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EFFECT OF STARTER BACTERIA ON PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF IRANIAN WHITE CHEESE

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ABSTRACT

The influence of the type of lactic starter inoculation on the physico-chemical and sensory properties of Iranian white cheese was investigated. Five treatments of starters were examined as follows: A) *Lactobacillus casei* 01+ aromatic mesophile CH-N-01 (1:1); B) Aromatic mesophile CH-N-01 + thermophile CH-1 (1:1); C) Thermophile CH-1; D) Thermophile CH-1 + mesophile 54 (1:1); E) Mesophile 54. The cheeses were analyzed for sensory characteristics moisture, pH, total protein, soluble nitrogen, acid degree value and hardness. Cheese samples treatments were taken in a completely randomized design. The results indicated that the type of starter bacteria and storage time had a significant effect on composition and hardness. The pH value in all treatments dropped drastically in such a way that curds made in intreatments B, C, and D reached their lowest pH during manufacturing whereas those made in treatments A and E reached their lowest pH during storage and ripening ($P<0.01$). The soluble nitrogen in treatment A was greater than all other treatments due to higher proteolytic activity. Results from acid degree value revealed that the contribution of lipolysis in such type of cheese was less than proteolysis but mesophilic starters particularly treatment A, gave higher value of lipolysis. The hardness of all cheeses decreased during storage and ripening. Sensory

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evaluation scores were the highest for starters A and B cheeses (P<0.01). Thus, a combination of commercially available mesophilic starters and *Lactobacillus casei* subsp. *casei* might be considered appropriate for Iranian white cheese production.

Key words: Iranian white cheese, Lactic starter, *Lactobacillus casei* subsp. casei. Sensory evaluation, Thermophilic and mesophilic starter.

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تاثیر آغازگرهای لاکتیک بر ویژگی های فیزیکو-شیمیایی و ارگانولپتیکی پنیر سفید ایرانی

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چکیده

در این پژوهش، تاثیر انواع آغاز گرهای لاکتیک بر ویژگی های فیزیکو-شیمیایی و ارگانولپتیکی پنیرسفید ایرانی بررسی شد. پنج تیمار مورد آزمایش شامل آغازگرهای لاکتیک عبارت بودند از: ۱) لاکتوباسیلوس کازیی 01 + آروماتیک مزوفیل (1:1)CH-N-01 ، ۲) آروماتیک مزو فیل CH-N-01 + ترموفیل

(1:1)CH-1، ۳) ترموفیل CH-1، ۴) ترموفیل CH-1+ مزوفیل 54 (1:1)، ۵) مزوفیل 54. پنیر حاصل از تاثیر آغازگرهای مختلف از نظر رطوبت، pH ، پروتیین کل، نیتروژن محلول و درجه اسیدی چربی و سفتی ارزیابی شد. ویژگی ارگانولپتیکی پنیر هم جداگانه ارزیابی شد. نمونه ای از تمام تیمارهای یاد شده مطابق الگوی آماری طرح کاملا' تصادفی و آزمون دانکن ویژگی های شیمیایی، فیزیکی و حسی در سطح آماری α=٪۱ آنالیز واریانس و مقایسه میانگین شدند. نتایج نشان دادند که نوع آغازگر و زمان نگهداری اثر معنی داری بر ویژگی های شیمیایی مانند رطوبت، pH ، پروتیین کل، نیتروژن محلول و درجه اسیدی چربی و همچنین بر ویژگی های فیزیکی مانند سفتی بافت داشت تغییرات pH نشان داد که روند در ابتدا نزولی و سپس صعودی است. تیمارهای ۲، ۳ و۴ در مراحل تولید پنیر و تیمارهای ۱و۵ در طول دوره نگهداری بیشترین کاهش درصد pH را داشتند و از نظر آماری در سطح ٪۱ معنی داربودند. در کلیه تیمارهای پنیر، روند تغییرات صعودی بود. این افزایش در تیمارهای ۱ و۵ بیش از تیمارهای ۲، ۳و۴ مشاهده گردید. نتایج آزمایش نیتروژن محلول افزایش قابل توجهی را در نتیجه تاثیر غازگر ۱ در مقایسه با سایرآغازگرها بر پدیده پروتئولیز نشان داد. بررسی میزان درجه اسیدی چربی تیمارهای مختلف پنیر بیانگر آن است که فرآیند لیپولیز کم دامنه تر از پروتئولیز بود و مقدار آن با کاربرد آغازگرهای مزوفیل بویژه آغازگر۱ افزایش بیشتری را نشان داد. میزان سفتی بافت به مرورزمان با گسترش پروتئولیز کاهش یافت. در نتایج حاصل از آزمون ارگانولپتیکی بالاترین امتیاز حسی به آغازگر ۱ و۲ تعلق گرفت ولی از نظر آماری اختلاف معنی داری نداشتند(P<0.01).

