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EFFECT OF SOME PROBIOTIC STRAINS ON THE PROTEOLYSIS OF EDAM CHEESE CURD SLURRY DURING RIPENING

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ABSTRACT: All aseptic cheese slurries were inoculated with 1% of Edam cheese starter containing *Lactococcus lactis spp lactis. Propionbacterium schermenii* PS-4, *Bifidobacterium bifidum* DSM 20082 and *Lb. acidophilus* ATTC4356 or their combination *Bifidobacterium bifidum* DSM 20082, *Lactobacillus acidophilus* ATTC4356 *and Propionbacterium schermenii* PS-4 (1:1:1) were aseptically inoculated to the cheese slurry at a rat of 1% to study the survival of the probiotic bacteria and the influence of these organisms on the proteolysis during ripening period of 20 days at 30°C.

All probiotic adjuncts strains survived the ripening process of Edam cheese slurry at high levels. After 20 days of ripening, cheese slurries maintained the level of probiotic organisms at 10^7cfu/g^{-1}. Results showed no direct influence of added probiotic organisms on the chemical composition (moisture, protein and fat) of Edam cheese slurry. However, cheese slurry inoculated with probiotic strains showed slightly higher acidity compared to control slurry. The rate of proteolysis measured (as concentration of soluble nitrogenous compounds) was found to be higher in probiotic cheese slurries than the control cheese slurry.

Each probiotic organism influenced the proteolytic pattern of Edam cheese slurry in different ways. Higher concentrations of soluble nitrogenous compounds were found in all probiotic cheese slurries. Moreover, cheese slurry containing *Bifidobacterium bifidum, Lactobacillus acidophilus and Propionbacterium schermenii* (1:1:1) had the highest concentration of these compounds and positively

influenced the flavor intensity and without detectable off flavour throughout the entire ripening. The obtained results thus suggested that *Lb. acidophilus* ATTC4356, *Bifidobacterium bifidum* DSM 20082 can be successfully applied in Edam cheese making.

Key words: Edam cheese curd, probiotic bacteria, proteolysis, cheese slurry, aseptic cheese slurry.

INTRODUCTION

Probiotic bacteria are defined as living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition (McFarland, 2000). A number of health benefits for product containing live probiotic bacteria have been claimed including alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, reduction of blood cholesterol and improvement in immunity (McFarland, 2000; Andersson *et al.*, 2001 and Shah, 2002). High levels of daily consumption of probiotic bacteria, however, are required to confer health benefits. For dietary cultures to be beneficial in food systems, they are expected to be viable in the food until the time of consumption and present at levels of at least 10^7 viable cells per gram or milliliter of a product (Shah *et al.*, 1995). For this reason, it is important to know changes in the numbers of viable bacteria during storage period.

Proteolysis plays a critical role in determining the typical sensory characteristics and represents a significant indicator of quality, as shown for Cheddar cheese (Fox *et al.*, 1996). Proteolysis is caused by enzymes contained in milk (plasmin) and rennet (pepsin and chymosin) or released by microorganisms. Probiotic strains *Lactobacillus acidophilus* 4962, *B. longum* 1941 were examined as a potential candidate for incorporation in Cheddar cheeses (Crittenden *et al.*, 2001).

Ong *et al.* (2007) investigated the proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei*, *Lb. casei* or *Bifidobacterium* sp. They found that all probiotic adjuncts survived the manufacturing process of Cheddar cheese at high levels without alteration to the cheese-making process.

The greatest contributing factor to cheese quality during ripening is proteolysis (Fox, 1989; Fox, and Law 1991 and Fox *et al.*, 1996). Cheese flavours have been attributed to degradation products of caseins, peptides, and amino acids. Specifically, flavor development has been associated with an increase in total amino acids (Ardo and Pettersson, 1988; Broome, *et al.*, 1990). Because of the lengthy ripening time required for flavor development in cheese, evaluation of each bacterial strain individually for its impact on cheese quality would be costly and time consuming. Cheese slurry systems that allow cheese to ripen at 30°C for 5 to 30 d have been used to rapidly evaluate flavor and proteolytic potential of starters and nonstarters (Kristoffersen *et al,.* 1967; Dulley, J. R. 1976; Harper *et al.*, 1978; Farkye *et al.*, 1995; Roberts *et al.*, 1995; and Wijesundera *et al.*, 1997).

