

## Effects of *Enterococcus faecalis* and *Enterococcus faecium*, Isolated from Traditional Lighvan Cheese, on Physicochemical and Sensory Characteristics of Iranian UF White Cheese

H. Rasouli Pirouzian<sup>1</sup>, J. Hesari<sup>1\*</sup>, S. Farajnia<sup>2</sup>, M. Moghaddam<sup>3</sup>, and Sh. Ghiassifar<sup>1</sup>

### ABSTRACT

The main objective of this study was to investigate the effect of enterococci isolated from traditional Lighvan cheese on the quality of Iranian UF white during ripening. Four samples of cheese were provided from four different cheese production units in Lighvan region. Strains of enterococci in these samples were isolated by standard microbiological methods and selective medium of Kanamycin Escilin Azide Agar and then identified by biochemical methods. In the second stage of research, the effect of adding isolated enterococci in traditional Lighvan cheese on the quality of Iranian UF white cheese was investigated in a 60-day period. Addition of *Enterococcus* spp. did not significantly ( $P > 0.01$ ) affect the pH and percentage of pH 4.6-Soluble nitrogen/total nitrogen. In the cheese produced with *E. faecalis* and *E. faecium* strains, lipolysis rate was higher and flavor properties were improved. Moreover, results of measuring percentage of soluble nitrogen at pH 4.6 and urea polyacrylamide gel electrophoresis indicated an increase in proteolysis rate in the cheese containing *E. faecalis* and *E. faecium* strains compared to the control cheese. Furthermore, the highest percentage of non- protein nitrogen was observed in the cheese containing *E. faecium*. In conclusion, the results showed the positive effect of the *E. faecalis* and *E. faecium* on secondary proteolysis during ripening. The proteolytic activity displayed by some enterococcal strains may contribute to cheese ripening and flavor development. Because of these interesting metabolic traits, enterococci have been proposed as part of defined starter culture combination for UF white cheeses.

**Keywords:** *Enterococcus faecalis*, *Enterococcus faecium*, Lighvan cheese, UF white cheese.

### INTRODUCTION

Nowadays, researchers are examining the potential of native microorganisms obtained from raw milks in the Mediterranean countries. Lighvan cheese is basically made from Ghezel sheep milk in the area of Sahand mountainside, which is located in the northwest Iran. It is the most popular traditional cheese made from raw sheep's milk in East Azerbaijan Province of Iran.

This variety of cheese is characterized by unique hardness (semi-hard), saltiness and spiciness. Milk coagulation is usually carried out at 23-25 °C for 120 minutes using animal rennet (pure chymosin (CHR HANSEN, Denmark) without deliberate addition of a starter culture.

It is well known that industrially produced cheeses do not have the traditional flavors, or at least lack some characteristic flavors, which is attributed to the pasteurization of

<sup>1</sup> Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Islamic Republic of Iran.

\* Corresponding author; e-mail: jhesari@tabrizu.ac.ir

<sup>2</sup> Tabriz Drug Applied and Biotechnology Research Center, University of Medical Sciences, Tabriz, Islamic Republic of Iran.

<sup>3</sup> Department of Agronomy and Plant Breeding, Faculty of Agriculture, Tabriz University, Tabriz, Islamic Republic of Iran. e-mail: Mmogadam@tabrizu.ac.ir



milk and the use of undefined commercial starters in cheese making (Ginzinger *et al.*, 1999). Thus, traditionally produced raw-milk cheeses exhibit a greater overall intensity of flavor and broader flavor profiles, and the typical sensorial properties of these cheeses are apparently a result of the diversity of species and strains of local and specific indigenous milk micro flora (Grappin and Beuvier., 1997).

Enterococci have important implication in the dairy industry. They play an important role in the development of sensory characteristics during ripening of many cheeses, probably through proteolysis, lipolysis, and citrate breakdown, hence, contributing to their typical taste and flavor (Foulquie Moreno *et al.*, 2006). Because of their role in ripening, flavor development, and bacteriocin production in cheese, it has been suggested that enterococci with desirable technological and metabolic traits could be included in starter cultures of various cheeses (Foulquie Moreno *et al.*, 2006). The aim of the present work was to evaluate the effect of *E. faecalis* and *E. faecium* strains when used as an adjunct starter on the proteolytic, lipolytic activity and sensory characteristics of Iranian UF white cheese.

