EFFECT OF H.T.S.T. PASTEURIZATION EXPOSURE ON BACTERIAL COUNT AND ON CERTAIN OTHER QUALITY FACTORS

by

Charles Hosmer McNaughton, B.S.A.
The University of Saskatchewan

A Major Thesis submitted to the
Faculty of Graduate Studies and Research
The University of Manitoba
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Master of Science
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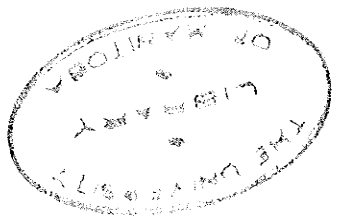

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Forty-eight one-thousand-pound-lots of raw milk, twelve of each of the four resazurin grades were pasteurized at H.T.S.T. exposures of 161.5°F., 166°F. and 170°F. for 16 sec. Bacterial counts of the raw and pasteurized milk were determined. Certain of the pasteurized samples were analysed for flavor and cream volume.

The bacterial counts of all resazurin grades of milk decreased as the pasteurization exposure was increased. This difference was statistically significant with milk of resazurin grades II, III and IV but not with that of gradeI.

A definite reduction in cream volume was noted at pasteurization exposures of 166 and 170°F. for 16 sec.

Pasteurization at 166 and 170°F. for 16 sec. imparted a cooked flavor to the milk. This flavor was not evident when the milk was held at 40°F. for 6 hours.

There was a correlation between the resazurin grade and the bacterial count of the raw milk, with some variations occurring.

Results of this study seem to indicate that a heat exposure of 161.5°F. is as satisfactory as higher exposures from the standpoint of bacterial count and is satisfactory for "cream-line" milk. Higher temperatures decrease cream volume.

It is suggested that the elimination of thermoduric organisms from milk to be pasteurized is desirable.

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INTRODUCTION

Some of the raw milk produced in the Winnipeg milk shed is of low quality, especially during the summer months. Due to the shortage of milk in this area it is necessary to accept this milk for processing, resulting in wide variation in the bacterial counts of pasteurized products.

These variations in certain cases make it difficult to conform with the requirements of certain Departments of Health that pasteurized milk must contain less than 30,000 bacteria per milliliter.

The results of a previous study (21) using "holder" pasteurization showed that low quality milk pasteurized at 145°F. for 30 min. did not meet this standard. However, pasteurization of the same quality milk at 155°F. for 30 min. was satisfactory from the standpoint of bacterial count.

Over fifty percent of the milk in this area is processed by the High-Temperature-Short-Time (H.T.S.T.) pasteurization system. At present the use of this system is limited to the larger plants but many small plants are changing to this method. It is felt that the problem of low quality milk not meeting this standard for

pasteurized milk should be evaluated using H.T.S.T. equipment. The study involved:

(a) the effect of H.T.S.T. pasteurization at three different temperatures on the bacterial count of the pasteurized product in milk of four grades -- the grade in each case being based on the resazurin reaction of the raw milk.

(b) the relationship between resazurin grade and bacterial count in raw milk.

(c) certain other problems relating to the quality of pasteurized milk.

REVIEW OF LITERATURE

Numbers Of Bacteria

The literature is replete with references concerning the effect of pasteurization at the generally recognized legal minimum of 161°F. for 16 sec. Very little work has been done using greater pasteurization exposures, especially on the bacterial flora.

Mycobacterium tuberculosis is considered to be the most heat resistant pathogen likely to be encountered in milk and has consequently been used to determine the proper time-temperature relationship for H.T.S.T. pasteurization (15).

It has been shown (3) that some haemolytic streptococci survive flash heating better than the tubercle bacilli.

Workman (24), using standard types of commercial H.T.S.T. pasteurizers, found that pasteurization at 160°F. for 16 sec. was effective in destroying 17 strains of M tuberculosis, 74 strains of Brucella abortus, B suis and B melitensis, 218 strains of streptococci of human origin and 186 strains of mastitic types of bacteria.

Hileman and Leber (12), in a study involving 125 samples,

found that low count milk pasteurized by the H.T.S.T. method gave counts of the same level as similar milk pasteurized by the holder method, whereas high count raw milk pasteurized by the H.T.S.T. method had higher counts than that pasteurized by the holder method.

Yale (25) found that H.T.S.T. pasteurization gave higher bacterial plate counts than the holder method in 13 out of 18 comparisons.