In most cases, fresh curds were mixed into slurries (Farkye *et al.*, 1995) and most recently, slurries were made aseptically using UHT-treated milk (Roberts et al., 1995 and Wijesundera *et al.*, 1997). Cheddar flavors were reported by 5 to 7 d (Kristoffersen *et al.*, 1967) or within 15 d in an aseptic system (Wijesundera *et al.*, 1997). Cheese slurries may be an efficient way to gather information; however, higher moisture and temperatures influence chemical and enzymatic reactions in the model. Therefore, it has been suggested that slurries can be used only to screen organisms for their potential and not to directly predict cheese ripening (Fox *et al.*, 1996). Use of UHT-treated milk could eliminate the influence of wild nonstarter lactic acid bacteria in slurries (Roberts *et al.*, 1995 and Wijesundera *et al.*, 1997).

The objective of this study was to evaluate probiotic bacterial strains for their influence on proteolysis of Edam cheese slurries made from aseptic curds manufactured under controlled conditions with a single starter, *Lactococcus lactis* ssp. *Lactis*.

MATERIALS AND METHODS

Bacterial Strains

Lactococcus lactis ssp. *Lactis* and *Propionbacterium schermenii* PS-4 were obtained from CHL Hansen Laboratories Copenhagen, Denmark. *Bifidobacterium bifidum* DSM 20082 and *Lb. acidophilus* ATTC4356 were obtained from Cairo Microbiological Center,

MICEN, Faculty of Agriculture, Ain Shams University, Egypt.

Strain preparation for slurries

Probiotic strains and propionic acid bacteria were activated several times before being used. Tubes were incubated at 30°C overnight, and 100 ml were inoculated into sterilized skim milk (11% NDM in distilled water, 121°C for 15min). The inoculated milks were held at 30°C for 24 h prior to addition to slurries.

Preparation of Edam cheese curd

Raw cow whole milk containing 3.5% fat was obtained from the Dairy Technology Unit at the Department of Food Science, Faculty of Agriculture, Zagazig University. The milk was pasteurized at 72°C for 15 sec, cooled to 32°C, and transferred to the sterile cheese vat in the laminar flow hood. Approximately 100 L of milk were used to make Edam cheese curd. Edam cheese curd was manufactured using small cheese making laboratory scale equipment, according to Kosikowski (1977). To hundred liters of warm (32°C) pasteurized cow's milk, 500 mL of a pure starter culture of *Lactococcus lactis* ssp. *lactis* was added till the acidity reach 0.19 and then the commercial rennet (50 mL) was added. Then, rennet-treated milk was left to coagulate under quiescent conditions. The coagulum was then cut and cooked to 38°C over 30 min (1°C raise per 5 min) after which the whey was completely drained. At this stage, the pH of the curd reached a value of 5.2 to 5.3 and was used for slurry preparation.

Preparation of Edam cheese slurry

Cheese slurry was prepared by modification of the method of Kristoffersen et al.(1967) as described by Farkye et al.(1995). The cheese curd was salted at a rate of 2% and transferred aseptically into a sterile wide mouth bottle and sterilized at 121°C for 15 min. All aseptic cheese slurries were aseptically inoculated with 1 % of Edam cheese starter. One part of cheese slurry was left without added probiotic stains and served as a control. The other part of cheese slurry was aseptically inoculated with either *Propionbacterium schermenii* PS-4; *Bifidobacterium bifidum* DSM 20082 and *Lb. acidophilus* ATTC4356 or their combination *Bifidobacterium bifidum* DSM 20082, *Lactobacillus acidophilus* ATTC4356 *and*

Propionibacterium schermenii PS-4 (1:1:1) to cheese slurry at a rat of 1% respectively. Each slurry preparation was replicated three times using freshly made starter cheese curd. Cheese slurries were placed into sanitized anaerobic chambers (chlorine at 200 ppm for 1 h) and incubated at 30°C. At 1, 5, 10, 15 and 20 d, cheese slurry bottles was removed from the chamber for analyses. The first analysis included enumeration of *Bifidobacterium bifidum, Lactobacillus acidophilus* and *Propionbacterium schermenii* organisms. The time zero samples were prepared immediately after slurry production.

