainly contributed by two classes of compounds, namely volatile sulfur compounds and fatty acids. A cheese with low fatty acid concentrations is considered young and un-ripened cheese (Collins *et. al.*, 2003; De Wit *et. al,* 2005).

Free fatty acids (FFA) of Ras and Bio-Ras cheese samples were determined and presented in Table (3) as mg/100g. The FFA composition of all samples varied and the most abundant in investigated Ras cheese samples were Palmitic, stearic, and meristic acids being highest level of saturated fatty acids in Ras cheese samples. FFA gradually increased with increasing the ripening time. Ras cheese with added probiotic lactobacilli cultures showed higher FFA values during ripening. However, cheeses containing *Lb. casei*, *Lb. rhamnosus* and *Lb. helveticus* exhibited a higher FFA value than that with other lactobacilli. The obtained results indicate that Probiotic Lactobacilli cultures contribute to lipolysis in cheese. High acid values in cheeses containing Lactobacilli have been previously reported by Madkor *et al.* (2000) and Alewijn *et. al.*, (2005) and attributed to the release of intracellular esterase and lipases.

Table 3. Free fatty acid (mg/100 g) content of fresh and 90 days ripened Ras and Bio-Ras cheese

Free fatty acid	Control	*Lb. acidophilus*	*Lb. casei*	*Lb. helveticus*	*Lb. reuteri*	*Lb. rhamnosus*
Fresh						
Caprylic	2.40	0.50	5.60	0.84	0.10	2.20
Capric	5.10	1.08	3.90	1.54	0.54	3.90
Lauric acid	2.00	0.79	1.90	0.91	0.20	2.00
Myristic	10.00	2.10	10.20	1.03	1.62	10.20
Pentadecanoic	ND	ND	ND	ND	ND	ND
Palmitic	0.70	2.63	0.60	2.65	3.97	0.40
Palmitoleic	1.00	0.21	1.00	0.10	0.18	1.10
Heptadecanoic	ND	ND	ND	ND	ND	ND
Cis -10 Heptadecanoic	ND	ND	ND	ND	ND	ND
Stearic	0.60	1.98	0.50	1.60	2.61	0.30
Oleic	7.90	0.97	11.70	3.25	6.84	11.20
Linoleic	7.80	0.43	11.90	0.91	0.23	11.40
Linolenic	ND	ND	ND	ND	ND	ND
Arachidic	ND	ND	ND	ND	ND	ND
Total free fatty acids	37.50	10.70	47.30	12.83	16.29	42.70
90 days of ripening						
Caprylic	3.90	1.58	3.80	1.46	0.55	3.70
Capric	4.00	4.05	11.60	5.26	1.04	5.10
Lauric acid	5.90	2.86	6.10	3.70	1.41	6.00
Myristic	6.20	9.48	17.90	13.75	9.24	15.80
Pentadecanoic	1.13	0.90	1.10	0.50	1.88	1.27
Palmitic	0.80	30.08	1.90	35.09	29.79	0.50
Palmitoleic	3.80	0.82	4.00	0.70	1.31	3.10
Heptadecanoic	0.75	0.72	0.76	0.99	1.14	0.08
Cis -10 Heptadecanoic	0.22	0.35	0.35	0.16	0.25	0.21
Stearic	0.40	16.60	0.30	10.98	18.29	0.60
Oleic	4.20	28.65	6.00	26.24	30.93	6.10
Linoleic	3.20	1.67	6.40	1.12	1.81	7.20
Linolenic	0.62	0.94	0.56	0.35	1.12	0.42
Arachidic	0.25	0.32	0.27	0.06	0.00	0.20
Total free fatty acids	35.37	99.02	61.04	100.36	98.76	50.28

ND: not detected

Microbiological analyses:

Fig. (3) shows the overall changes in the total viable aerobic bacterial counts Ras cheese during the ripening period (90 days/15 ±2 °C). Generally, throughout the first month of ripening, all types of cheese showed gradual increase in Total Viable Bacterial (TVB) count. However, towards the end of the cheese ripening period, there was a slight decrease in the TVB count. The Bio-Ras cheese with *Lb. helveticus* had the highest TVB count, followed by *Lb. rhamnosus* and control Ras cheese. However, by the end of the ripening period, the *Lb. rhamnosus* treatment had the highest TVB count, followed by the *Lb. helveticus* treatment. This is constant with the acidity values shown in Table (1) and agrees with previous research of (Abd-Ellah, 2008) and (Ibrahim, *et. al*., 2011).

