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Comparison of Petrifilm™ and Plate Count Methods for Enumerating Molds and Yeasts in Cheese and Yogurt

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ABSTRACT

The Petrifilm™ method for enumerating yeasts and molds was evaluated on 52 samples of fresh cheese and yogurt. A conventional plate method on glucose yeast extract medium with oxytetracycline was used as reference. Counting the repeatability a value of 0.229 log units was obtained for the Petrifilm™, compared to 0.175 for the conventional plate method. The correlation coefficient was 0.9978 with a residual standard deviation of 0.094 log units. The Petrifilm™ method is practical and fast, and its results may be compared to those of the conventional plate method.

Key Words: Molds, Petrifilm™, plate count methods.

Media applied to a portable film are already being used to determine the total count and coliforms (*4-6*). They have been accepted by the Association of Official Analytical Chemists (AOAC) as an official method (*2*). Up to now, only one Petrifilm™ evaluation for molds and yeasts has been documented (*3*). A general purpose medium with acidification was used, e.g., acidified potato dextrose agar and chloramphenicol supplemented plate count agar (CPCA), as reference. Preference is given to the addition of antibiotics instead of acidification. In this study the Petrifilm™ method to determine yeasts and molds in yogurt and fresh cheese is compared to a conventional pour plate count technique. A general selective medium, the glucose yeast extract medium (*1*) with added oxytetracycline antibiotic was used as reference.

MATERIALS AND METHODS

Experimental samples.

Fifty-two fresh cheese and yogurt samples have been examined for the presence of molds and yeasts. The samples were mainly farm products, including both full-fat and low-fat cheese and yogurt types, with or without added fruit.

The Petrifilm™ method

The Petrifilm™ method was developed by 3M (3M Company, St. Paul, MN). It is a sterile, ready-to-use system. To determine the population of yeasts and molds, the medium consists of a modified Sabouraud medium, enriched with 3% dextrose and provided with a second layer, in which a soluble swelling agent is applied. The upper film contains chlortetracycline and choramphenicol, which diffuse in the medium and inhibit bacterial growth. A color indicator is added to the system to simplify counting. For the analysis, the upper film has to be lifted and 1 ml of a representative dilution of the sample made in one-fourth strength Ringer (2.25 g NaCl; 0.105 g KCl ; 0.06 g $CaCl_2$; 0.05 g $NaHCO_3$ per liter, pH 6.9) on the lower film, (e.g., the medium), has to be applied and equally spread. The surface to be inoculated is about 30 cm^2. Incubation takes three days at 25°C. Yeasts form small blue-green or yellow colonies. Mold colonies are normally larger and show black, yellow, green or other pigmentation, depending on the species. The differentiation is confirmed by microscopic investigation (*1*). All determinations were carried out in duplicate. Numbers between 15 and 150 were counted. Only in a few exceptional cases higher or lower numbers were counted.

Reference methods.

Parallel enumerations of molds and yeasts were carried out using the method described in the International Dairy Federation 94B: 1991 standard (*1*). For each sample, 10 g is mixed with 90 ml of one-fourth strength of Ringer and a dilution series (one-tenth) is made. The standard prescribes glucose yeast extract with chloramphenicol (pH 6.6) as medium. A pour plate technique is used and 1 ml of the respective dilutions is inoculated. Incubation takes five days at 25°C. All enumerations have to be made in duplicate. In case of doubts, colonies must be examined microscopically. In this study, chloramphenicol was replaced by chlortetracycline.

Statistical analysis.

The repeatability of both Petrifilm™ and conventional methods was determined. The repeatablility r is the 95% reliability interval for the difference between two duplicate determinations.

$$r = 2 \times \sqrt{2} \times s_r = 2.83 \times s_r$$

The standard deviation s_r for the repeatability was calculated using the following formula:

$$s_r = \frac{\sqrt{d_n^2}}{2n} \quad \text{with}$$

$d_n = X_n - X$ (deviation from the individual average results with respect to the overall average) and n: the number of samples.

Comparison between the Petrifilm™ and the conventional method.

Statistical analysis was carried out using the normal numeric data and the logarithmised data. A standard regression analysis was carried out, and the slope and intercept, as well as the

correlation coefficient, were calculated. Y = a + bx (Y = numeric value of conventional reference method, x = numeric value of Petrifilm method). Both, direction coefficient *b* and correlation coefficient provide an indication of the correspondence between both methods.

The residual standard deviation (s_{yx}) was calculated as well. This is the standard deviation between the actual average Y-values (Y_n) and the average Y-values calculated using the regression line (y_n). When S_{yx} <0.2 log units, the method is considered to be acceptable (*7*).

