A CASE STUDY: SYNERESIS OF COLD-PACK CHEESE FOOD RELATED TO GROWTH OF LACTIC ACID BACTERIA

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Abstract

Commercial Cold-Pack Cheese Food which exhibited marked syneresis in consumer packages had a pH of 4.60 to 4.80. Bi-weekly microbial examination of commercial Cold-Pack Cheese Food stored at 5, 10, 15, and 20 C indicated a marked growth of lactobacilli and streptococci at all temperatures above 5 C and a slight decease in staphylococci at all temperatures. It is assumed that the lactose added in the form of skim-milk or whey powders provided the carbohydrate nutrient source for these organisms so that the pH was reduced from 5.05 to 4.60. The minimum hydration of casein occurs at pH 4.60 and this lowered pH caused syneresis in the packages. Cooling the Cold-Pack Cheese Food quickly to 5 C and keeping it at or below this temperature eliminated the problem.

Cold-Pack Cheese Food[1] is the product made by comminuting one or more lots of cheese into a homogeneous plastic mass without the aid of heat. The optional ingredients may be cream, milk, solids derived from milk, and other minor ingredients. The fat content shall be not less than 23% and the product shall not contain more than 46% moisture. The U. S. Cheese Food Standards of Identity[2] are similar to the Canadian Standards.

Cheese Food is a common item on the Canadian food market. In 1971 many packages of this product were returned from supermarkets because of brittleness of the body and presence of free liquid in the package. This Cheese Food usually possesses a smooth texture and does not show any free serum. The pH of the defective samples ranged from 4.50 to 4.90 which was lower than the normal fresh product (5.00 to 5.20). Because of the decrease in pH, the nature of the food, and the optional ingredients which were high in lactose, lactic acid bacteria were believed to be involved in causing this defect. The effect of temperature and time of storage on the possible growth of lactic acid bacteria was investigated.

Materials and Methods

Manufacture of Cold-Pack Cheese Food

The cheese for this product was a blend of 6 months, and 1 and 2 years old Cheddar cheese. The cheese and other ingredients (Table 1) were thoroughly mixed in a silent cutter[3] until a smooth homogeneous mass was obtained. During the comminuting the mixture attained a temperature of 42 C. The mixture was placed in 45 kg polyethylene lined boxes and held overnight at 5 C for firming. The Cheese Food was cut into 226-g portions and were placed in pre-formed vacu-formed cryovac pouches (P540) of unsupported polyethylene saran, which were then evacuated and heat sealed.

Collection of samples

The samples were obtained from the manufacturing plant immediately after packaging and transferred to the laboratory in insulated boxes. The packages of Cheese Food were stored at 5, 10, 15, and 20 C. Packages were removed and analyzed at regular intervals (every 3rd and 7th day) for a period of 42 days. An unopened package was used for each set of analyses.

Analytical determinations

Eleven grams of cheese were blended for 3 min with 99 ml sterile phosphate buffer (pH 7.20) in a sterile blendor. After suitable dilutions the following microbiological analyses were carried out: *(a)* total aerobic count on Standard Plate Count Agar at 32 C for 48 hr; *(b)* coliforms on Violet Red Bile Agar at 37 C for 24 hr; *(c)* yeast and mold on Potato Dextrose Agar acidified to pH 3.5 with sterile 10% tartaric acid, incubated at 25 C for 5 days; *(d)* staphylococci on Tellurite Polymyxin Egg Yolk Agar at 37 C for 48 hr; a representative number of colonies were streaked on Plate Count Agar and incubated at 37 C for 24 hr; the cultures were then examined by gram stain and for coagulase by the slide method *(1)* using lypholized bacto-coagulase plasma; *(e)* lactobacilli on Rogosa's medium *(6)* at 37 C for 72 hr using the double layer plate method; and *(f)* streptococci on Nutrient Agar containing 0.05% thallous acetate *(3)* at 32 C for 72 hr; the double layer plate method was used. All medium ingredients and reagents were obtained from Difco.