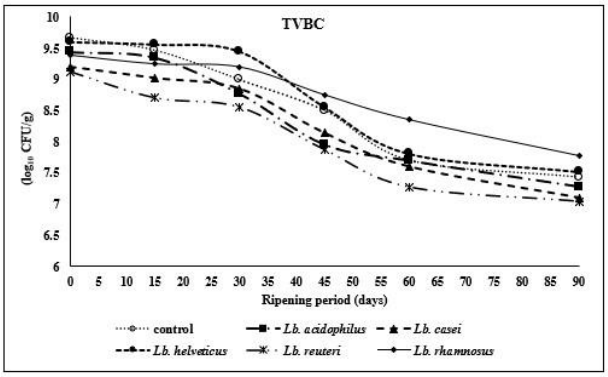

Fig. 3. Total viable bacterial count (log_{10} CFU/g) of Ras and Bio-Ras cheese during ripening for 90 days at 15 ±2 °C.

The results of Lactobacilli and Streptococci counts were constant with the total viable bacterial count shown in Fig. (4). The count of Lactic acid bacteria increased till the first month of the ripening period, after which it gradually decreased till the end of ripening period. The highest count of Lactobacilli in treated Bio-Ras cheese compared to Control cheese indicates that Lactobacilli were well-preserved in the experimental cheeses. The Lactobacilli count in Bio-Ras cheese with *Lb. helveticus* was the highest, followed by *Lb. rhamnosus* and *Lb. casei*. Throughout the ripening period, *Lactobacilli* and *Streptococci* counts were consistently higher than 7 log_{10} CFU/g, indicating that Bio-Ras cheese has probiotic properties for humans.

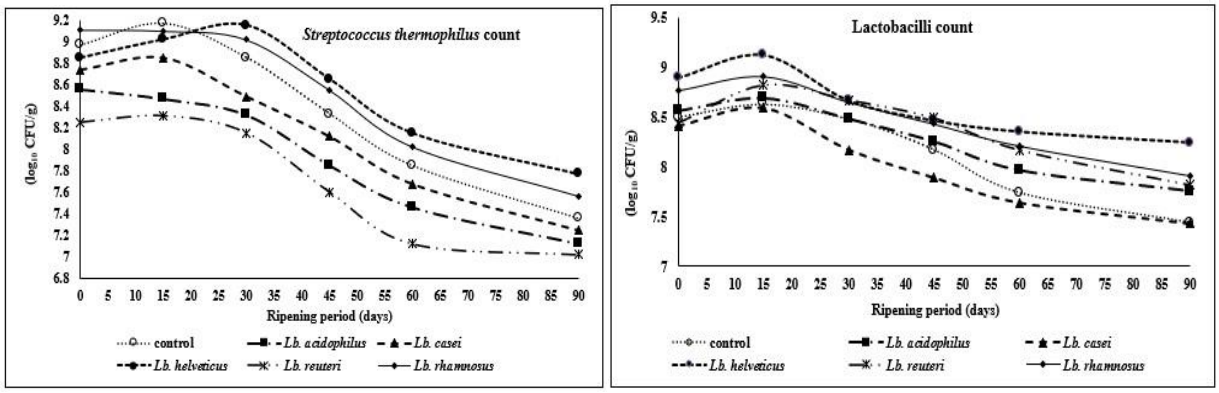

Fig. 4. Lactobacilli and Streptococci counts (log_{10} CFU/g) of Ras and Bio-Ras cheese during ripening for 90 days at 15 ±2 °C.