Hiscox (14), in routine weekly bacterial tests made over a period of two years on commercial samples from a single plant, showed that H.T.S.T. pasteurization greatly reduced the number of thermophilic bacteria and that thermoduric organisms were destroyed to a degree equal to that expected of the holder process. This view was supported by Workman (24) and Yale and Kelly (26).

Hileman et al (13) claimed that high counts in milk pasteurized by the H.T.S.T. method are primarily due to thermoduric organisms. In a study made on 484 samples, from 49 producers, laboratory pasteurized at 161°F. for 16 sec., these investigators found that 79.3% of the surviving bacteria were micrococci, 7.4% streptococci, 8.1% sarcinae and 5.2% bacilli. They found also that certain species of micrococci survived the H.T.S.T. method

in greater numbers than they did the holder method. The most common of these were M. candidus, M. epidermicus, M. luteus and M. varians. They considered these to be the predominant micococci in the thermoduric flora of improperly cleaned milking utensils.

Galesloot (9) found in studying high temperature laboratory pasteurization that a high thermoduric count was due chiefly to the presence of certain species of the genus Streptococcus -- St. thermophilus, St. bovis and St. durans. He showed that these thermoduric bacteria were present in pasteurized milk only when the milk was pasteurized at the minimum temperature-time combination required to give a negative phosphatase reaction but were not present in milk pasteurized at greater exposures.

Corash (4) reported that thermoduric bacteria found in properly pasteurized milk were not pathogenic. Both Macy (19) and Doetsch (6) found excessive numbers of thermoduric bacteria in milk to be significant because they indicate unsanitary conditions of production.

Mallmann et al (20) collected milk samples from milking machines which received little or no inspection. A large percentage of the bacteria in these samples were resistant to

pasteurization exposures. Further, they examined milk produced under strict sanitary inspection and found little evidence of heat resistant organisms.

Finally, it is a well known fact that the rate of destruction curve of a bacterial culture will have the same slope but will project over a greater time when the initial bacterial content is high.

Cream Volume and Flavor

The creaming ability of milk may be permanently impaired by excessive heat treatment. It has been found (7) that no appreciable advantage in cream volume formation could be attributed to either H.T.S.T. or holder pasteurization if the H.T.S.T. exposure was 161°F. for 16 sec.

Holland and Dahlberg (15) measured impairment of creaming ability caused by various pasteurization exposures. These investigators found comparable cream volumes in milk pasteurized at 143°F. for 30 min., 160°F. for 16 sec. and 170°F. for 2.5 sec. Any temperature-time exposure greater than any of these treatments reduced the cream volume.

Excessive pasteurization exposure may cause the development

of a cooked flavor. This may be partly due to the liberation of the sulph-hydryl groups of the milk protein (11).

Dahlberg (5) obtained no evidence of cooked flavor in milks heated to temperatures ranging as high as 177.5°F. when the time of raising from 145°F. to the temperature used was not greater than 24 sec.

Other investigators (7), (3) reported that milk pasteurized by the H.T.S.T. method had less cooked flavor than had vat pasteurized milk.

The Resazurin Grade and the Plate Count

Frayer (8) compared the resazurin grade with the plate count on a number of samples of raw milk. After incubation at 37°C. for 1 hr. the samples were graded according to permanent standards. The resazurin grade showed a poor correlation with the plate count.

Brannon (2) found a correlation between the resazurin grade and the bacterial plate count although a considerable overlapping of counts occurred. The milk was graded I, II, III or IV, in each case according to the time required for the milk dye mixture to reach a definite color. The plate count of Grade I milk

varied from 1,000 -- 180,000 per ml.; Grade II from 1,000 -- 1,800,000; Grade III from 2,100 -- 7,600,000; Grade IV from 9,400 -- 10,200,000 per ml.

Golding and Gorgenson (10) incubated the milk dye mixture for 60 min. and made color readings with a Lovibond comparator. The resazurin test correlated fairly well with the plate count.

OKulitch, Millard and Fleming (22) used a color standard designated as Munsell P.R.P. 7/8 to compare the plate count with the resazurin grade. Readings were made after incubation for one and three hours. In poor quality milk the resazurin grade correlated with the standard plate count in more cases than did the methylene blue grade and plate count.