## MATERIALS AND METHODS

### Microbiological Analysis

Four samples of cheese were provided from four different cheese producers in Lighvan region. Five grams of each sample were homogenized with 10 ml of a sterile solution of Phosphate buffer saline (PBS) at 40-45°C for 1 minute in a Stomacher 400 Lab Bender, thus making a  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  dilutions. Strains of enterococci in these samples were isolated by standard microbiological methods and selective medium of Kanamycin Esculin Azide Agar (KAA) (Suzzi *et al.*, 2000) and, then, identified by biochemical methods. To identify species of enterococci, sugar

fermentation tests were performed using six sugar types including *Arabinose* (L), *Raffinose* (D), *Lactose* (D), *Sorbitol* (D), *Sorbose* (D) and *Melibiose* (D) (Lopez-Diaz *et al.*, 2000). Afterwards, catalase and curd formation tests (Fox *et al.*, 2000) were done on isolated strains. Some of *E. faecium* and *E. faecalis* strains, which were incubated in milk powder solution at 37°C for 16 hours, separated curd from whey.

### Antibiotic Susceptibility Testing and Haemolytic Activity

The minimum inhibitory concentration (MIC) of ten antibiotics including penicillin and vancomycin was determined for all the tested strains. Strains were reactivated overnight at 37°C both in LB broth and Mueller-Hinton medium. Inoculation of MIC microtiter test plates containing different antibiotics and preparation of inoculua to reach a final inoculum density of  $10^4$  bacteria  $\text{ml}^{-1}$  were performed. After aerobic incubation of the inoculated plates at 37°C for 24-48 hours, the MIC of a given antibiotic was visually evaluated as the lowest concentration at which no growth was observed (Maietti *et al.*, 2007).

For testing haemolytic activity, fresh culture of *E. faecium* and *E. faecalis* strains was streaked on Cplumbia agar plates, containing 5% (w/v) sheep blood, and incubated for 48 hours at 30°C. Blood agar plates were examined for signs of  $\beta$ -haemolysis (clear zones around colonies) (Ghraiiri *et al.*, 2008).

### Cheese Making

Experimental UF white cheeses were made in four trials at four separate days. The retentate was provided by Iran Dairy Industry Inc., Pegah Co (Tabriz, Iran) and used for production of Iranian UF white cheese. Raw milk of high microbial quality was standardized to 3.5% fat and, after bactofugation in two steps, pasteurized at

72°C for 15 seconds and then ultra-filtered at 50°C. The membrane cartridges were of the spiral wound type (no UFPH20 Invensys APV, Silkeborg, Denmark) and the membrane had a nominal molecular weight cut-off of approximately 20 kg mol<sup>-1</sup> with a surface area of 16.9 m<sup>2</sup>. The ultra-filtration unit was operated at an inlet pressure of 5.3 bar and an outlet pressure of 1.7 bar. The retentate was pasteurized at 78°C for 60 seconds and then cooled to 35°C. A mixture of mesophilic (G3 mix, composed of *Lactococcus cremoris* and *L. lactis*) and thermophilic (Joghurt 709, composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*) cultures (both produced commercially by Laboratorium Visby, Tender Aps, Denmark) in the ratio of 7:1, used as starter, immediately filled (450 g) into containers. Four types of cheeses were produced: (1) combination A contained the control starter cultures (2% w/v) plus *E. faecalis* strain as an adjunct starter (1% w/v), (2) combination B contained the control starter cultures (2% w/v) plus *E. faecium* strain as an adjunct starter (1% w/v), (3) combination C contained the control starter cultures (2% w/v) plus *E. faecalis* and *E. faecium* strains as adjunct starters (0.5% w/v) and (4) combination D was used as the control sample and contained *L. lactis* (G3mix6, g3mix7), *L. cremoris*, *L. bulgaricus* (yoghurt 709) and *Streptococcus thermophilus* (2% w/v) (5 ml of 1% (w/v) aqueous extract from strains was added to 450 g retentate). Then, samples were left to coagulate at 30-32°C room for 20 minutes. A parchment paper was placed on top of the coagulum and dry salt (3%) was added. The containers were sealed with aluminum foil. Salt gradually adsorbed moisture from curd and a layer of brine formed around the cheese in the containers. Cheese packs were held at 26-28°C for 24 hours and then transferred to a cool room (7°C); the next day was considered as the first day of ripening and the samples were ripened for 60 days. One cheese of each trial was sampled and

analyzed at days 1, 15, 30, 45 and 60 of ripening.

#### Chemical Composition

The pH and moisture were determined following Freital *et al.* (1997). Salt was measured by a potentiometric method (Alonso *et al.*, 1987) and fat according to the Gerber-van Gulik method (Ardo and Polychroniadou, 1999). Total protein was determined using the Kjeldahl method (Ardo and Polychroniadou, 1999). All analyses were repeated three times and results were reported as means±standard deviations.