INTRODUCTION

Cheese manufacturing is a combination of microbial (lactic acid bacteria) fermentation and an enzymatic hydrolysis of the milk protein κcasein by an added proteinase, chymosin or chymosin substitutes such as bovine pepsin, or *Rhizomucor miehei* proteases. Addition of starter bacteria to milk leads to lactic acid formation, which enhances curd formation and subsequently causes the curd to shrink. The inoculation of milk with starter culture also affects the elasticity of the final curd, helps suppress the growth of undesirable organisms and affects the nature and extent of enzymatic changes during ripening of the cheese (30). Mesophilic and thermophilic–strains of starter cultures of lactic acid bacteria are most commonly used in the manufacturing of various types of cheeses, including pickled white cheeses (6). Several investigators have studied the effect of mesophilic and thermophilic starters and mixtures of both on the physico-chemical and sensory properties of pickled white cheeses. Kehagias *et al.* (22) reported that the use of thermophilic starter cultures, increases the rate of pH reduction in the first 24 h, whereas after 2 months, the pH and TCA soluble N of cheeses made with thermophilic starters were higher than other treatments. They also noticed that the hardness, fractureability as well as the sensory properties of cheese made with thermophilic starters had lower acceptability due to the change in some chemical properties such as pH and moisture content. The effect of four types of starter bacteria on the biochemical and sensory properties of white brined cheese from goat's milk was studied by Tzanetakis, *et al.* (40). The level of N soluble in phosphotungstic acid (PTA-N) and the sensory properties were higher in cheeses using *Lactococcus lactis* subsp. lactis, *Lactobacillus casei* subsp. casei, *Entrococcus durans* and *Leuconostoc mesenteroides* subsp. cremoris than cheeses made with starters lacking entrococcus and leuconostoc. This could be due to the presence of *E. durans* and higher counts of mesophilic lactic acid bacteria. Leuconostoc has been found to have intracellular as well as extracellular peptidases. Thus, a combination of lactococcus subspecies with *E. durans* or *L. cremoris* as starter culture improve the flavor of cheese mainly due to the production of high levels of PTA-N, and hence, free amino acids and ammonia which are known to be flavor precursors (9, 10). Characterization of lactococci and lactobacilli isolated from goat cheese showed that *Lactococcus lactis* possessed higher proteolytic activity than *Lactobacillus casei* or *Lactobacillus plantarum* due to higher growth rate of lactococci (35). The rate of flavor production in cheese is believed to be proportional to the level of soluble N fractions and free amino acids mainly glutamic acid, methionine and leucine (34).

The objective of this study was to investigate the effect of different starters on the physico-chemical and sensory characteristics of Iranian white cheese from pasteurized cow's milk. Knowledge of the impact of different starter cultures on quality could be of assistance to cheese manufacturers.

MATERIALS AND METHODS

Milk

Whole milk containing 3.3% fat (determined according to Gerber method) (5), was delivered from Leurak farm, Isfahan University of Technology to the pilot plant on the day of cheesemaking.