Lewton, Markland and Babel (18) compared the bacterial count of raw milk with the triple reading resazurin test using a Munsell P. 7/4 color standard end point. They found that the resazurin test placed 41% of the samples in the same grade as the plate count, 20% one grade lower and 17% three grades lower. Many of the samples that showed the largest differences in classification had plate counts of less than 50,000 per ml. It was suggested that the reduction of the dye in these samples might have been due to pathological conditions.

Johns (17) found a high correlation between resazurin (triple reading) reduction time and plate count at 32°C. on raw milk and on the same milk after laboratory pasteurization. This was true particularly during the warmer months.

PROCEDURE

(a) Bacterial counts on raw milk of different resazurin grades and on the same samples pasteurized at different temperature-time exposures

Forty eight one-thousand-pound-lots of raw milk, twelve of each of the four resazurin grades, were obtained from commercial plants. Each lot was placed in a storage tank and well mixed before pasteurization.

The milk was pasteurized in a commercial type 2000 lb. per hr. H.T.S.T. unit at 170°F., 166°F. and 161.5°F. for 16 sec. Each lot was pasteurized in the temperature sequence shown above. The holding time at the pasteurizing level was checked by the salt conductivity method (16), (23). In order to simulate commercial conditions the added effect of heating during bringing the temperature up to the pasteurizing level and during cooling was neglected.

After the milk had been thoroughly mixed in the storage tank the raw sample was removed aseptically. The pasteurized sample was removed from a sample cock at the outlet of the cooling section of the plate heat exchanger. A series of samples were obtained. The first sample was heated at 170°F. for 16 sec.

Then the temperature was lowered to 166°F. The second sample was heated at 166°F. for 16 sec. Again, the temperature was lowered to 161.5°F. and a third sample heated at 161.5°F. for 16 sec. obtained. The samples were placed in an ice-water bath.

The analytical work was begun immediately after pasteurization and conducted according to standard methods (1).

The raw milk was graded by the triple reading resazurin test. One ml. 0.005% resazurin solution was added to 10 ml. milk and the mixture incubated in a water bath at 37°C. for 1 hr.

The milk color was compared with a Munsell P. 7/4 color standard and milk that was reduced beyond this color standard was placed in grade IV. Milk which was not reduced beyond this color was inverted once to redistribute the cream and bacteria and replaced in the water bath for one hour. The inspection and inversion was repeated at the end of the second hour. Milk which was reduced during the second hour was placed in grade III. Milk which was not reduced was returned to the water bath for a third hour. Milk which was reduced during the third hour was placed in grade II. If it was not reduced beyond a Munsell P. 7/4 color standard after three hours of incubation it was placed in grade I.

Bacterial counts on raw and pasteurized samples were made by plating in duplicate in the appropriate dilutions in Tryptone Glucose Yeast Agar and incubating at 32°C. for 48 hrs. The count used represented the average count on two replicate plates made from one dilution in each case. The counts obtained on the twelve trials on each grade at the three temperature-time exposures were submitted to analysis of variance study. As an additional procedure and for purposes of summarizing data on counts at the different pasteurizing temperature-time exposures, average counts on each sample were transposed to logarithms. The logarithms on all samples at each temperature-time exposure were averaged and the averages retransposed to plate counts.

(b) Coliform Counts on Pasteurized Milk

Coliform counts were determined by plating 1 ml. of the pasteurized samples in Violet Red Bile Agar and incubating at 37°C. for 24 hrs.

(c) Cream Volume in Relation to Pasteurizing Exposure

The effect of different pasteurizing exposures on cream volume was determined by holding replicates of certain samples in quart bottles at 40°F. for 24 hrs. and measuring the depth of cream.

(d) Flavor in Relation to Pasteurizing Exposure

Similarly the effect of different pasteurizing exposures on flavor was determined by testing replicates of certain pasteurized samples immediately after pasteurizing and cooling and after holding for 6 to 12 hrs. at 40°F. In each case at least two persons shared in this test.

(e) Relation between Bacterial Counts and Resazurin Grades of Raw Milk

The forty eight thousand-pound-lots of raw milk were placed in four grades on the basis of bacterial counts, and into four grades on the basis of resazurin reactions. These data were submitted to a chi square test.