RESULTS AND DISCUSSION

Repeatability.

The results of the enumerations of molds and yeasts carried out on 52 fresh cheese and yogurt samples are listed in Table 1. Repeatability was calculated based on the data from the duplicate determinations. For the conventional method, the repeatability was 0.175 log units. For the Petrifilm method a value of 0.229 log units was obtained. This means that the repeatability of the Petrifilm™ method is somewhat smaller compared with the conventional plate method (OGYE). The standard deviation for repeatability was 0.062 log units for the plate count and 0.081 log units for the Petrifilm™. The s_r was also counted for different classes of samples in function of the level of contamination as shown in Table 2. The standard deviation for repeatability was significantly higher for a lower level of contamination.

Comparison of results obtained by both methods.

Figure 1 shows the relationship log Y = 0.133 + 0.969 log X, between the Petrifilm™ method (x-axis) and the conventional plate method (y-axis) for the determination of yeasts and molds. A correlation coefficient of 0.9978 was found between both methods while Beuchat et al. (*3*) found a value of 0.994 between Petrifilm™ and pour-CPCA. For s_{yx} a value of 0.094 was found, which is certainly acceptable for microbiological methods (*7*).

TABLE 1. *Means of duplicate enumerations of yeasts and molds in 52 cheese and yogurt samples using OGYE agar and Petrifilm™.*

No. sample	log CFU/g OGYE	log CFU/g Petrifilm	No. sample	log CFU/g OGYE	log CFU/g Petrifilm
1	3.41	3.32	27	6.06	6.01
2	2.85	2.59	28	4.17	4.19
3	4.54	4.69	29	4.09	3.94
4	6.19	6.34	30	4.98	5.02
5	5.48	5.39	31	6.67	6.63
6	4.61	4.62	32	5.00	5.07
7	2.08	1.96	33	3.39	3.15
8	5.08	5.15	34	3.25	3.04
9	4.95	4.88	35	6.40	6.55
10	6.23	6.38	36	5.81	5.74
11	3.44	3.46	37	4.33	4.44
12	7.77	7.84	38	6.54	6.63
13	5.74	5.80	39	1.95	2.05
14	4.93	4.94	40	3.75	3.70
15	5.78	5.78	41	2.63	2.59
16	4.83	4.95	42	4.15	4.32
17	5.42	5.48	43	5.16	5.08
18	2.84	2.93	44	3.29	3.39
19	3.14	3.13	45	2.28	2.28
20	3.15	3.07	46	3.56	3.46
21	3.28	3.32	47	7.01	7.08
22	5.62	5.77	48	5.47	5.50
23	4.02	4.08	49	6.22	5.87
24	5.58	5.61	50	4.34	4.18
25	6.16	6.13	51	3.37	3.41
26	6.41	6.47	52	5.37	5.51

TABLE 2. *Standard deviation for repeatability (s_r) for the OGYE and the Petrifilm™ in function of the level of contamination.*

Colony count (CFU/ml)	n	Plate method (OGYE) s_r	Petrifilm™ s_r
All samples	52	0.062	0.081
< 1,000	6	0.103	0.113
1,000 ≤ x < 10,000	11	0.068	0.111
10,000 ≤ x < 100,000	11	0.057	0.080
100,000 ≤ x 1,000,000	14	0.036	0.062
x ≥ 1,000,000	10	0.053	0.024

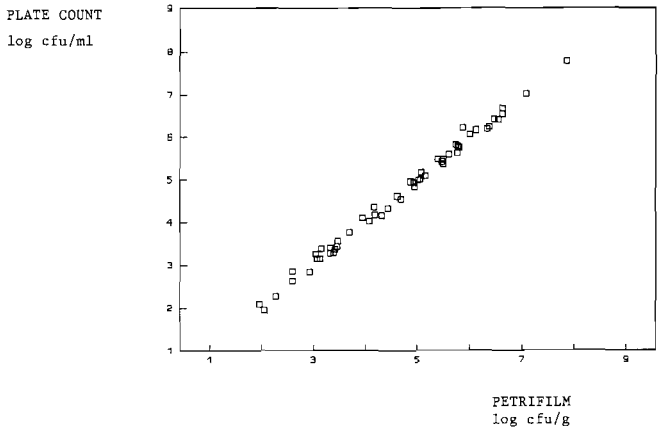

Figure 1. *Relation between log plate count CFU (OGYE) and log Petrifilm™ for the determination of molds and yeasts (linear regression).*

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