Analysis of Cheese Slurry

Microbiological analyses

Samples for microbiological analyses were aseptically taken from cheese slurry at 0, 5, 10, 15 and 20 days of incubation at 30°C. The cheese slurry samples were homogenized, serial dilutions of homogenized cheese slurry were prepared with 0.9% NaCl solution, and 0.1 mL of each dilution was spread onto the de Man-Rogosa-Sharpe (**MRS**) agar medium (Oxoid Ltd., Basingstoke, and Hampshire, U.K.). The plates were incubated at 37°C for 2 d in an incubator. For determination of the lactobacilli count grown on MRS were selected (Mikelsaar *et al.*, 2002; Annuk *et al.*, 2003).

Sensory evaluation

The flavor intensity of aseptic cheese slurries with and without inoculation of probiotic bacterial strains was assessed by the method of King and Cleeg (1979).

Chemical analysis of cheese slurry

All cheese slurry samples were analysed for acidity, moisture, fat and protein at each stage of cheese slurry ripening. The moisture, protein, fat contents and titratable acidity of cheese slurries were determined in duplicate by the method described by Ling (1963).

Ripening indices

Water soluble nitrogen (WSN), 12% TCA-soluble nitrogen (NPN) and amino acid nitrogen (AN) were determined by the method described by Gripon *et al.*, (1975).

RESULTS AND DISCUSSION

Composition of Slurries

Table 1 shows the average chemical compositions of Edam cheese slurries containing

probiotic bacterial strains during ripening at 30°C. Results showed that addition of both individual probiotic bacterial strains and in combination did not influence the chemical composition of cheese slurries during ripening for 20 days. This indicated that preparation of slurries was almost uniform between samples.

Moistures were expected to be higher than those found in cheeses, typically <40%. Higher moistures found in slurries, relative to cheese, could increase microbial growth, particularly enhance nonstarter growth, and increase enzyme activities (Fox, 1989). These reactions, along with high ripening temperature, are the factors that promote accelerated ripening. Similar results were reported by Ong *et al.* (2007).

Table 1. Changes in chemical composition of cheese slurry containing probiotic bacterial stains during ripening

Components %	Ripening period (days)	Bacterial strains added				
		A	B	C	D	E
Moisture	0	60.25	60.03	60.47	60.95	60.22
	5	60.30	60.09	60.44	60.76	60.09
	10	60.20	60.13	60.23	60.64	60.16
	15	60.21	60.14	60.20	60.51	60.12
	20	60.15	60.05	60.23	60.55	60.20
Fat	0	18.50	18.42	18.00	18.20	18.20
	5	18.55	18.51	18.07	18.35	18.36
	10	18.60	18.60	18.21	18.20	18.49
	15	18.80	18.62	18.40	18.25	18.50
	20	18.70	18.60	18.35	18.50	18.50
Protein	0	19.77	19.52	19.26	19.45	19.45
	5	19.84	19.74	19.90	19.69	19.70
	10	19.65	19.58	19.52	19.77	19.84
	15	19.90	19.77	19.33	19.39	19.71
	20	20.67	19.90	20.28	19.90	19.77
Acidity	0	1.03	1.01	1.90	0.99	1.08
	5	1.20	1.17	1.25	1.32	1.35
	10	1.46	1.41	1.43	1.45	1.48
	15	1.65	1.70	1.80	1.52	1.86
	20	1.80	1.75	1.87	1.65	1.92

A: *Lactococcus lactis sup lactis* (control) **B:** *Propionibacterium schermenii*
C: *Bifidobacterium bifidum* **D:** *Lactobacillus acidophilus*
E: *Propionibacterium schermenii* +*Bifidobacterium bifidum* + *Lactobacillus acidophilus*

The acidity of aseptic cheese slurries ranged from 0.99 to 1.92. A slightly higher acidity in slurries containing probiotic strains compared with cheese was found. This could be explained by the high moisture content about 60% and high incubation temperature, which facilitated rapid utilization of residual lactose in the slurries. Differences in titratable acidity were found between the control slurries and those with added probiotic bacterial strains throughout ripening. This result indicated that the added probiotic bacterial strains contribute significant acid producing capabilities within the slurries.