RESULTS

The data on bacteria in milk pasteurized at each temperature -time exposure appear variously in Tables I to IV. Resazurin grade I milk shows no significant difference in count when pasteurized at the three temperature-time exposures. On the contrary, milk of resazurin grades II, III and IV shows differences on this basis. However, in general in grades II, III and IV, as the pasteurizing temperature is increased the bacterial counts are decreased. This is indicated in Fig. I. In grade I milk this decrease is only slight.

Coliform bacteria were not present in any of the forty-eight lots of milk pasteurized at any of three exposures used.

In each of five trials on different lots of milk the cream volume in milk heated at 166°F. was appreciably less than in the same milk heated at 161.5°F., and the volume in milk heated at 170°F. wasfurther decreased. This is shown in Table V, in which the reduction is expressed as a percent of the volume of cream in the milk heated at 161.5°F.

In each of the five trials on the same lots of milk as used in the cream volume studies and heated respectively at 161.5°F. 166°F. and 170°F. a cooked flavor was evident in the freshly

cooled milk after having been heated at 166°F. and at 170°F., but not in that heated at 161.5°F. This cooked flavor had disappeared in the samples when tested at 6 hr. and at 12 hr.

On the basis of the limited data available there was a highly significant correlation between the four grades of milk based on the bacterial counts and the four resazurin grades of the milk. This is shown in Table VI. This correlation is more evident in lots of low and high bacterial counts. Unfortunately the data involve only 6 lots of milk in the intermediate bacterial count grades.

Table I.

Bacteria in different lots of resazurin Grade I milk pasteurized for 16 sec.

Raw	170°F.	166°F.	161.5°F.
8,150	225	350	395
25,500	3,250	4,150	4,350
8,650	270	280	250
19,150	255	255	360
6,650	240	225	285
5,400	190	155	170
201,000	450	295	400
6,700	600	350	400
11,000	950	1,000	1,050
18,700	450	350	300
6,050	220	210	135
23,900	1,210	1,330	3,350

Analysis of Variance

	d.f.	S.S.	M.S.	F (calc)	F(0.01)
Samples	11	40,290,224	3,662,747	25.08	3.18
Treatments	2	460,434	230,217	1.5	5.72
Error	22	3,211,966	145,998	--	--
Total	35	43,962,624	---	--	--

Table II

Bacteria in different lots of resazurin
Grade II milk pasteurized for 16 sec.

Raw	170°F.	166°F.	161.5°F.
154,000	13,800	15,350	19,100
168,500	4,700	5,500	8,150
127,500	13,600	15,750	19,350
140,000	8,250	10,950	15,150
16,700	555	685	640
9,400	680	620	860
990,000	4,800	5,150	5,750
10,800	360	260	280
170,000	13,900	16,400	19,900
24,950	2,500	3,600	5,400
291,000	3,100	2,830	1,720
7,500	220	220	260

Analysis of Variance

	d.f.	S.S.	M.S.	F (calc)	F (0.01)
Samples	11	1,456,496,789	132,408,799	60.9**	3.18
Treatments	2	38,716,710	19,358,355	8.9**	5.72
Error	22	47,773,107	2,171,504	--	--
Total	35	1,542,986,506	---	--	--

Table III

Bacteria in different lots of resazurin
Grade III milk pasteurized for 16 sec.

Raw	170°F.	166°F.	161.5°F.
4,050,000	3,050	3,950	6,350
1,300,000	13,000	11,050	13,400
300,000	7,350	9,250	13,500
775,000	9,050	10,100	12,200
245,000	15,150	29,850	28,500
325,000	8,500	8,000	10,400
1,910,000	5,500	9,750	10,450
130,000	3,500	3,150	3,400
2,080,000	4,350	6,750	8,750
500,000	7,550	7,650	8,400
495,000	34,100	42,800	38,550
625,000	7,050	10,900	15,250

Analysis of Variance

	d.f.	S.S.	M.S.	F(calc)	F(0.01)
Samples	11	3,191,880,000	290,170,000	41.9**	3.18
Treatments	2	113,760,000	56,880,000	8.2**	5.72
Error	22	152,310,000	6,920,000	--	--
Total	35	3,457,950,000	---	--	--

Table IV

Bacteria in different lots of resazurin
Grade IV milk pasteurized for 16 sec.