Starter Bacteria

The following starters were inoculated (at 1% level) into pasteurized standardized milk held at 35°C: A) *Lactobacillus casei* 01+ aromatic mesophile CH-N-01 (containing *L. lactis* subsp. *lactis*, *L. lactis* subsp. cremoris, biovar. *diacetylactis* and *L. mesenteroides* subsp. cremoris in a ratio of 2.5, 75, 15, and 7.5%, respectively (1:1), B) Aromatic mesophile CH-N-01 + Thermophile CH-1 (containing *L. delbruckii* subsp. *bulgaricus* and *S. thermophilus* in an equal ratio (1:1), C) Thermophile CH-1, D) Thermophile CH-1 + Mesophile 54 (containing *L. lactis* subsp. lactis and subsp. cremoris in a ratio of 5:95, respectively (1:1), E) Mesophile 54. All starters were purchased from Hansen's Laboratories, Copenhagen, Denmark.

Clotting Enzyme

Fungal Hanilase supplied by Chr. Hansen Co., Denmark, was used as rennet (3 g 100 kg^{-1} milk).

Chemical Reagents

Unless otherwise mentioned, all chemicals used in this study were analytical grades from Merck Co., Germany.

Cheesemaking

Cheese was manufactured from pasteurized cow's milk adjusted to 3% fat. Starter cultures (1%) were added to milk at 35°C. When the acidity of milk increased by 2 D°, calcium chloride (0.02 g 100 kg^{-1}) and rennet (3 g 100 kg^{-1}) were added. Curd was subsequently cut (2 cm thick slices) and transferred into a vat with perforated inner walls and bottom and was pressed by a weight equal to that of the curd for 12 h. Cheese blocks (5x7x7 cm) were then immersed in saturated brine at 20°C for 5 h and transferred to 8% brine, packed and stored at 11-13°C for 2 mo.

Chemical Analysis

The pH of the cheese was determined electrometrically (EYELA, PHM 200 pH meter, Tokyo Rikakiki, Japan), the moisture content was measured by heating at 105°C to a constant weight and the salt content was determined by the method of the International Dairy Federation (18), and Iranian Standards (19). Total nitrogen (TN) and water-soluble nitrogen were determined according to Kjeldahl method (24). Fat hydrolysis was determined by acid degree value (7).

Texture Properties

Hardness of the cheeses was determined at the end of production and after 10, 30, and 60 days of storage by measuring the shearing strength using an Instron Universal Testing Machine (Model 1140), equipped with 0.5 to 5 kg compression load cell. Identical dimensions of cheese sample cubes (30x30x20 mm) were used for all tests. The crosshead speed was 20 cm min^{-1} in all cases. Samples were stored at 11-13°C prior to and during testing. Hardness was defined as the peak force exerted when a cylindrical metal probe (13 mm in diameter) was driven into the cheese to a depth of 1.5 cm. Hardness was then calculated using the following formula:

$S = F/NDT$ in which:

S= Resistance to fracture (g cm^{-2})

F= Shearing force (g)

D= Diameter of cylindrical probe (cm)

T= Thickness of cheese cube (cm)

Flavor Assessment

A trained taste panel of 20 persons judged the quality of the cheeses and scored their sensory properties at 60 days. The sensory data were statistically analyzed using a 5 point hedonic method with 1 being devoid of attribute and 5 being extremely strong. Judges were selected by their ability to differentiate the main attributes such as flavor, color, texture, and total acceptability on dairy products by sequential triangle method (17).

Experimental Design and Statistical Analysis

A completely randomized design was used to evaluate the influence of the type of lactic starter (5 levels) and storage time (4 levels) at 3 replicate trials on physico-chemical and sensory properties of cheese. Data were submitted to analysis of variance and means were compared using Duncan's test (36). Treatment differences with $P<0.01$ under the null hypothesis were considered to be significant.