#### Proteolysis

The nitrogen fractions of the cheese samples, including pH 4.6-soluble nitrogen (SN) and soluble nitrogen in 12% trichloroacetic acid (TCA) considered as non-protein nitrogen (NPN), were obtained by a slight modification of the procedure of Kuchroo and Fox (1982) as described by Sousa and McSweeney (2001). Urea-polyacrylamide gel electrophoresis (PAGE) of the pH 4.6-insoluble fractions of the cheeses was performed using a vertical slab gel unit (Akhtariyan-Tehran-Iran) according to the method of Andrews (1983) as modified by Shalabi and Fox (1987). The gels were stained directly with Coomassie Brilliant Blue G250, as described by Blakesley and Boezi (1977).

#### Lipolysis

The percentage of free fatty acids (FFA) was measured using the method of Nunez (1996). Results were expressed as milli equivalents FFA (meq/100g sample).

#### Sensory Evaluation

Sensory evaluation was performed at days 1, 15, 30, 45 and 60 of ripening by a sixteen-member non-professional tasting panel familiar with UF and Lighvan cheeses.



Sensory evaluation was assayed on a scale of 1 to 5 (1: Low value, 5: High value) (Institute of Standards and Industrial Research of Iran, 1998). Every calculated average (from values given by each of the members of the jury of sensory analysis) was integrated into the statistical analysis in conformance with an analytical value.

The evaluation forms included a table of five rows and six columns. In column one, the cheeses were numbered one to four, and, in columns two to six, sensory evaluations were ranged from very good to very bad. The sensory evaluation was performed for flavor, aroma, and texture.

### Statistical Analysis

The experimental design was split plot based on randomized complete blocks. The main plots included four experimental cheeses (made with *E. faecalis* strains, *E. faecium* strains, *E. faecalis* and *E. faecium* strains, and the control cheese) and the subplots were the days of ripening. After analysis of variance, means were compared

by the 1-way ANOVA method. Statistical analyses were carried out using SPSS Version 9 for Windows 2003 (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

### Microbiological Analysis

The final results demonstrated that the dominant strains in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> cheese samples were, respectively, *E. faecalis*, *E. faecium* (four of the five strains), *E. faecalis* (four of the five strains), and *E. faecium* (Table 1). Also, the number of *Enterococcus* in UF cheese was not significant. The results indicated that the 19 isolated strains from four types of cheese were catalase negative and only one strain was catalase positive. Both enterococci strains had no haemolytic properties when tested on sheep or human blood, and exhibited no resistance against vancomycin and penicillin.

**Table 1.** Number of isolates and biochemical criteria used to identify species of enterococci.

| Sugar fermentation |                    | Lactose | Sorbitol | Sorbose | Raffinose | Melibiose | Arabinose |
|--------------------|--------------------|---------|----------|---------|-----------|-----------|-----------|
| Cheese number 1    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | -         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
| Cheese number 2    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | -         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | +         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | +         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | -         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | +         |
| Cheese number 3    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | -         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | +         |
|                    | <i>E. faecium</i>  | +       | +        | -       | -         | -         | -         |
|                    | <i>E. faecalis</i> | +       | +        | -       | -         | -         | -         |
| Cheese number 4    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | -         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | -         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | +         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | -         |
|                    | <i>E. faecium</i>  | +       | -        | -       | -         | -         | +         |

### Composition

The compositions of 1-d old tested Iranian UF white brined cheeses are shown in Table 2. There were no significant ( $P < 0.05$ ) differences among the gross compositions of the cheeses. These results showed that changes in the starter did not have significant effect on the gross composition of cheeses as reported by other workers (Karakus and Alperden, 1995).

### pH

The levels of pH of UF Iranian white cheeses during ripening are shown in Figure 1. The level of pH decreased during 60 days of ripening. It was noticed that pH levels of the four cheese types were not significantly ( $P > 0.01$ ) different. In the cheeses made with *E. faecalis* and *E. faecium* strains, pH levels

were lowest at day 60, while the cheeses made with *E. faecium* and the control cheese had the higher pH. In the cheese samples inoculated with *E. faecium* strains, the pH levels were slightly higher than the cheeses made with *E. faecalis* strains. The pH decreased rapidly during ripening, due to the rapid growth of the starter cultures and *Enterococcus* strains and acid production.