Raw	170°F.	166°F.	161.5°F.
7,000,000	6,700	6,900	10,400
4,180,000	2,700	4,400	4,600
10,100,000	6,900	5,500	7,000
34,900,000	7,500	8,700	12,100
46,800,000	4,500	7,800	10,900
20,000,000	4,200	4,800	5,700
20,050,000	6,450	10,700	14,200
47,450,000	5,250	8,850	17,200
6,350,000	7,800	8,600	12,800
9,500,000	6,950	12,450	16,450
7,295,000	12,750	23,250	33,500
5,330,000	2,150	5,550	12,750

Analysis of Variance

	d.f.	S.S.	M.S.	F (calc)	F(0.01)
Samples	11	803,430,000	73,040,000	8.5**	3.19
Treatments	2	297,080,000	149,040,000	17.3**	5.72
Error	22	189,390,000	8,610,000	--	--
Total	35	1,289,900,000	---	--	--

FIG. I

THE AVERAGE BACTERIAL COUNT OF FOUR RAW MILK GRADES AT DIFFERENT PASTEURIZATION EXPOSURES

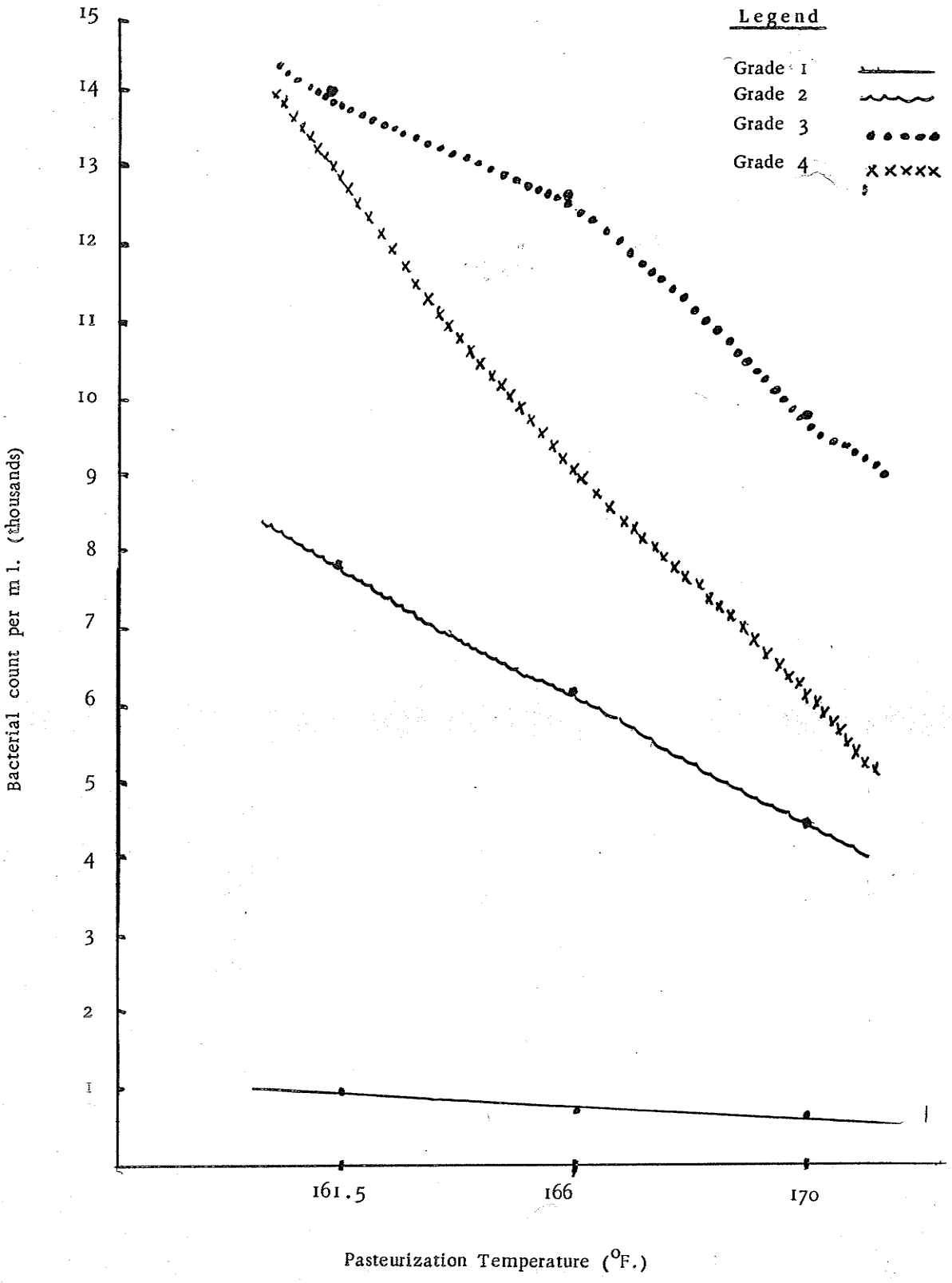

Table V

Relationship between pasteurizing temperature[1] and cream volume

Trial	161.5°F.	166°F.	170°F.
1	0	43.9 [2]	71.0 [2]
2	0	44.0	72.0
3	0	32.3	75.0
4	0	31.0	74.0
5	0	47.0	70.0

1 Exposure 16 sec.

2 Reduction in percent of the volume at 161.5°F.

Table VI

Correlation between resazurin grade and bacteria count of raw milk

Resazurin Grade

Bacterial Count	I a	I t	II a	II t	III a	III t	IV a	IV t	, X^2	d.f.	P value at
0 - 500,000	12	7	11	7	5	7	0	7	53.69	9	0.001 21.67
500,000 - 1,000,000	0	1	1	1	3	1	0	1			
1,000,000 - 2,000,000	0	0.5	0	0.5	2	0.5	0	0.5			
Over 2,000,000	0	3.5	0	3.5	2	3.5	12	3.5			

DISCUSSION

Bacterial counts of pasteurized milk may be more dependent upon the type of bacteria present in raw milk than upon the heat exposure. This may be shown by the fact that a raw milk sample with a count of 46,800,000, yielded a plate count of 10,900 when heated at 161.5°F. for 16 sec. (Table IV), whereas lower count raw milk was reduced only to 38,550 with the same heat exposure (Table III) and even higher temperatures did not reduce this count significantly. This is shown also in the fact that the average count of resazurin Grade III milk at three pasteurization exposures was greater in each case than that of Grade IV milk. The average raw count of Grade III milk is much lower than that of Grade IV milk. Evidently these Grade III lots contained larger numbers of thermoduric bacteria.

Elimination of thermoduric bacteria from raw milk may be more preferable, from a public health standpoint, than increased pasteurization exposures as the additional heat treatment may affect the nutritional properties of milk.