The changes in pH during storage were determined on the Cheese Food at the same time intervals using a Fisher Accumet (Model 310) pH meter and a Beckman combination electrode.

At the end of the 42 day storage period the free liquid of unopened packages of cheese at the various temperatures was collected by gravity in tubes and the average volume and pH determined.

Results and Discussion

The ingredients, their corresponding pH, and respective microbial flora are shown in Table 1. It is interesting that coagulase-positive staphylococci were only found in trimmings from the cutting operations.

[1]Trade Information Letter No. 370, June 7, 1972, Dept. of National Health and Welfare, Ottawa, Ontario, Canada.

[2]Code of Federal Regulations, Title 21, Food and Drug 19. 787 U. S. Govt. Printing Office, Washington, D. C. 1967.

[3]Rasant cutter—Seydelmann, Stuttgart (also called Buffalo Silent cutter).

TABLE 1. MICROBIAL COUNT PER GRAM OF INGREDIENTS USED IN THE MANUFACTURE OF COLD-PACK CHEESE FOOD

Ingredients	pH	Coliforms	Lacto-bacilli	Strepto-cocci	Staphy-lococci	Total count	Molds	Yeasts
6-Months old Cheddar	5.10	4	1×10^6	6×10^6	7×10^1	5.5×10^6	6	120
1-Year old Cheddar	5.03	<2	14×10^6	10×10^6	4×10^1	16×10^6	2	<2
2-Years old Cheddar	5.23	<2	4.2×10^6	4.5×10^6	78×10^1	6.3×10^6	12	>3000
3-Years old Cheddar	5.20	<2	5.3×10^6	5×10^6	32×10^1	9.3×10^6	12	50
Butter	5.67	<2	<10	<10	20×10^1	<10	<2	110
Trimmings from cutting operation	5.35	<2	11×10^6	12×10^6	47×10^{1}[a]	10×10^6	200	>3000
Water	6.90	<2	1×10^1	8×10^1	<10	29×10^3	<2	<2
Sorbic acid	—	<2	<10	<10	<10	18×10^2	<2	<2
Whey powder	5.60	<2	<10	<10	<10	15×10^2	<2	<2
Skimmilk powder	6.10	2	92×10^1	2×10^2	32×10^1	28×10^2	6	120
Kling[b]	6.27	<2	50×10^2	28×10^2	6×10^1	52×10^2	24	2

[a]60% were coagulase-positive
[b]A calcium reduced skimmilk powder (Standard Spice Co., Toronto)

The cheese contributed most of the microflora. The pH of the mixture and the changes occurring during storage at different temperatures over a period of 42 days are given in Table 2. The decrease of the pH with time was more pronounced at the higher temperatures. This was also reported by Park et al. *(5)* on a cheese food. There was no change in pH when the product was stored at 5 C.

The degree of syneresis was related to the temperature of storage and was also accompanied with lower pH values (Table 3). The amount of exudate at the low pH is not surprising as this is close to the iso-electric point of casein or the point of minimum hydration.

The lactobacilli and streptococci constituted the predominant microflora. Their number increased slightly during storage and was affected by the higher temperatures (Tables 4-7). Once the maximum concentration was reached it was maintained at this level for the remainder of the storage period.

The total count seemed to parallel the counts of the lactobacilli. Microscopic examination and catalase reaction of isolated colonies from the total plate count showed that the majority were gram-positive and catalase-negative rods and some cocci. The pH of the cheese favors growth and survival of acid-tolerant organisms such as the lactic acid bacteria. The initial staphylococci population was low and showed no tendency to increase at any of the storage temperatures. This is in agreement with Dahiya and Speck *(2)* who observed inhibition of *Staphylococcus aureus* by cell-free extracts of lactobacilli and lactic streptococci. Coliforms were not detected. Yeasts were isolated only during the first 7 days of storage while molds were detected during the 42 days of storage with a tendency to decrease, probably because of the effect of sorbate in the mixture. Sorbate at the concentration used in the mixture had