A rapid decrease in pH during the initial steps of cheese preparation is of crucial importance in cheese making process, since it is essential for coagulation and the prevention or reduction of the growth of adventitious microflora (Sarantinopoulos *et al.*, 2001).

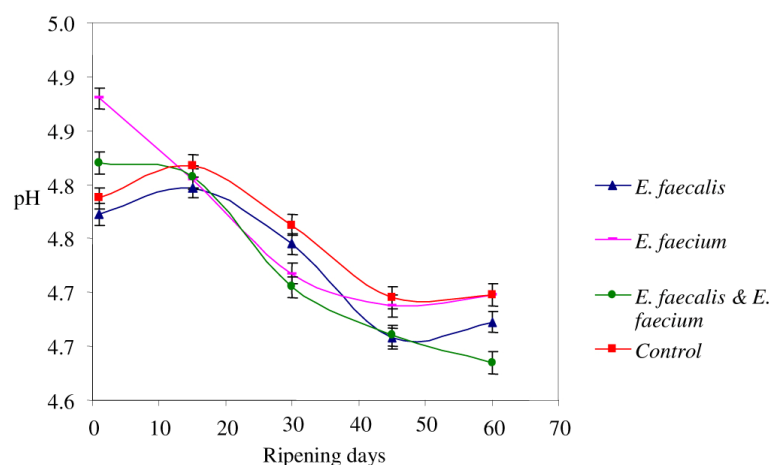
Sixty eight enterococci isolates collected from dairy products in north-west Italy were characterized with respect to their technologically relevant biochemical properties (Morandi *et al.*, 2006). The results of the study showed that *E. faecalis* was the

**Table 2.** Composition of 1-d-old experimental ultra-filtered (UF) Iranian white cheeses.

| Cheeses                                | NaCl (%)  | Moisture (%) | Fat (%)   | Protein (%) |
|--|-----------|--------------|-----------|-------------|
| <i>E. faecalis</i>                     | 3.80±0.12 | 62.39 ±0.8   | 15.5±0.39 | 13.55±0.32  |
| <i>E. faecium</i>                      | 3.75±0.14 | 63.37±0.36   | 15.5±0.41 | 14.03±0.51  |
| <i>E. faecalis</i> + <i>E. faecium</i> | 3.72±0.11 | 62.50±0.23   | 15.5±0.42 | 13.95±0.38  |
| Control                                | 3.66±0.13 | 62.59±0.25   | 15.5±0.40 | 14.30±0.25  |
| F value                                | NS        | NS           | NS        | NS          |

NS: Not significant ( $P < 0.05$ ).

Results are presented as average of data from three independent replicated Trials±Standard deviations.



**Figure 1.** pH curves of UF Iranian white cheese made without (control cheese) or with added *Enterococcus* strains during ripening. The values reported are the mean pH of all samples.



most commonly isolated *Enterococcus* species from the specific media used (51%), followed by *E. faecium* (40%) and *E. durans* (11.4%). The majority of the strains displayed weak acidification activity in milk. Most of the strains studied reduced the pH to 4.5-5 after 24 hours of incubation in skim milk. This can lead enterococci to be proposed as adjunct starter or protective cultures in the cheese.

### Lipolysis

The extent of lipolysis of experimental cheeses during ripening, as indicated by total free fatty acids levels, is shown in Figure 2. The lipolysis index increased gradually until 60 days of ripening. It is noticed that lipolysis indexes were significantly different ( $P < 0.01$ ) among the cheeses inoculated with the two types of strains. The cheeses with adjunct starters, in general, exhibited significantly ( $P < 0.01$ ) higher levels of lipolysis index by the progress of ripening compared to the control cheese. Cheese made with *E. faecalis* and *E. faecium* strains involved the highest level of FFA. These results indicated that adjunct enterococci contributed to lipolysis in cheese.

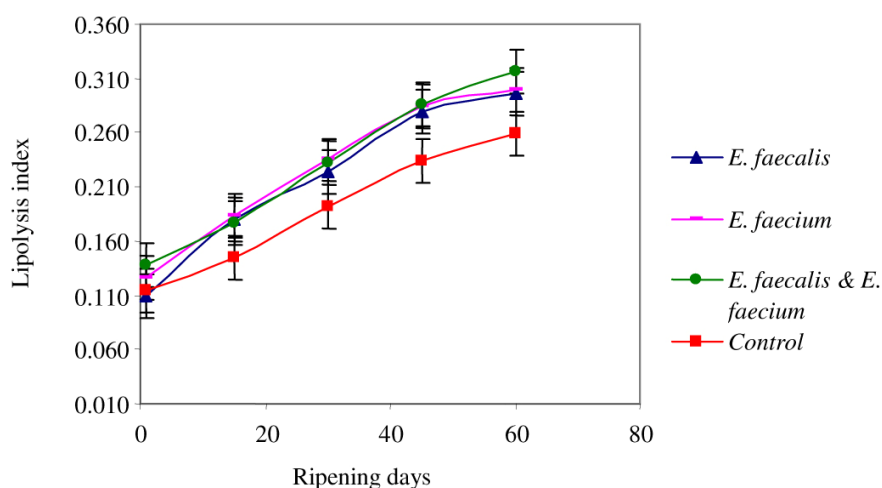
Milk fat hydrolysis during cheese manufacture and ripening is due to the