Cheese slurry containing *Bifidobacterium bifidum* DSM 20082 + *Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 strains (1:1:1) indicated that more lactic acid was formed than in the control slurry. Similar results were found with slurries containing the other Examined strains.

Ripening Indices

Changes in water soluble nitrogen (WSN), 12% TCA soluble nitrogen (NPN) and 5% phosphotungestic acid soluble nitrogen amino acid nitrogen (AN) were taken as indices of testing Edam cheese slurry.

Water soluble nitrogen

Fig. 1 illustrates that the WSN/TN contents of Edam cheese slurries containing probiotic bacteria strains and control slurry increased gradually during ripening. However, the rate of increases during ripening was more pronounced in experimental cheese slurries than the control. Cheese slurry containing *Bifidobacterium bifidum* DSM 20082 + *Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 strains (1:1:1) showed the highest concentration of water soluble nitrogenous compounds compared with control slurry and slurry inoculated with other tested strains. Similar results were obtained of other kinds of cheese by some research workers (Dulley, 1976; Muehlenkamp-Ulate and Warthesen 1999 and DING *et al.*, 2001). The increase in soluble nitrogen Tab.2 during the ripening period indicates that the slurries treated with either *Bifidobacterium bifidum* DSM 20082+ *Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 or their (1:1:1) mixture showed higher levels of water-soluble nitrogen (WSN) compared to the control.

It was also of interest to notice that *Bifidobacterium bifidum* showed

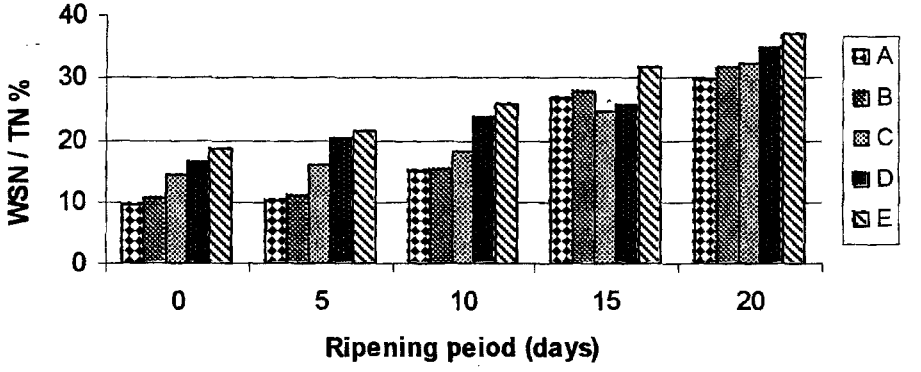

Fig. 1. Changes in WSN/TN % of cheese slurry containing probiotic bacterial stains during ripening

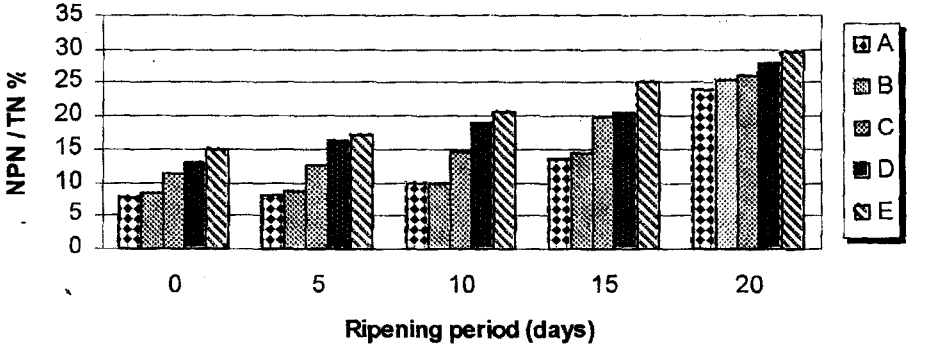

Fig. 2. Changes in NPN/TN % of cheese slurry containing probiotic bacterial stains during ripening

the highest increase in water-soluble nitrogen when compared to *Lactobacillus acidophilus* and *Propionibacterium schermenii*.