TABLE 2. PH OF COLD-PACK CHEESE FOOD STORED AT 5, 10, 15 AND 20 C FOR 42 DAYS

Days of storage	Temperature of storage C			
	5	10	15	20
0	5.05	5.05	5.05	5.05
3	5.04	5.06	5.10	4.90
7	5.04	5.08	5.14	4.83
10	5.07	5.02	5.12	4.82
14	5.09	5.27	5.18	5.04
17	5.15	5.17	5.25	4.75
21	5.11	5.19	5.22	4.87
24	5.05	5.23	5.21	4.75
28	5.03	4.82	4.75	4.69
31	5.02	5.06	4.80	4.80
35	5.04	4.90	4.75	4.78
38	5.04	4.84	4.83	4.80
42	5.05	4.78	4.67	4.60

TABLE 3. THE AVERAGE VOLUME AND PH OF EXUDATE FROM 5 PACKAGES OF COLD-PACK CHEESE FOOD AFTER 42 DAYS OF STORAGE AT 5, 10, 15, AND 20 C

Temperature of storage °C	Exudate (ml)	pH of exudate
5	0	—
10	9	4.88
15	12	4.44
20	27.2	4.45

TABLE 8. RELATIONSHIP BETWEEN PH, TYPE OF ORGANISM AND THE TEMPERATURE OF STORAGE AS EXPRESSED BY CORRELATION COEFFICIENTS

Interaction	Temperature of storage °C		
	10	15	20
pH × lactobacilli	−.64	−.88	−.59
pH × streptococci	.04	−.42	−.47 N.S.
pH × total count	−.66	−.86	−.62
Streptococci × lactobacilli	.39	.67	.96
Lactobacilli × total count	.98	.98	.97
Streptococci × total count	.43	.70	.89

N.S. not significant

TABLE 4. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 5 C

Days of Storage	Coliforms	Lactobacilli (×10⁶)	Streptococci (×10⁶)	Staphylococci (×10²)	Total count (×10⁶)	Molds (×10²)	Yeasts
0	<10	4.1	5.1	13.0	4.4	19	250
3	<10	1.3	2.1	23.0	2.0	0.7	10
7	<10	2.5	2.4	11.0	3.8	90	<2
10	<10	2.6	2.1	9.1	4.3	35	<2
14	<10	2.7	1.6	3.0	3.4	15	<2
17	<10	3.5	1.5	1.2	5.0	60	<2
21	<10	2.4	2.4	5.4	3.1	12	<2
24	<10	1.5	2.5	1.8	2.9	27	<2
28	<10	2.3	2.6	6.4	2.4	18	<2
31	<10	2.7	0.8	1.3	4.0	35	<2
35	<10	2.4	0.5	2.2	3.2	LA[a]	LA
38	LA	LA	LA	LA	LA	LA	LA
42	<10	1.8	0.4	1.7	1.8	0.4	<2

[a]Laboratory accident

TABLE 5. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 10 C

Days of Storage	Coliforms	Lactobacilli (×10⁶)	Streptococci (×10⁶)	Staphylococci (×10²)	Total count (×10⁶)	Molds (×10)	Yeasts
0	<10	4.1	5.1	13.0	4.4	190.0	250
3	<10	0.6	0.9	4.5	1.0	2.0	15
7	<10	3.3	4.9	7.6	6.4	20.0	<2
10	<10	16.0	9.4	14.0	39.0	190.0	<2
14	<10	22.0	17.0	7.0	43.0	5.0	<2
17	<10	0.5	0.4	0.8	2.0	3.5	<2
21	<10	0.6	0.5	0.8	0.9	1.0	<2
24	<10	0.4	0.4	0.7	0.7	27.0	<2
28	<10	20.0	0.1	6.7	25.0	38.0	<2
31	<10	0.2	0.1	0.4	0.6	2.5	<2
35	<10	30.0	LA[a]	18.0	45.0	LA	LA
38	<10	51.0	19.0	4.3	210.0	15.0	<2
42	<10	71.0	8.7	1.6	180.0	38.0	<2

[a]Laboratory accident

little effect on the lactobacilli although yogurt bacteria have been reported to be inhibited by 0.1% potassium sorbate *(4)*.