RESULTS AND DISCUSSION

Effect on Biochemical Characteristics

Cheese made with thermophilic lactic starter (starter cultures B, C, and D) exhibited significant ($P<0.05$) change in pH at the curd stage and beginning of storage. Mesophilic lactic starter (starter cultures A and E) lowered the pH during storage due to the favorable temperature existed for each group (Table 1). For all starter culture systems used, the pH of the cheese during storage was lower than 5 (4.54-4.87). This trend is in agreement with those found for other starters on brined cheeses (31, 41). However, pH of cheese increased ($P<0.05$) slightly after 30 d of storage (Table 1), apparently due to the formation of ammonia and related compounds resulting from the continuous proteolysis of caseins, and reduction in lactic acid content by utilization as a substrate and lactate formation (neutralization). Similar trends were previously observed for brined cheeses (31, 41).

Significant effect ($P<0.01$) of starter culture inoculation towards lower moisture content occurred in cheeses made with thermophilic starters (B, C, and D) than mesophilic starters (A and E). The lower water content of

Table 1. pH, moisture and salt content of Iranian white cheese made with different lactic starters†.

Time (d)	pH					Effect of starters on Moisture (%)					Salt –in moisture (g 100 g^{-1})				
	A§	B¶	C††	D§§	E¶¶	A	B	C	D	E	A	B	C	D	E
Curd1†††	6.30	6.20	6.00	6.25	6.3										
Curd2§§§	5.18^{a}¶¶¶	5.08^{b}	4.93^{c}	5.00^{b}	5.21^{a}	54.6^{a}	52.4^{f}	50.1^{d}	52.8^{b}	54.4^{a}	4.83^{a}	4.04^{f}	3.67^{d}	3.65^{d}	4.23^{b}
10	4.83^{a}	4.72^{a}	4.63^{b}	4.76^{b}	4.87^{a}	57.7^{a}	56.2^{c}	54.3^{e}	55.6^{d}	57.4^{b}	5.01^{b}	5.03^{b}	3.94^{d}	4.26^{c}	5.40^{a}
30	4.68^{c}	4.63^{c}	4.56^{d}	4.71^{b}	4.76^{a}	58.7^{a}	57.4^{b}	56.9^{f}	56.8^{c}	58.8^{a}	6.15^{a}	5.46^{c}	5.35^{d}	5.33^{d}	6.08^{b}
60	4.78^{a}	4.71^{b}	4.65^{c}	4.76^{a}	4.80^{a}	59.9^{a}	58.1^{b}	57.7^{f}	57.6^{c}	59.9^{a}	6.25^{d}	6.33^{c}	6.14^{f}	6.60^{a}	6.43^{b}

† Average values of three cheesemaking trials.

§ *Lactobacillus casei* 01+ aromatic mesophile CH-N-01.

¶ Aromatic mesophile CH-N-01 + thermophile CH-1.

†† Thermophile CH-1.

§§ Thermophile CH-1 + mesophile 54.

¶¶ Mesophile 54.

††† Immediately after cutting.

§§§ Before packaging.

¶¶¶ Means of the same row with different superscripts differ significantly ($P<0.01$).

cheese made with thermophilic starters coincided with lower pH values (Table 1). Moisture fluctuations throughout cheese ripening (2, 31) were observed in this study. The ability of the cheese to take up water from the brine can be attributed to the cleavage of peptide bonds, in particular those of α_{s1}casein (which form the protein network of the cheese), and thus the formation of new ionic groups, which bind to water. Storage at a temperature less than 15°C, as is the case in our study, could help in take up of water by cheese (3, 28, 29, 32, 38).

The salt-in moisture of cheese increased in all samples during ripening to over 5%, which is the low spoilage limit for cheese (22, 39, 40). However, there was a significant ($P<0.01$) difference in the salt content (Table 1). Salt concentration gradient, cheese block dimension, stage of salt addition, pH and water content of curd affected the NaCl content of cheese. It is reported that the rate of salt absorption increases as the water content in samples with the same acidity increases (14, 33).