A count of less than 30,000 bacteria per milliliter is the pasteurized milk standard set by many health departments. Only

two of the forty-eight samples of milk showed counts greater than this standard after pasteurization at the legal minimum. In one of these, Table III, the increased heat exposure did not substantially reduce the count. It would appear from these trials that a pasteurization exposure of 161.5°F. for 16 sec. produces as satisfactory a bacterial count as increased exposure.

There is one additional point in favor of the minimum pasteurization treatment. Cream volume trials clearly indicate that pasteurization exposures of 166 or 170°F. for 16 sec. drastically reduce the creaming ability of milk. This is of no consequence if the milk is to be homogenized.

None of the pasteurization exposures used would have a detrimental effect on the milk flavor. Normally, milk is not consumed for at least six hours after pasteurization. The flavor trials showed no cooked flavor in milk at any of the three heat exposures after the milk was held at 40°F. for six hours.

Absence of coliform bacteria in all samples of pasteurized milk indicates that the milk was produced under excellent sanitary conditions or that the coliform types present were not thermoduric.

SUMMARY AND CONCLUSIONS

Forty eight one-thousand-pound-lots of raw milk, twelve of each of the four resazurin grades were pasteurized at H.T.S.T. exposures of 161.5°F., 166°F. and 170°F. for 16 sec. Bacterial counts of the raw and pasteurized milk were determined. Certain of the pasteurized samples were analysed for flavor and cream volume.

The bacterial counts of all resazurin grades of milk decreased as the pasteurization exposure was increaed. This difference was statistically significant with milk of resazurin grades II, III and IV but not with that of grade I.

A definite reduction in cream volume was noted at pasteurization exposures of 166 and 170°F. for 16 sec.

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Results of this study seem to indicate that a heat exposure

of 161.5°F. is as satisfactory as higher exposures from the standpoint of bacterial count and is satisfactory for "cream-line" milk. Higher temperatures decrease cream volume.

It is suggested that the elimination of thermoduric organisms from milk to be pasteurized is desirable.

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