# THE KEEPING QUALITY OF PASTEURIZED MILK

by

MING-fang Li

A Thesis Submitted to

The Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfilment of the Requirements

For the Degree of

Master of Science

May 1957

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Experiments have been carried out to determine the effect of the method of pasteurization, the storage temperature and the number of days in storage on the keeping quality of pasteurized milk. Milk obtained from the University dairy was pasteurized by the H.T.S.T. and vat methods and samples were stored at 35° F., 40° F., and 50° F. for a period up to 18 days. Samples were analyzed at three day intervals for bacterial count, flavor, pH and acidity.

Milk pasteurized by the H.T.S.T. method at 161° F. for 16 seconds produced a better product from the standpoint of bacterial count, pH and flavor than milk pasteurized at 143° F. for 30 minutes.

The storage temperature had a significant effect on the bacterial count, pH, acidity and flavor of the pasteurized product. Storage at high temperatures tend to inhibit the development of oxidized flavor. However, storage at 50° F. over a long period of time induced the development of acidity and the souring of milk. Pasteurized milk with a low bacterial count should perhaps be stored at 40° F. rather than at 35° F. Milk with a high bacterial count should be stored at lower temperatures (35° F.). The proper storage temperature seems to be determined by the quality of the pasteurized milk as well as by the length of storage period desired before consumption.

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# INTRODUCTION

Many changes have taken place in the dairy industry in Manitoba during the last five years. Paper containers have largely replaced glass bottles for the distribution of milk. Roads throughout the province have been greatly improved and a large part of the milk is now transported by truck. These changes have resulted in a centralization of the milk plants in the province. A few widely scattered plants now process almost all of the fluid milk used in the province. Milk is now being delivered from the City of Winnipeg to all parts of Manitoba and even to Saskatchewan. This may involve transportation over distances of 600 miles for example to Flin Flon, Manitoba. This milk may be up to 14 days old before it is finally consumed.

The following project was conducted to determine the effect of storing pasteurized milk at various temperatures for periods up to 18 days. Milk was pasteurized by the High Temperature Short Time method (161° F. for 16 seconds) and by the vat method (143° F. for 30 minutes) and samples were stored at 35° F., 40° F., and 50° F. for periods up to 18 days. Samples were analyzed at three day intervals for bacterial count, flavor, pH and acidity.

This thesis reports the results of this investigation.

## REVIEW OF LITERATURE

Bacteriological Deterioration of Pasteurized Milk.

Thermoduric and Thermophilic Bacteria: Bacteriological deterioration of pasteurized milk is caused either by bacteria which survive pasteurization or by bacteria which enter the milk after pasteurization. Those bacteria which survive pasteurization in considerable numbers are considered by the Dairy Industry to be thermoduric bacteria. These bacteria are capable of growing rapidly in milk over a wide range of temperature. Those bacteria which grow and multiply at pasteurization temperatures are called thermophilic bacteria. They grow best at temperatures between 50° C. and 60° C. Above and below this range reproduction is retarded, Olson (48).

These organisms gain entrance to the milk from the udder, from the equipment with which the milk comes in contact and from the dust in the air at the time of milking. Hileman (36) and Abdel-malek (1) considered that the cows udder was the main source of thermoduric bacteria. Levowitz (42), however, believed that the udder did not contribute to the thermoduric count sufficiently to cause it to exceed municipal milk standards. Dirty milking machines and utensils, especially if dried milk solids were present, were shown to be excellent sources of contamination by Galesloot (24) and Thomas (58). The importance of proper cleaning and sanitizing of utensils was also indicated by Dotterer (17). Dust in the stable air at milking time was reported by Mack (43) as another vital source of contamination since feed, bedding and soil harbored thermophilic organisms.

Alexander (2) reported that microbacteria and micrococci together with streptococci, aerobic sporeforming bacilli and non-sporing rods survived H.T.S.T. pasteurization. Fabian (22) classified the most common bacteria which survived pasteurization as Micrococcus albus, M. aureus, M. candidus, M. conglomeratus, M. epidermis, M. luteus, M. varians, Streptococcus thermophilus, S. liquefaciens, S. bovis, S. glycerinaceous, S. inulinaceous, S. fecalis, S. zymogens, Sarcina lutea, S. rosa, Bacillus cereus, and B. subtilis. The most common thermophilic bacteria were B. stearothermophilus, Clostridium thermosaccharolyticum and C. nigrificans. This view was supported by Galesloot (25, 27) and Abdel-malek (1).