The type of starter cultures significantly ($P<0.01$) affected the formation of proteolysis products as shown in Table 2. Generally, proteolysis products significantly increased with storage or aging. The highest degree of proteolysis during the course of storage (60 days) was found in cheese made with starter culture A, which is a mixture of *Lactobacillus casei* and aromatic mesophile. The proteolytic activity of lactobacilli seems to be stronger than lactococci and the proteolytic activity of *Lactobacillus casei* is stronger than other lactobacilli (25, 29, 34, 35). Although *Lactobacillus casei* and its subspecies are not used solely as starter cultures during the manufacture of most cheese varieties, these bacteria which have been isolated from fairly wide variety of ripened cheese, can have a significant effect on microflora development and may hasten ripening (16). A comparative study of the intracellular peptidases of *L. casei* subspecies was conducted by Arora and Lee (4), who concluded that the specific activities were significantly different among the subspecies. In another study, Habibi-Najafi and Lee (15) concluded that *L. casei* strains exhibit debittering effects during cheese ripening apparently due to strong activity of proline-specific peptidases. Thus, many workers have suggested the incorporation of lactobacilli with lactococci to shorten the ripening process and improve the sensory properties of cheese (34, 38). As indicated in Table 2, starter A exhibits more change in soluble protein during storage. In general, gross proteolysis by starter culture at the early stage of ripening is retarded in cheese with low pH (31). Such trend on proteolytic activity of different starter cultures is in agreement with previous experiments (21, 31). The ADV of all cheeses increased significantly ($P<0.01$) during the storage (Table 2). Higher ADV was recorded in mesophilic starter cultures (A, B and E) than thermophilic starters (C). Starter C (thermophile CH-1) cheese had the lowest ADV. Generally lactic starters (Lactococcus and lactobacillus) possess lower lipolytic and esterase activities than leuconostoc and entrococcus (20, 23, 31). The highest ADV in starter A and B cheeses might be due to the leuconostoc and *Lactococcus lactis* incorporation.

Effect on Physical Properties

Significant ($P<0.01$) variations in hardness (body and texture) throughout cheese ripening were observed in all treatments at different rates (Table 3). Body and texture developed better in mesophilic starter cheeses (particularly starter A cheese) than thermophilic starter cheeses apparently due to storage temperature (8). In general, fluctuation in the hardness during cheese ripening is associated with two distinct stages. In the initial stage that takes place during 10-15 days of ripening, the breakdown of casein network leads to a decrease in hardness due to the degradation of α_{s1}-casein to α_{s1}-I casein by rennet. In the second stage breakdown of α_s–casein and other caseins mainly by starter proteases and peptidases, liberated small peptides and free amino acids which were soluble in aqueous phase. This leads to increase in the shearing strength of the cheese (12, 13, 22, 27, 28, 37). There is also a strong correlation between the hardness development in the cheese and its respective pH (27). However, texture developments were not similar for brined and hard cheeses (38, 11).

Effect on Sensory Characteristics

Typical flavor and texture appearances of all cheeses were judged after 60 days of storage (Table 4). Based on the scores awarded for flavor intensity, all cheeses were certified to be of good quality. Starters A, B, C, and D significantly ($P<0.01$) affected cheese flavor; mean scores were 4.85, 4.80, 4.50, and 4.60, respectively. The judge scores of cheese A and B were

Table 2. Proteolysis and lipolysis content of Iranian white cheese made with different lactic starters[†]