12% TCA-soluble nitrogen

Fig. 2 shows the changes in 12% TCA-SN as a percent of TN of Edam cheese slurry containing probiotic bacterial strains during ripening. Generally, the intensity of proteolytic activity of *bacterial strains* varies considerably. Results showed that the level of 12 % TCA-soluble nitrogen contents of Edam cheese slurries containing probiotic bacteria strains and control slurry increased gradually during ripening. However, the rate of increases during ripening was more pronounced in experimental cheese slurries than the control.

Cheese slurry containing *Bifidobacterium bifidum* DSM 20082 + *Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 strains (1:1:1) had the highest concentration of 12 % TCA-soluble nitrogen compounds compared with control slurry and slurry inoculated with other tested strains. Similar results were obtained of other kinds of cheese by some research workers (Dulley, 1976; Muehlenkamp-Ulate and Warthesen1999 and Ding *et al.*, 2001).

Amino acid nitrogen (AN/TN)

Fig 3 illustrates the changes in 5% PTA soluble nitrogen of Edam cheese with added probiotic strains during ripening. The liberation of free amino acids during the ripening of the slurries indicating that the addition of probiotic bacterial strains led to higher values of free amino nitrogen AN/TN compared to the control. Cheese slurry made with *probiotic bacterial and Propionibacterium schermenii* PS-4 *strains* developed higher amino acid nitrogen AN/TN than both control and *Propionibacterium schermenii* and *lactobacillus acidophilus* treated cheese slurries. This suggests that probiotic bacteria *may* be added to cheese to increase the release of free amino acids during ripening.

Our results in that respect are comparable with the work of previous authors for other genera of lactic acid bacteria. Bartels *et al.* (1987) used whole cells of *Lb. helveticus* CNRZ32 which were freeze-shocked at –24 C before being added to milk for Gouda cheese manufacture in an attempt to enhance flavour development. Substantial increases in water-soluble peptides and amino acids were observed in experimental cheese compared to controls.

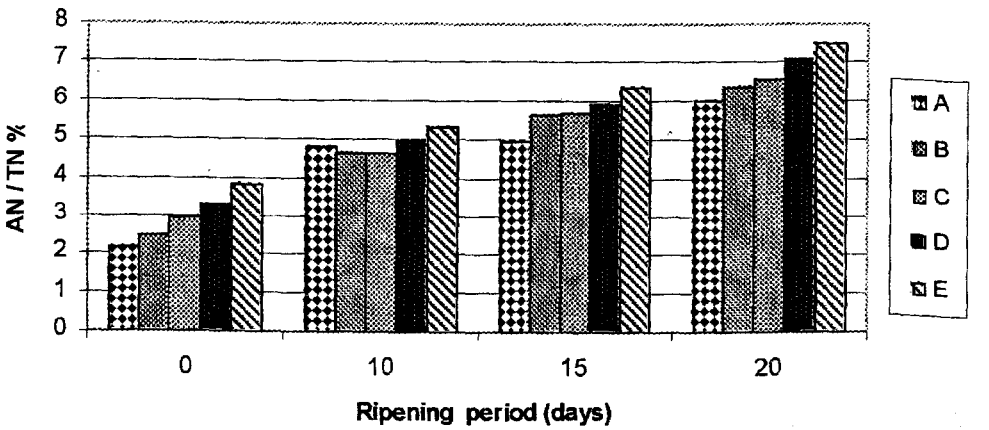

Fig. 3. Changes in AN/TN % of cheese slurry containing probiotic bacterial stains during ripening

The obtained results are also comparable to the findings of Spangler *et al.* (1989), El-Shafei (1994) and Johnson *et al.* (1995).

These authors reported that the incorporation of freeze-shocked cells of *lactobacilli*, *lactococci*, *Leuconostoc* and *Bifidobacterium* increased the levels of proteolysis in the cheese. We would also like to point out that the model system comprising cheese slurries containing probiotic bacterial strains gave promising results. Addition of probiotic bacterial strains to starter cheese curd gave a good indication of their contribution to proteolysis during ripening. However, cheese making using conventional procedures is still needed to confirm these findings.

Changes in Probiotic Bacteria

Figs. 5 and 6 show the changes in probiotic bacterial counts of Edam cheese slurries inoculated with probiotic strains during ripening. The obtained results showed that all probiotic adjuncts strains survived the ripening process of Edam cheese slurry at high levels. The probiotic strains increased gradually reaching their maximum level after 10 days of cheese slurry maturation and then decreased gradually until the end of incubation period.