Galesloot (24) reported that microbacteria were more prevalent during the summer months and thermoduric streptococci were more prevalent during the winter months. Thomas (59), however, found that the thermoduric organisms were more numerous in summer and early autumn than during the winter and early spring months.

There is some doubt as to the relationship between the Standard Plate Count of pasteurized milk and the keeping quality as indicated by Ashton (5) and Galesloot (26). Thomas (57) found that a high proportion of milk samples of good keeping quality had low thermoduric counts and nearly half the samples of poor keeping quality had high thermoduric counts.

Psychrophilic Bacteria: Those bacteria which have optimum growth rates at comparatively low temperatures are considered to be psychrophilic. Incubation temperatures below 7.5° C. for 15 days or longer were recommended where conducting standard plate counts on such organisms, since at 10° C. thermoduric organisms which are not considered to be true psychrophiles began to grow, Atherton (8) and Boyd (11). However, in milk refrigerated at 35° F. - 40° F. Nelson (46) obtained the highest counts where the plates were incubated between 21° C. and 25° C. Watrous (64), Atherton (7) and Van Der Zant (63) confirmed this viewpoint.

Sherman (54) and Olson (49) have reported that psychrophilic bacteria im milk are in general Gram negative, non-sporeforming rods. Erdman (20) classified 190 psychrophilic cultures isolated from milk and cream as Pseudomonas, Lactobacillus, Streptococcus, Aerobactor, Flavobacterium, and Escherichia in descending order of importance. Within a group of 41 psychrophilic cultures isolated from water and butter, 28 were classified as belonging to the genus Pseudomonas, 5 to the genus Flavobacterium, 6 to the genus

Alcaligenes, one to the genus Achromobacter, and one was a non-lactose fermenting yeast, Jezeski (38).

Burgwald (12) found that the psychrophilic bacteria which developed in milk during refrigerated storage were primarily responsible for the deterioration of the product. This view was supported by Davis (16).

The general opinion is that the majority of true psychrophiles are destroyed at pasteurization temperatures, Thomas (56), Rogick (53), and Olson (50). Consequently, their presence in pasteurized milk in relative high numbers could be considered as an index of post-pasteurization contamination, Trout (62) and Hempler (35). Contamination could be prevented by the use of sanitary equipment England (19) and by the use of potable water supplies, Parker (51).

The Keeping Quality of Pasteurized Milk.

Boyd (10) stated that in his experiments the average keeping quality of commercially pasteurized and homogenized milk stored at 40° F. was found to be from 13 to 18 days based on a flavor score of 33, (from the U.S.D.A. Bureau of Dairy Industry Score Card for milk) and from 8 to 11 days based on a standard of not over 50,000 bacteria per ml. When duplicate samples were stored at 33° F., the average keeping quality of the milk was extended to 11 to 14 days.

The flavor deterioration was correlated with the growth of psychrophilic bacteria.

Atherton (8) found that the first perceptible indication of deterioration of milk at low temperatures seemed to be a loss of stability of the casein. There was little change in the acidity and a slow change in flavor even with a rapidly increasing bacterial count.

Development of Off Flavors during the Storage Period.

Oxidized Flavor: Roland (52) found that oxidized flavor was the predominating flavor encountered in pasteurized milk. Milk of a high fat content seemed to be more susceptible to this flavor. Thurston (60) claimed that lecithin rather than butter fat appeared to be the constituent affected. By the removal of lecithin and related substances by separation, Mucha (44) was able to decrease the sensitivity to the development of this off flavor.

Iron and copper were found by Greenbank (29) to be ideal catalysts for the development of this flavor. Light may inhibit, promote, or have no effect on the development of the flavor depending on the metallic contamination of the milk and the intensity of irradiation.

Homogenization and prolonged agitation at low temperatures reduce the susceptibility to the development of oxidized flavor, Thurston (61) and Guthrie (32). The experimental evidence of Larsen (41) indicated there was no correlation between the Eh and the inhibiting effect of homogenization.

