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EFFECT OF PSYCHROTROPHIC BACTERIAL CONTAMINATION OF RAW MILK ON ORGANIC ACID CONTENT AND FLAVOROF AGED CHEDDAR CHEESE

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SUMMARY

Cheddar cheese was made from milk which had been inoculated with psychrotrophs 48hr. prior to pasteurization. This was compared to control cheese.The cheese was stored at 7 °C and evaluated at 0, 5, 30, 60 and 180 days. All cheese made with psychrotroph-treated milk had significant lower flavor score than control cheese. The predominant flavors of the 180 day-treated cheese were bitter and unclean.

High performance liquid chromatography (HPLC) analysis indicated significant increases of citric, pyruvic, lactic, formic, acetic and propionic acids with aging of Cheddar cheese. Only orotic acid decreased with aging. The control cheese had lower values than the treated cheese for pyruvic, lactic and formic acids. The control cheese had higher propionic acid value than did the treated cheese.

The aroma and flavor quality of Cheddar cheese was attributed to a delicate balance of organic acids

produced as metabolites by culture bacteria during fermentation.

INTRODUCTION

Storage of raw milk for long period at refrigeration temperature has resulted in new quality problems for the dairy industry. These problems are related to growth and metabolic activities of psychrotrophs which are the common contaminants of milk (Thomas and Thomas, 1973 and Cousin, 1982). Most psychrotrophs isolated from milk are gram negative, nonspore-forming rods and are species of pseudomonas (Buchanan, 1974).

As these psychrotrophs increase in number throughout refrigeration, their enzymes are also synthesized during microbial growth in the milk. These enzymes many of which are heat-stable, biochemically alter the milk, eventually causing their spoilage. These enzymes are carried into the cheese, and, in excess, could produce bitterness (Moskowitz, 1980).

Although the quality of Cheddar cheese can be attributed to many factors in processing, this research centers on the effect of experimental psychrotrophic contamination of raw milk on organic acid content and flavor development of Cheddar cheese.

MATERIALS AND METHODS

Raw Milk and Cheese Preparation and Storage:

Raw milk was inoculated experimentally with one of two levels (10,000 and 100,000/ml) of one of the two psychrotrophic strains (*Ps. fluorescens* 27; *Ps. fluorescens* 103) and incubated at 7°C for 48 hr. The contaminated and control milk were pasteurized at 73.9°C

for 17 sec. The Cheddar cheese was made in 378.4 liter vats following the normal cheese manufacturing procedure (Kosikowski, 1978). The cheese was stored at 7 °C and evaluated at 0, 5, 30 and 180 days.

Values obtained and analysis performed included: sensory evaluation (flavor and body/texture) and (HPLC).

High Performance Liquid Chromatography (HPLC)

Samples for organic acid analysis were prepared as described by Marsili (1981). The following were the equipment and operating conditions utilized in this study: Varian U.V. detector set at 220nm and detector slit width of 8nm; Varian 8000 Automatic sampler; Cation exchange column (300 x 78.8 i.d.) packed with Aminex HPX-87 from Bio-Rad Laboratories (Richmond, California); mobile phase of 0.009 N H_2SO_4 prepared by diluting reagent grade sulfuric acid with distilled water; isocratic analysis at column temperature of 65°C and flow rate of 0.7 ml/min; and attenuations of 32 and 526. HPLC results were calculated by the external standard technique.

Sensory Evaluation

Cheddar cheese from each vet was subjected to sensory evaluation by a four-member trained panel at 5, 30, 60 and 180 days. Modified ADSA Cheddar cheese score card (Angevine, 1958) was used to score the flavor (1-10 scale), while the body/texture was scored with 1-5 scale. A flavor score of less than 5 was regarded as unacceptable (poor), 5-6 fair, 7-8 good and 9-10 excellent.

Statistical Analysis

The results of chemical and flavor determinations were analyzed by the Analysis of Variance (ANOVA) Technique in a randomized block design, blocked on batches of cheese with a split plot in time arrangement treatment (Steel and Torrie, 1960). The F test was used to determine if significant differences existed among sources of variation. When differences were indicated, means were separated using Duncan's Multiple Range Test to determine where differences occurred (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Effect of Psychrotrophic Contamination on Flavor, Body and Texture of Cheddar Cheese

Mean flavor scores for the control and high psychrotrophic inoculation level (Table 1) revealed that control cheese had significant superior flavor scores than all psychrotrophic treated cheese. In addition, cheese treated with *Ps. fluorescens* 27 had significantly lower flavor scores ($P < 0.001$) than *Ps. fluorescens* 103. The lower flavor score caused by *Ps. fluorescens* 27 could be attributed to its high proteolytic activity.