The low pH (5.05) of the Cheese Food would inhibit growth of most organisms even at storage temperatures of 15-20 C. However, growth of lactic acid bacteria, some of which are both psychrotrophic and acid tolerant, is possible. Improper refrigeration would enhance the possibility for their growth.

The data indicate that to overcome the defect of syneresis in this type of food, the product has to be stored at 5 C. Addition of sorbate at proper concentration may provide additional protection especially at the higher storage temperatures.

The correlation coefficients indicating the relationships between pH, total count, lactobacilli, and streptococci at the various temperatures are shown in Table 8. The pH is inversely influenced by the numbers of organisms as reflected by the population of lactobacilli and the total count. It would appear that the lactobacilli have more influence on pH than streptococci. The total count is highly correlated with number of lactobacilli which might indicate that the majority of the microbial flora were lactic acid bacteria or a lack of selectivity of the lactobacillus medium. It is assumed that the added source of lactose in the whey, skimmilk powder and Kling (see Table 1) provided the carbohydrate source for the streptococci and lactobacilli. With the nutrient addition, these organisms flourished and produced lactic acid which lowered the pH to the iso-electric point of casein, producing the exudate in the packages.

The companies producing this Cheese Food had a high level of returns because of the unsightly exudate. Cooling the Cheese Food rapidly to 5 C and holding at or below this temperature eliminated the problem.

The microbiological flora does not appear to have public health significance but it should be noted that coagulase-positive staphylococci were found. In considering any public health significance Tatini et al. *(7)* found enterotoxin A production was stimulated in Swiss cheese with cooking temperatures of 50 C. The temperature of this Cheese Food attained 42 C

TABLE 6. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 15 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^6$)	Streptococci ($\times 10^6$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^6$)	Molds ($\times 10$)	Yeasts
0	<10	4.1	5.1	13.0	4.4	190.0	250
3	<10	3.7	3.7	16.0	6.5	4.0	54
7	<10	33.0	19.0	9.4	48.0	170.0	50
10	<10	<1.0	0.5	1.6	4.5	0.4	<2
14	<10	1.0	1.0	1.3	2.0	5.0	<2
17	<10	<1.0	0.2	0.6	1.5	3.0	<2
21	<10	0.6	0.5	1.5	1.0	2.0	<2
24	<10	0.4	0.4	0.4	0.8	1.0	<2
28	<10	35.0	0.1	5.6	0.3	39.0	<2
31	<10	83.0	13.0	6.4	140.0	44.0	×2
35	<10	55.0	15.0	18.0	47.0	LA[a]	LA
38	<10	150.0	57.0	3.8	280.0	4.0	<2
42	<10	94.0	12.0	15.0	230.0	39.0	<2

[a]Laboratory accident

TABLE 7. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 20 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^8$)	Streptococci ($\times 10^8$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^8$)	Molds ($\times 10$)	Yeasts
0	<10	0.04	0.05	13.0	0.04	190	250
3	<10	0.33	0.19	17.0	0.57	26	10
7	<10	1.20	0.79	8.6	1.80	95	50
10	<10	2.20	1.60	3.9	3.10	25	<2
14	<10	2.40	1.30	6.0	3.00	120	<2
17	<10	2.30	1.30	1.6	2.30	8	10
21	<10	4.20	4.00	4.8	2.30	8	<2
24	<10	3.30	3.10	1.0	3.20	17	<2
28	<10	2.40	0.83	7.8	2.30	20	<2
31	<10	3.50	1.70	4.1	5.00	9	<2
35	<10	4.20	2.00	17.0	4.20	—	—
38	<10	5.00	2.40	3.6	5.90	4	<2
42	<10	3.00	1.00	5.1	2.70	3	<2

during comminuting, without external application of heat.

It should also be pointed out that Park et al. (5) found viable salmonellae in an inoculated Cheese Food after 23 weeks at pH 4.70. It is therefore of utmost importance to use high quality ingredients in the manufacture of Cheese Food and to keep the temperature low during processing and storage.

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