endogenous milk lipase, the lipolytic enzymes of starter and non-starter bacteria, lipases from psychrotrophic bacteria, and exogenous enzyme preparations. Fatty acids can be further converted to methylketones and thioesters, which have been implicated as cheese flavor compounds (Sarantinopoulos *et al.*, 2001).

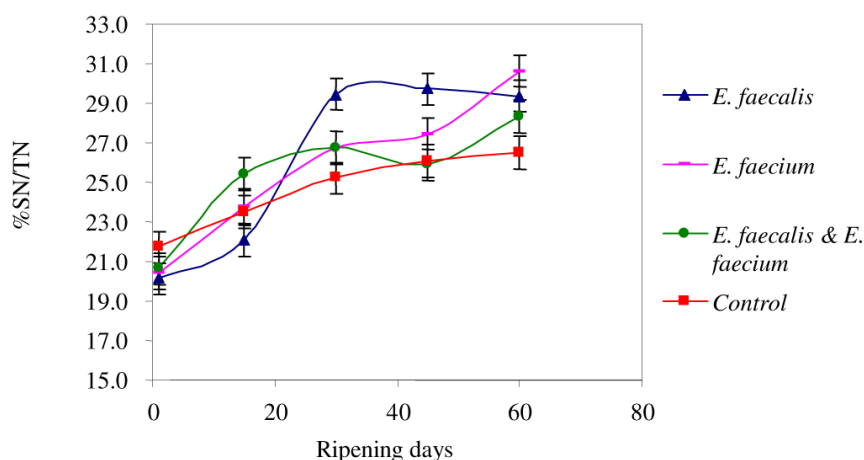
Centeno *et al.* (1999) reported that the content of volatile free fatty acids (VFFA) was higher in the samples containing *E. faecalis* var *liquefaciens* than in the *E. faecalis* var *faecalis* samples. The lowest amounts were found in the control batches. In all of the *E. faecalis* var *faecalis* batches, the highest amounts of VFFA were noted on 15 d of storage, whereas in the *E. faecalis* var *liquefaciens*, the FFA of days 10 and 15 were similar.

Level of pH 4.6-soluble Nitrogen as % of Total Nitrogen (pH 4.6-SN/TN)

The levels of pH 4.6-SN/TN in the tested Iranian UF white cheeses during ripening are shown in Figure 3. The concentrations of water-soluble nitrogen showed a gradual increase in all cheeses up to the end of ripening period, with no significant difference ( $P > 0.01$ ) among cheeses made in the presence or absence of enterococci. *E. faecium* strains showed higher levels of SN/TN as compared to the control or other adjunct-treated cheeses after the 60 days of



**Figure 2.** Amount of total free fatty acids during ripening of UF Iranian white cheese made without (control cheese) or with added *Enterococcus* strains.



**Figure 3.** Amount of pH 4.6-soluble nitrogen as a percentage of total nitrogen (SN/TN) in UF Iranian white cheeses made with *Enterococcus faecalis*, *E. faecium*, *E. faecalis* and *E. faecium*, and control samples during ripening.

ripening.

The proteolytic and esterolytic activities displayed by some enterococcal strains, as well as their ability to metabolize citrate, may contribute to cheese ripening and flavour development. Because of these interesting metabolic properties, enterococci have been proposed as part of defined starter culture combinations for different European cheeses, such as Feta, water-buffalo Mozzarella and Cebreiro cheeses (Morandi *et al.*, 2006).

Some authors claim that the enterococci used as adjunct starters in cheese manufacture contribute to increased breakdown of casein and, thus, to soluble nitrogen production (Centeno *et al.*, 1999). However, other studies have shown that proteinase activity in enterococci is low, with *E. faecalis* being the most proteolytic species (Suzzi *et al.*, 2000).