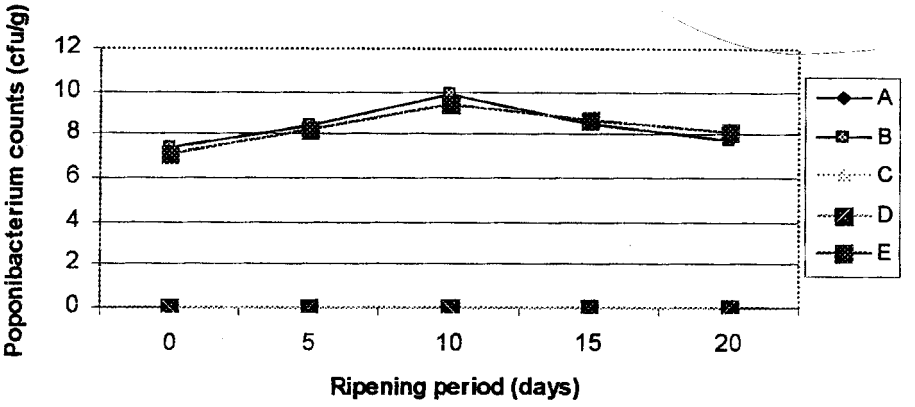

Fig. 4. Changes in Proponibacterium counts of cheese slurry containing probiotic bacterial stains during ripening

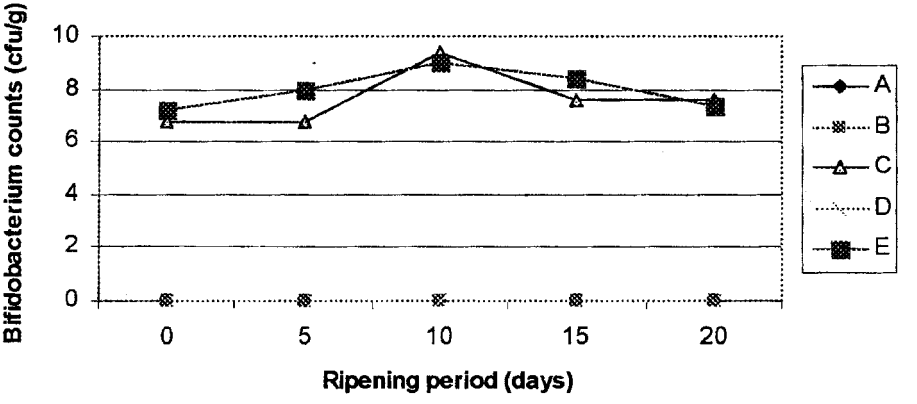

Fig. 5. Changes in Bifidobacterium counts of cheese slurry containing probiotic bacterial stains during ripening

However, their numbers after 20 days of ripening maintained the level of probiotic organisms at 10^7cfu/g^{-1} (Shah *et al.*, 1995). For dietary cultures to be beneficial in food systems, they are expected to be viable in the food until the time of consumption and present at levels of at least 10^7 viable cells per gram or milliliter of a product (Shah *et al.*, 1995). For this reason, it is important to know the changes in the numbers of viable bacteria during storage period. Similar results have been reported by Ong *et al.* (2007). They evaluated the proteolytic pattern of probiotic Cheddar cheese containing probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei*, *Lb. casei* or *Bifidobacterium* sp. found that all probiotic adjuncts survived the manufacturing process of Cheddar cheese at high levels without alteration to the cheese-making process.

Sensory Evaluation

Sensory analysis and water soluble nitrogen analysis confirm that *Bifidobacterium bifidum* DSM 20082 + *Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 (1:1:1) provides high proteolytic activity that is associated with cheese flavor enhancement without any detectable off flavour. The results demonstrate the differences in performance between *Lactobacillus acidophilus* and *Bifidobacterium bifidum* or *Propionibacterium schermenii.*

Table 2. Organoleptic properties of Edam cheese slurry as affected by different probiotic bacterial strains

Ripening period (days)	Cheese slurry inoculated with				
	A	B	C	D	E
10	4.20	4.30	4.50	4.65	4.85
15	4.50	4.65	4.75	4.90	5.25
20	5.00	5.25	5.50	5.60	5.80

A: *Lactococcus lactis sup lactis* (control) **B:** *Propionibacterium schermenii*
C: *Bifidobacterium bifidum* **D:** *Lactobacillus acidophilus*
E: *Propionibacterium schermenii+Bifidobacterium bifidum + Lactobacillus acidophilus*
*The maximum point of evaluation is 6 degrees.