Time (days)	Effect of starters														
	Protein (g 100 g^{-1} dry weight)					Soluble-N (g 100 g^{-1} dry weight)					Lipolysis (ADV)				
	A[§]	B[¶]	C[††]	D[§§]	E[¶¶]	A	B	C	D	E	A	B	C	D	E
Curd[†††]	44.5[c§§§]	44.3[d]	45.3[a]	44.5[c]	44.9[b]	7.00[a]	7.02[a]	6.93[b]	6.95[b]	6.56[c]	1.30[a]	1.29[a]	1.19[d]	1.21[c]	1.34[b]
10	35.14[e]	38.91[d]	44.55[a]	42.89[b]	40.32[c]	9.03[a]	8.52[b]	8.00[f]	8.68[d]	7.99[f]	1.61[a]	1.44[c]	1.22[d]	1.52[b]	1.49[c]
30	32.71[c]	33.79[d]	39.91[a]	38.58	36.32[c]	16.49[a]	12.49[c]	10.92[c]	9.57[e]	9.93[b]	1.66[b]	1.83[a]	1.38[e]	1.64[c]	1.58[d]
60	30.24[e]	32.12[d]	37.22[a]	35.50[b]	33.68[c]	17.04[a]	13.01[c]	12.35[e]	12.99[d]	11.87[f]	2.16[a]	2.05[b]	1.53[d]	1.68[c]	2.00[b]

† Average values of three cheesemaking trials.

§ *Lactobacillus casei* 01+ Aromatic mesophile CH-N-01.

¶ Aromatic mesophile CH-N-01 + thermophile CH-1.

†† Thermophile CH-1.

§§ Thermophile CH-1 + mesophile 54.

¶¶ Mesophile 54.

††† Before packaging.

§§ § Means of the same row with different superscripts differ significantly ($P<0.01$).

significantly higher ($P<0.01$). The starter cultures used in these cheeses were more proteolytic and had a lower pH. High levels of water-soluble nitrogen are significantly correlated with flavor intensity and may be precursors to other components essential for characteristic flavor (1, 26). Cheese E differed significantly ($P<0.01$) from all other cheeses.

Table 3. Changes in hardness of Iranian white cheese made with different starters during ripening[†]

Starter	Effect of starter on hardness ($g\ cm^{-2}$) Time(d)			
	0	10	30	60
A[§]	580 [c†††]	329 [d]	556 [b]	314 [c]
B[¶]	611 [b]	469 [c]	518 [c]	375 [b]
C[††]	678 [a]	859 [a]	923 [a]	639 [a]
D[§§]	617 [b]	661 [b]	435 [d]	368 [b]
E[¶¶]	573 [c]	467 [c]	298 [e]	284 [c]

† Average values of three cheesemaking trials.

§ *Lactobacillus casei* 01+ aromatic mesophile CH-N-01.

¶ Aromatic mesophile CH-N-01 + thermophile CH-1.

†† Thermophile CH-1.

§§ Thermophile CH-1 + mesophile 54.

¶¶ Mesophile 54.

††† Means of the same column with different superscripts differ significantly ($P<0.01$).

The body and texture of starter A and B cheeses developed better than C and E cheeses. It seems that cheese made with solely thermophilic (C) or mesophilic (E) starter developed weak texture and so is not recommended to be used alone as starter culture.

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Table 4. Flavor, body and texture[†] of Iranian white cheese manufactured with different lactic starters after 60 d of storage.

Starter	Effect of starters (1%) on	
	Flavor[§]	Body and texture[§]
A[¶]	4.85[a§§§]	4.9[a]
B[††]	4.80[a]	4.85[a]
C[§§]	4.50[b]	3.60[a]
D[¶¶]	4.60[b]	4.76[b]
E[†††]	4.64[a,b]	3.67[c]

† Average values of three cheesemaking trials.

§ Flavor score 1-5, body and texture score 1-5.

¶ *Lactobacillus casei* 01+ aromatic mesophile CH-N-01.

†† Aromatic mesophile CH-N-01 + thermophile CH-1.

§§ Thermophile CH-1.

¶¶ Thermophile CH-1 + mesophile 54.

††† Mesophile 54.

§§§ Means of the same column with different superscripts differ significantly (P<0.01).

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