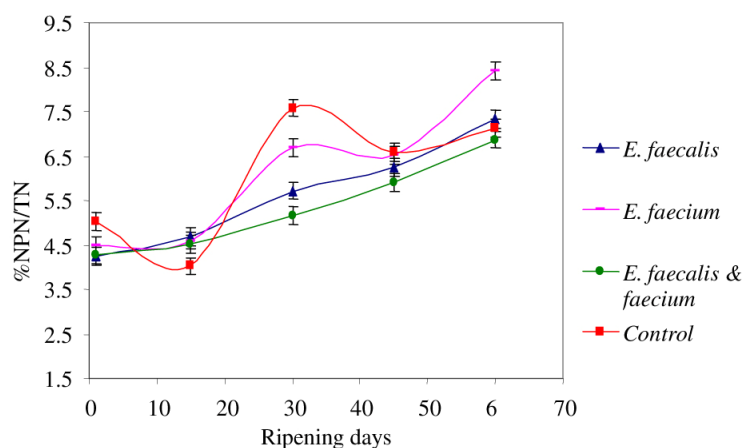
In the research of Centeno *et al.* (1999), in all of the batches made with enterococci, the percentages of soluble nitrogen were higher than in the control batches, which indicated, in general terms, a greater proteolysis in the batches made with enterococci. The highest values were observed in the samples containing *E. faecalis* var *liquefaciens*. The increase in soluble nitrogen over the period

of maturation of fermented dairy products made with *E. faecalis* has also been noted by other authors (El-Samragy *et al.*, 1988).

#### Level of Non-protein Nitrogen (NPN) as a Percentage of Total Nitrogen (NPN/TN)

The levels of NPN/TN in the tested UF Iranian white cheeses during ripening are shown in Figure 4. The levels of NPN/TN were significantly ( $P < 0.01$ ) different in the four types of cheeses. The degree of secondary proteolysis in terms of NPN was higher in cheeses produced using *E. faecium* strains. In samples produced by *E. faecium* strains, NPN/TN% increased from 5.03% at the beginning of ripening to 8.42% at 60 days of ripening, while in cheeses made with *E. faecium* and *E. faecalis* strains, NPN/TN% was lower during ripening.

Addition of adjunct cultures can improve the flavor of reduced- and low-fat cheeses mainly through increased proteolysis, particularly aminopeptidase activity, reducing bitterness, and increasing the concentration of small peptides and amino acids, which contribute directly to, or can act



**Figure 4.** Amount of non-protein nitrogen (NPN) in 12% TCA as a percentage of total nitrogen (NPN/TN) in UF Iranian white cheeses made with *Enterococcus faecalis*, *E. faecium*, *E. faecalis* and *E. faecium*, and control samples during ripening.

as precursors of, flavor compounds (Ardo, 1997; Engels and Visser, 1994).

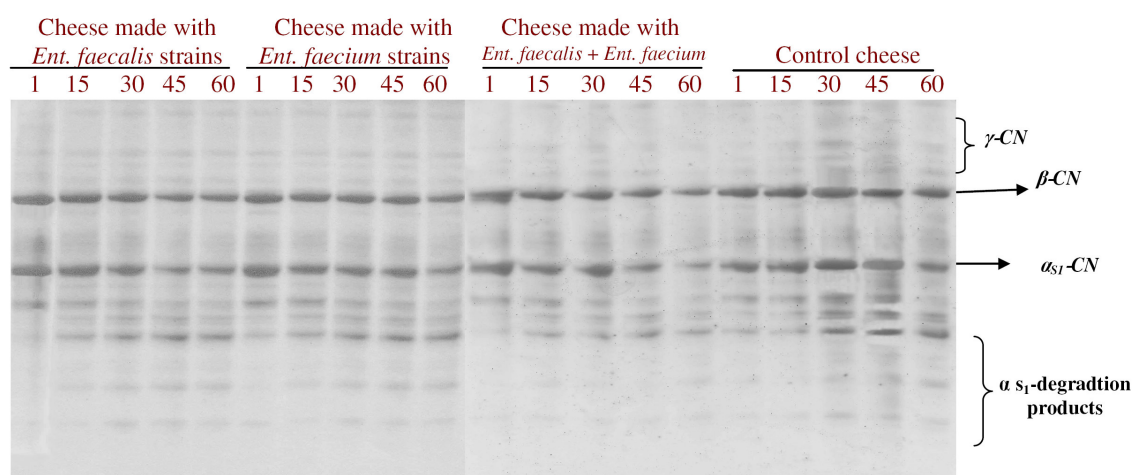
High and medium molecular mass peptides and caseins are gradually broken down by rennet and starter culture enzymes to lower molecular mass peptides and amino acids (O’Keeffe *et al.*, 1976), which are soluble in 12% TCA (Kuchroo and Fox, 1982). Therefore, the amount of 12% TCA-soluble nitrogen increases with the age of cheese (Reville and Fox, 1978).