The predominant flavor criticisms in the cheese made from psychrotrophic contaminated raw milk were bitter and unclean after 60 days of refrigerated (7°C) storage (Table 2). By 180 days, bitterness was detected in 38% of the cheeses inoculated with both medium and high levels of psychrotrophic; non of the control samples were criticized as bitter (Table 3). This indicates probable production of bitter peptides by the psychrotrophic bacteria. By 180 days unclean flavor was detected in 25% of the cheese inoculated with both medium

Table 1. Effect of Psychrotrophic Contamination of raw milk on the flavor of Cheddar cheese

Treatment	Mean Flavor Score
1. Control Cheese	7.95 A*
2. *Ps. fluorescens* Ps. 103-10,000/ml	7.20 B
3. *Ps. fluorescens* Ps. 103-100,000/ml	6.90 B C
4. *Ps. fluorescens* Ps. 27-10,000/ml	6.65 C
5. *Ps. fluorescens* Ps. 27-100,000/ml	6.55 C

* Means not followed by the same letter differ significantly ($P < 0.001$).

Table 2. Most frequently noticed flavor versus time

	Time (d)			
Treatment	5	30	60	180
Control	Flat	Flat	Flat	Acid
Psychrotroph	Flat	Flat Slight Bitter	Flat Bitter Unclean	Bitter Unclean Acid Sulfide

Table 3. Frequency distribution of flavor criticisms of control and psychrotroph treated cheese at 180 days.

Treatment	No. of Samples	Flavor frequency (%)*				
		Flat	Bitter	Unclean	Acid	Sulfide
I. Control	5	1(20)	0	0	4(80)	0
II. *Ps. fluorescens* 103	10	2(10)	6(30)	5(25)	6(30)	1(5)
III. *ps. fluorescens* 27	10	3(14)	8(38)	5(24)	0	5(24)

* Percent values based on total number of defects and not total number of samples. More than one criticism could be used for each sample.

and high levels of psychrotrophs. On the same days, non of the control samples were criticized as unclean (Table 3).

Table 4 showed mean body/texture scores for the control and high psychrotrophic inoculation level, revealed that cheese made from psychrotrophic contaminated raw milk had significantly lower body/texture scores ($P < 0.001$) than the control. In addition, cheese treated with *Ps. fluorescens* 27 had significantly lower body/texture score ($P < 0.001$) than *Ps. fluorescens* 103.

Table 4. Effect of psychrotrophic contamination of raw milk on the body of Cheddar cheese

Treatment	Mean Body Score
1. Control	4.20 A*
2. *Ps. fluorescens Ps.* 103-10,000/ml	3.65 B
3. *Ps. fluorescens Ps.* 103-100,000/ml	3.60 B
4. *Ps. fluorescens Ps.* 27-100,000/ml	3.35 C
5. *Ps. fluorescens Ps.* 27-10,000/ml	2.90 C

* Means not followed by the same letter differ significantly ($P < 0.001$).

The predominant body/texture critisms noted after 60 and 180 days were weak, open and gassy (Tables 5 and 6). Schormuller (1968) has demonstrated that proteases and peptidases are involved in the flavor and texture development of both soft and hard types of cheeses. The degradation of proteins by microbial proteases contributes to a softening of the cheese (Arun, 1978).

Table 5. Most frequently noticed body/texture criticism over time for control and psychrotrophic treated Cheddar cheese.

	Time (d)			
Treatment	5	30	60	180
1. Control	Open Curdy	Open Curdy	Open	Open
2. Psychrotroph	Weak Open Curdy Crumbly Gassy	Weak Open Curdy Gassy	Weak Open Curdy Gassy	Weak Open Gassy

Table 6. Frequency distribution of body/texture criticisms of control and psychrotrophic treated Cheddar cheese at 180 days.

Treatment	No. Samples	Criticism Frequency (%)*				
		Weak	Open	Curdy	Crumbly	Gassy
1. Control	5	0	1(100)	0	0	0
2. *Ps. fluorescens* P 103	10	3(22)	10(71)	0	0	1(7)
3. *Ps. fluorescens* P 27	10	2(14)	10(72)	0	0	2(14)

* Percent values based on total number of defect and not total number of samples. More than one criticism could be used for each sample.

Effect of Aging and adding Psychrotrophs to Raw Milk on Organic Acid Content and Flavor of Cheddar Cheese

Results of HPLC Analysis were shown in Table 7 and 8 which indicate significant increases ($P < 0.001$) of citric, pyruvic, lactic, formic, acetic and propionic acids with aging of the cheese. Only orotic acid decreases significantly ($P < 0.001$) with aging. These results were consistent with those of Marsili (1981) who also reported a decline in concentrations of orotic acid during storage of Cheddar at 7°C.