Fig. (5) shows that there is no yeast and mould count during the first 15 days of ripening, then appeared in the control Ras cheese treatment after 15 days. After 30 days of ripening, yeast and mould count appeared in all Bio-Ras cheese. During the ripening period, the number of yeasts and molds decreased slightly in all treatments. The highest number of yeast and mold counts was found in the control and Bio-Ras cheese with *Lb. reuteri* followed by *Lb. acidophilus*. This indicates that the incorporation of Lactobacilli bacteria used in making Ras cheese helped to avoid and decrease the growth of fungi. Caplice and Fitzgerald (1999).

Lactobacilli bacteria may have created facultative anaerobic conditions in the cheese or produced lactic and organic acids, leading to a decrease in the pH of the growth environment, which inhibits the growth and sporulation of molds. Additionally, natural compounds with antifungal

properties produced by The LAB can be used effectively as a bio-preservative to protect against the growth of phytopathogenic and food-spoilage fungi., as shown by the results of Nagy *et.al*., (2009) and Zabouri *et al.* (2021).

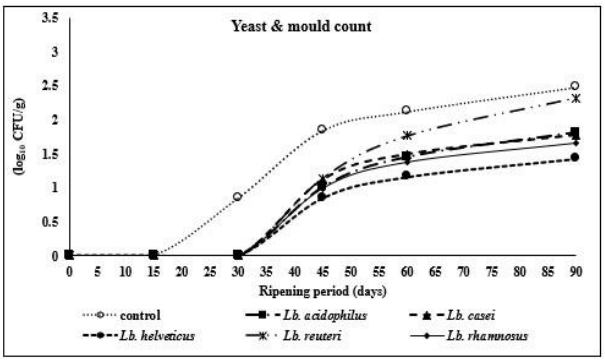

Fig. 5. Yeast & mould counts (log_{10} CFU/g) of Ras and Bio-Ras cheese during ripening for 90 days at 15 ±2 °C.

Organoleptic evaluation:

As in Table (4) all organoleptic parameters increased in control Ras and Bio-Ras cheese treatments by increasing the ripening period 30, 60 and 90 days.

Previous studies have shown that addition of probiotic Lactobacilli to hard cheese can improve its taste and Body & texture (El-Abboudi *et. al.,* 1991; Muir *et. al.*, 1996). Table (4) summarizes the sensory scores for Ras and Bio-Ras cheese at 30,60 and 90 days of ripening. The results indicate that addition of probiotic Lactobacilli cultures can positively impact the acceptability of cheese which explained by its flavor, texture, appearance, and overall appeal. The Bio-Ras cheese treatment with *Lb. helveticus* received the highest score at 60 days of ripening ($P \leq 0.05$). According to a report by Madkor *et al*. (2000), the enhanced flavor of Ras cheese can be attributed to the increased proteolytic and lipolytic activity induced by the probiotic Lactobacilli.

Table 4. Organoleptic evaluation of Bio-Ras cheese among ripening for 90 days at 15 ±2 °C.

	Ripening period (days)		
	30	**60**	**90**
	Flavor (50 points)		
Control	31.1^{Cc}	37.0^{Bb}	45.1^{Aa}
Lb. acidophilus	36.5^{Ab}	38.7^{Ab}	41.5^{Ab}
Lb. casei	33.2^{Ac}	35.6^{Ab}	37.5^{Ac}
Lb. helveticus	39.8^{Ba}	42.5^{Aa}	46.1^{Aa}
Lb. reuteri	32.5^{Bc}	36.5^{Bb}	43.0^{Ab}
Lb. rhamnosus	38.7^{Aa}	41.1^{Aa}	45.8^{Aa}
	Body & Texture (40 points)		
Control	27.0^{Cb}	30.0^{Bb}	34.0^{Aa}
Lb. acidophilus	31.1^{Ba}	32.5^{Ba}	35.7^{Aa}
Lb. casei	30.7^{Ba}	33.0^{Ba}	35.2^{Aa}
Lb. helveticus	32.6^{Aa}	34.5^{Aa}	37.1^{Aa}
Lb. reuteri	30.8^{Aa}	33.0^{Aa}	35.5^{Aa}
Lb. rhamnosus	31.8^{Aa}	33.7^{Aa}	36.1^{Aa}
	Appearance (10 points)		
Control	8.1^{Bb}	9.0^{Ac}	9.0^{Ab}
Lb. acidophilus	9.0^{Aa}	9.3^{Ab}	9.7^{Aa}
Lb. casei	9.1^{Aa}	9.3^{Ab}	9.6^{Aa}
Lb. helveticus	9.1^{Aa}	9.6^{Aa}	9.8^{Aa}
Lb. reuteri	8.6^{Ab}	9.0^{Ac}	9.4^{Ab}
Lb. rhamnosus	9.2^{Aa}	9.7^{Aa}	10.0^{Aa}