The increase in phosphotungstic acid soluble nitrogen levels in cheeses made with

enterococci has been linked with the activity of the peptidases of these microorganisms and associated with low pH levels (Litopoulou-Tzanetaki *et al.*, 1993).

### Urea-PAGE

Urea-PAGE electrophoretograms of the pH 4.6-insoluble fraction of experimental UF white cheeses of the first replication after 1, 15, 30, 45 and 60 days of ripening are shown in Figure 5. Results of other trials



**Figure 5.** Urea polyacrylamide gel electrophoretograms of the tested UF Iranian white brined cheeses made without (control cheese) or with added *Eenterococcus* strains after 1, 15, 30, 45 and 60 d of ripening.



were similar (not shown). Some differences were observed in electrophoretic patterns among the four cheese types. In samples made with *E. faecalis* and *E. faecium* strains,  $\alpha_{s1}$ -casein had the highest hydrolysis, a fact which may be attributable both to a possible action of proteolytic enzymes (intracellular) of strains (Menendez *et al.*, 1998) and to a greater retention of rennet as a result of the less intense whey drainage during the processing of these samples.

The rennet remaining in the curd after whey drainage may be responsible for the initial degradation of the caseins (especially of the  $\alpha_{s1}$ -casein) forming high molecular weight peptides (Gripon *et al.*, 1975).

Sarantinopoulos *et al.* (2002) studied urea-page electrophoretic patterns of casein fractions in cheese and found that the residual  $\alpha_{s1}$ - and  $\beta$ -caseins in all vats continuously decreased during aging. The rate of  $\alpha_{s1}$ -casein degradation was high during the first stage of ripening and then continuously decreased. The combination of the two enterococci strains significantly enhanced the degradation of  $\alpha_{s1}$ -casein; on the contrary,  $\beta$ -casein degradation proceeded much slower and less extensively than the degradation of  $\alpha_{s1}$ -casein. Since  $\beta$ -casein is only slightly sensitive to the action of the rennet, its major degradation should be attributed to action of microbial enzymes and particularly to the specificity of the proteolytic system of *Enterococcus* strains.

### Sensory Characteristics

The sensory assessment of the tested

Iranian UF white cheeses during ripening is presented in Table 3. There were significant ( $P < 0.01$ ) differences among cheeses inoculated with the two types of strains in terms of sensory characteristics. Cheeses prepared with the *E. faecalis* and *E. faecium* as adjunct starters received better grades as compared to the other three samples. Cheeses made with *E. faecium* received lower scores than cheeses made with other adjuncts. The panelist's comments indicated that cheeses prepared with *E. faecium* strains lacked good flavor after two months and were scored low due to some inappropriate and pasty texture and weaker aroma at the end of ripening period. In contrast, cheeses made with *E. faecalis* and *E. faecium* obtained the highest flavor score and received no comment concerning bitter flavors.

Some bacteria produce extra cellular proteolytic and lipolytic enzymes that may cause undesirable texture and flavor defects (Hatzikamari *et al.*, 1999). The degradation of casein plays an important role in the development of texture in cheese. In addition, some peptides lead to the formation of flavor, whereas other undesirable bitter-tasting peptides can lead to off-flavor formation. Bacterial cell wall associated proteinases and intracellular peptidases released after cell lysis in the curd are considered to play an important role in casein hydrolysis during cheese preparation (Wilkinson *et al.*, 1994). Moreover, the significant increase in cheese flavor can be linked to the high levels of free amino groups in cheese made with the adjunct culture that possess considerable

**Table 3.** Sensory evaluation of UF Iranian white cheeses made with *E. faecalis*, *E. faecium*, *E. faecalis* and *E. faecium*, and control samples during ripening.