Table 7. Effect of aging on the orotic, citric, pyruvic and lactic acid content of Cheddar cheese

Days	Orotic -ppm-	Citric -ppm-	Pyruvic -ppm-	Lactic -ppm-
0	16.99 A*	1089.4 A	24.35 A	5950.7 A
5	13.10 B	1150.8 A	25.56 A	6487.4 B
30	9.80 C	1255 B	36.52 B	6967.4 C
60	6.36 D	1105.3 C	37.67 B	6608.5 B
180	3.95 E	1841 D	65.96 C	8626.4 D

* Means in a column not followed by the same letter differ significantly ($P < 0.001$).

Table 8 Effect of aging on the formic, acetic and propionic acid content of Cheddar cheese.

Days	Formic -ppm-*	Acetic -ppm-	Propionic -ppm-
0	139.3 A**	100.3 A	465.4 A
5	161.40 A	132.8 B	522.3 B
30	212.3 B	176.5 C	617.95 C
60	266.54 C	239.2 D	679.7 D
180	239.54 B	319.44 E	751.95 E

* ppm = part per million.

** Means in a column not followed by the same letter differ significantly ($P < 0.001$).

Mikolajcik (1979) reported that the breakdown products produced by enzymes from psychrotrophic bacteria stimulate culture acid production, which in excess produces body and flavor defects. Cheddar cheese ripening showed a dramatic decrease in lactose during pressing with subsequent increases in lactic and citric acids. (Irwin *et al.*, 1984).

Results in Table (9) reveal significantly higher levels ($P < 0.001$) of lactic and formic acids and lower levels of propionic acid in cheeses made from raw milk cultured with psychrotrophs than the control cheese. Although differences between the control and psychrotrophic contaminated cheeses were not statistically significant for the rest of organic acids. The control cheese had lower values than the

psychrotrophic treated cheese for pyruvic acid content, and not trend were noted for acetic, citric and orotic acids (Table 9).

Table 9. Effect of added psychrotrophs to raw milk on organic acid content of Cheddar cheese.

Organic Acid	Control Cheese	Treated Cheese[1]
	-ppm-	-ppm-
Orotic	10.10	9.81-10.22 NS[2]
Citric	1280	1255-1322 NS
Pyruvic	33.10	35.0-43.10 NS
Lactic	6307	6938-7352 ***
Formic	173.6	198.5-231.0 ***
Acetic	185.20	179.71-231.6 NS
Propionic	742.8	494.6-656.1 ***

1 Range of treated cheese for all four psychrotrophs (*ps. fluorescens* 27 and 103)

2 NS = non significamnt.

*** Significant ($P < 0.001$).

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تأثير تلوث الحليب الخام بالبكتريا المحبة للبرودة على كمية الحوامض العضوية والنكهة لجبن الجدر

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الخلاصة

يشمل البحث دراسة تلوث الحليب الخام مختبريا بالبكتريا المحبة للبرودة وتأثيره على نوعية جبن الجدر. تمت صناعة جبن الجدر من الحليب الخام الذي سبق وان حقن بالبكتريا المحبة للبرودة خلال (٤٨) ساعة قبل اجراء عملية البسترة وقورن مع نظيره الغير معامل بالبكتريا المحبة للبرودة. خزنت الاجبان من اجل انضاجها في غرف مبردة وعلى درجة حرارة ٧م واجريت التحليلات عليها بعمر ٠، ٥، ٣٠، ٦٠و ١٨٠ يوما. وبعد اجراء التحليلات الاحصائية تميزت الاجبان التي صنعت من الحليب الغير ملوث بالبكتريا المحبة للبرودة من حيث النكهة والقوام بقياسات اعلى من نظيرتها التي صنعت من الحليب الملوث. كانت النكهة السائدة على الاجبان المعاملة بالبكتريا المحبة للبرودة غير نظيفة وفيها مرارة بعد مرور فترة ١٨٠ يوما على انضاجها.

اكدت التحليلات الكروماتوكرافية (HPLC) زيادات معنوية في تراكيز الاحماض العضوية التالية: الستريك، البايروفيك، اللاكتيك،

الفورميك، الخليك والحامض البروبيوني مع تقدم فترة انضاج الجبن بينما على العكس من ذلك انخفض تركيز حامض الاوروتيك مع مرور عامل الزمن للانضاج. بالاضافة الى ذلك تميز الجبن ذو النوعية الجيدة باحتوائه على مستويات اقل من كل من حامض البايروفيك واللاكتيك والفورميك وعلى مستويات اعلى من حامض البروبيوني في الجبن ذو النوعية الرديئة. تعزى نكهة جبن الجدر الى التوازن الدقيق في تراكيز الاحماض العضوية الناتجة عن وجود الاحياء المجهرية فيه خلال فترة الانضاج.