Means with the same superscript capital letter in the same row along the ripening period is not significantly different ($P \leq 0.5$). The total Means with the same superscript small letter in the same column among treatments is not significantly different ($P \leq 0.5$).

The results of the body and texture score showed that the Bio-Ras cheese produced with probiotic Lactobacilli (*Lb. helveticus* and *Lb. rhamnosus*) improved was compared to the Control Ras cheese after 30 days of ripening. This could be due to the influence of Lactobacilli with high intracellular peptidase activity, which leads to increased casein hydrolysis and affects the cheese. This leads to the formation of smaller peptide fragments and free amino acids, which can affect the conformation of secondary structures such as the α-helix. Shehata *et. al*., (2004b). This may impact changes in the curd firmness, resulting in an enhancement and improvement in the body and texture quality of cheese with an adjunct starter culture.

Therefore, it could be recommended that Bio-Ras cheese with acceptable organoleptic, microbiological, and chemical properties could be achieved with the use of *Lb. helveticus, Lb. rhamnosus* and *Lb. casei* as probiotic starter cultures.

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خواص جودة الجبن الجاف الحيوي المصري

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الملخص

تمت صناعة جبن راس كنترول باستخدام بادئ اليوجهورت *Streptococcus thermophiles, Lactobacillus delbrueckii* subsp. *bulgaricus* وكذلك جبن راس حيوي باستخدام سلاله نشطه من بكتريا بروبيوتيك هي *Lb. acidophilus*، *Lb. casei*، *Lb. helveticus*، *Lb. reuteri* وكذلك *Lb. rhamnosus* باستخدام لبن بقرى معدل التركيب. تم تغطية قوالب الجبن الناتجة بمادة بلاستيكية ووضعها فى غرف التسوية على 15°م/ 90 يوم، تم أخذ العينات لعمل التحليلات الكيماوية والميكروبيولوجية والحسيه المطلوبة كل (0، 15، 30، 45، 60، 90 يوم) وقد أظهرت النتائج التالي: حدوث زيادة تدريجية في النسبة المئوية للمادة الجافة وحموضة الجبن ونسبة الملح في المادة الرطبة وكذلك الرماد في كل المعاملات بزيادة فترة التسوية، وكانت اعلاها في الجبن الراس الحيوي الناتج من استخدام *Lb. casei* يليها *Lb. helveticus* ثم *Lb. rhamnosus*. وبتقدير دلائل التسوية للجبن الراس كانت النسبة المئوية SN/TN والاحماض الامينية الحرة FAA وكذلك الأحماض الدهنية الحرة منخفض فى بداية التسوية ثم ازداد فى نهايتها، كما كان أفضلها الجبن الراس الحيوي المحتوى على *Lb. helveticus* يليه *Lb. rhamnosus* مقارنة بالجبن الراس الكنترول. وقد حصلت الجبن الراس الحيوى الناتج من *Lb. rhamnosus* يليها *Lb. helveticus* على أعلي درجات التحكيم الحسي (النكهة والقوام والتركيب والمظهر) ، كما كان محتواها من بكتريا البروبيوتيك اعلا من النسبة المطلوب تواجدها في الجبن في نهاية التسوية. وبالتالي يمكن التوصية بإنتاج جبن راس حيوي ذو خواص كيماوية وميكروبيولوجية وحسية مرتفعة باستخدام سلالات *Lb. helveticus* ثم *Lb. rhamnosus*.