| Ripening day | ( <i>E. faecalis</i> ) | ( <i>E. faecium</i> ) | ( <i>E. faecalis</i> and <i>E. faecium</i> ) | Control           |
|--------------|------------------------|-----------------------|--|-------------------|
| 1            | 3.08 <sup>a*</sup>     | 3.45 <sup>bd</sup>    | 4.03 <sup>c</sup>                            | 3.63 <sup>d</sup> |
| 15           | 3.37 <sup>abd</sup>    | 3.09 <sup>b</sup>     | 4.00 <sup>cd</sup>                           | 3.74 <sup>d</sup> |
| 30           | 3.39 <sup>abd</sup>    | 3.31 <sup>bd</sup>    | 4.00 <sup>c</sup>                            | 3.62 <sup>d</sup> |
| 45           | 3.59 <sup>abd</sup>    | 3.70 <sup>bd</sup>    | 4.09 <sup>c</sup>                            | 3.70 <sup>d</sup> |
| 60           | 3.80 <sup>abd</sup>    | 3.72 <sup>bd</sup>    | 4.23 <sup>c</sup>                            | 3.74 <sup>d</sup> |

\*Values in the same row with different subscript are significantly ( $P < 0.05$ ) different.



levels of aminopeptidolytic activity. Several studies have emphasized that a relationship exists between amino N content and flavor in cheese (Muir *et al.*, 1996).

Litopoulou-Tzanetaki *et al.* (1993) reported that body and texture was developed better in Feta cheese made with *E. durans* strains than in the control batches.

## CONCLUSIONS

The majority of the studied strains exhibited low milk acidifying effect, thus suggesting a possible role of enterococci as adjunct cultures for cheese production rather than as starter microorganisms. Lipolytic activity was detected in *E. faecalis* and *E. faecium* strains and the flavor properties were improved. Also the cheeses containing *E. faecium* strains indicated the highest percentage of non- protein nitrogen.

Many reports indicate the desirable role of enterococci in cheese production and quality. It could be suggested that application of enterococci in foods could proceed after determining which strains are not pathogenic on the basis of careful selection criteria.

Based on the overall evaluation of the results obtained from the physicochemical and sensorial analysis, the most pronounced impact of the enterococci strains on Iranian UF white cheese was observed on the lipolysis index, the levels of NPN/TN, and the organoleptic properties of the ripened cheese. The present work demonstrates the technological potential of *Enterococcus* strains to be used as adjunct starters in the production of UF cheese. They appear to have the potential metabolic characters involved in cheese ripening and in aroma and flavor development.

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## اثرات انتروکوکوس فکاليس و انتروکوکوس فاسيوم جداسازی شده از پنير سنتی لیقوان بر ویژگیهای فیزیکی-شیمیایی و ارگانولپتیکی پنیر سفید فراپالایشی ایرانی

ح. رسولی پیروزیان، ج. حصاری، س. فرج‌نیا، م. مقدم، و ش. قیاسی‌فر

### چکیده

در این تحقیق تاثیر افزودن انتروکوکوس‌های جداسازی شده از پنیر سنتی لیقوان بر کیفیت پنیر سفید فراپالایشی طی یک دوره ۶۰ روزه رسیدن مورد بررسی قرار گرفت. نتایج حاصل از تجزیه واریانس داده‌ها، تفاوت معنی‌داری را در سطح ۱٪ طی دوره رسیدن بین تیمارها از نظر pH و ازت محلول در pH ۴/۶ نشان نداد. زمان رسیدن تاثیر معنی‌داری بر روی تمامی صفات اندازه‌گیری شده داشت. در نمونه پنیر تهیه شده با سویه‌های انتروکوکوس فکاليس و انتروکوکوس فاسيوم، شدت لیپولیز بیشتر بود و خواص حسی بهبود یافت. ارزیابی فرآیند پروتئولیز با اندازه‌گیری درصد ازت محلول در pH= ۴/۶ و الکتروفورز روی ژل پلی آکريل آمید نیز نمایان‌گر افزایش شدت پروتئولیز در نمونه پنیر حاوی سویه‌های انتروکوکوس فکاليس و فاسيوم در مقایسه با نمونه پنیر کنترل (فاقد سویه‌های انتروکوکوس) بود. همچنین بیشترین درصد ازت غیرپروتئینی در نمونه پنیر تلقیح شده با سویه انتروکوکوس فاسيوم مشاهده شد که نشان‌دهنده تاثیر سویه بکار رفته در پروتئولیز ثانویه پنیر طی دوره رسیدن بود. فعالیت پروتئولیتیکی مشاهده شده توسط برخی سویه‌های انتروکوکوسی می‌تواند منجر به رسیدگی پنیر و بهبود عطر و طعم آن شود. به علت همین ویژگی‌ها و رفتارهای متابولیکی، کاربرد انتروکوکوسی به عنوان کمک آغازگر در ترکیب با سایر آغازگرها در تولید پنیر سفید فراپالایشی پیشنهاد می‌شود.