Table 2 shows that incorporation of adjunct lactobacillus acidophilus + *Bifidobacterium bifidum Propionibacterium schermenii* (1:1:1) in Edam cheese curd slurry positively influenced the flavor intensity throughout the entire ripening period. A taste panel indicated that adjunct-treated cheese rapidly developed a typical flavor and highest flavor intensity after 15 days of ripening compared to control cheese slurry. Table 2 showed that incorporation of adjunct *Bifidobacterium bifidum DSM 20082 + Lactobacillus acidophilus* ATTC4356 and *Propionibacterium schermenii* PS-4 (1:1:1) in Edam cheese curd slurry positively influenced the flavor intensity and gained the highest score points for flavour intensity throughout the entire ripening period.

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تأثير بعض السلالات الحيوية علي التحلل البروتيني لمعلق خثرة جبن الايدام أثناء التسوية

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في هذا البحث تم تلقيح جميع خثرات جبن الايدام المعقمة بـــ ١% من بادئ جبن الايدام المحتوي علي *Lactococcus lactis spp lactis.* . ثم تم تلقيح الخثرات المعقمة بكلا من السلالات *Propionbacterium schermenii* PS-4, *Bifidobacterium bifidum* DSM 20082 and *Lb. acidophilus* ATTC4356 أو الخليط ما بين *Bifidobacterium bifidum* DSM 20082, *Lactobacillus acidophilus* ATTC4356 *and Propionbacterium schermenii* PS-4 بنسبة (١ : ١ : ١) لدراسة البكتيريا الحيوية الحية و تأثير هذه الميكروبات علي التحلل البروتيني أثناء فترة التسوية لمدة ٢٠ يوم علي درجة حرارة ٣٠ °م.

وقد أوضحت النتائج أن كل السلالات الحيوية المضافة كانت حية أثناء عملية تسوية معلق جبن الايدام عند مستويات مرتفعة. بعد ٢٠ يوم من التسوية ، فان خثرات الجبن الملقحة حافظت علي مستوي الميكروبات الحيوية عند مستوي $10^7 cfu/g^{-1}$. وقد وجد أشارت النتائج انه لا يوجد تأثير مباشر من إضافة الميكروبات الحيوية علي التركيب الكيماوي (رطوبة - بروتين - دهن) لمعلق جبن الايدام. و مع ذلك ، فان خثرة الجبن الملقح بالسلالات الحيوية اظهر زيادة طفيفة في الحموضة مقارنة بخثرة الجبن المقارن (الكونترول). وقد كان معدل التحلل البروتيني المقدر كتركيز المركبات النيتروجينة الذائبة والتي كانت اعلي في خثرة جبن الايدام الحيوي عن خثرة جبن الايدام المقارن (الكونترول).

وقد أثرت كل سلالة من سلالات البكتيريا الحيوية علي التحلل البروتيني بطريقة مختلفة. وقد كانت التركيزات عالية في المركبات النيتروجينة في كل خثرات الجبن الحيوي (المحتوي علي سلالات حيوية). فضلا علي ذلك، معلق الجبن المحتوي علي خليط من كل من *Bifidobacterium bifidum, Lactobacillus acidophilus and Propionbacterium schermenii* بنسبة (١: ١: ١) أظهر أعلي التركيزات من هذه المركبات وقد كان تأثيرها ايجابيا علي شدة الرائحة و بدون ملاحظة أي روائح غير مرغوبة في كل مراحل التسوية. ومن النتائج المتحصل عليها يتضح انه يمكن إضافة كل من سلالات *Lb. acidophilus* ATTC4356, *Bifidobacterium bifidum* DSM 20082 يمكن استخدامها بنجاح في صناعة جبن الايدام.