Weinstein (65) added 35 mg. of ascorbic acid per litre and retarded the development of the oxidized flavor for 72 hours in milk stored at 45° F. Bell (9) found the addition of ascorbic acid lowered the Eh and greatly deferred, but did not prevent the development of an oxidized flavor in frozen milk. Chilson (13) stated that no oxidized flavor developed in ascorbic acid fortified milk (1.5 gm. per 100 lbs.) after 5 or 7 days' storage but rapid reduction of ascorbic acid in milk, either by exposure to direct sunlight or through the addition of 3% $H_2O_2$, resulted in no oxidized flavor over a 5-day storage period. He believed that ascorbic acid was a contributing factor in the development of this off flavor. Guthrie (31) also noted that when the ascorbic acid was eliminated oxidized flavor did not develop.

Greenbank (29, 30) believed that this off flavor was the result of an intermediate oxidation product and that the development of the flavor in milk may be inhibited by reducing or by oxidizing agents. Such an oxidation may be represented as follows:

| R------ | RO----- | $RO_2$----- |
|---|---|---|
| No oxidized flavor | Oxidized flavor | No oxidized flavor |

Roland (52) found that the bacterial counts on milk with an oxidized flavor were generally lower than counts on milk free from this defect. When Cone (14) added a large inoculation of oxidase producing gram-negative bacteria to a milk sample the development of oxidized flavor was markedly inhibited.

Greenbank (29) claimed that the oxidized flavor developed by milks held in storage increased in intensity with decreased storage temperatures. The probable explanation may be related to the dissolved oxygen content at different temperatures upon the relative rate of two or more successive reactions that may be involved in the formation and destruction of the flavor.

Other Factors Affecting the Keeping Quality.

Method of Pasteurization: Yale (66) found that none of the evidence collected showed any significant difference between the two methods of pasteurization on the cream layer, flavor, reduction of bacterial counts or destruction of Escherichia-Aerobactor types of bacteria present.

Fabian (21) stated that we should expect to find more thermophilic bacteria in milk pasteurized at 142° F. to 145° F. for 30 minutes than in the milk pasteurized at 160° F. to 161° F. for 15 to 16 seconds, and conversely we should expect to find more thermoduric bacteria in milk pasteurized at 160° F. to 161° F.

Hileman (37) found that H.T.S.T. pasteurization resulted in higher bacterial counts than pasteurization of the same milk in the laboratory at 143° F. to 144° F. for 35 minutes. Ashton's (5) investigations confirm these findings, but the keeping quality of vat pasteurized milk was inferior to that of the H.T.S.T. pasteurized milk.

Production and Processing Sanitation: Corash (15) and Hammer (33) stressed that pasteurization could not convert low quality milk into high quality milk. The sale of a high quality product depended on clean milk production and sanitary processing.

Harding (34) and Kasli (40) found that a better quality of milk could not be obtained unless clean utensils and bottles were used, and special attention was paid to proper cooling, before as well as after pasteurization. Moisture left in the cleaned cans caused marked increases in bacterial counts. According to Atherton (6) the elimination of cans and the improvement of cooling by conversion to bulk tanks and bulk tank pick up at the farm contributed to improved flavor and lower bacterial counts in milk.

Improperly cleaned and sanitized pipelines constituted an important source of general and thermoduric contamination of raw milk. Milk pipelines could be sanitized effectively by the use of procedures which included either chlorination or hot water at 185° F. as a germicidal agent, Alexander (3).

Circulation through the equipment of 100 to 125 p.p.m. available chlorine solution for at least 5 minutes, and spraying the surfaces of vat walls with 400 p.p.m. of quaternary ammonia solution was advised by England (19). The quaternary ammonia compounds generally were more effective against Gram-negative species, Johns (39). Mueller (45) found that iodine liquid and iodine detergent-sanitizer products were equally effective in bactericidal properties when 25 p.p.m. of available iodine were compared with 100 p.p.m. of available chlorine in killing *E. coli* *S. Typhosa*, *M. pyrogenes*, and *Ps. aeruginosa*.

Investigations by Alexander (3) and Fortney (23) indicated that a high degree of sanitation could be achieved by the installation of cleaned-in-place pipelines thus eliminating the need for dismantling the pipelines.

# METHODS

## Samples.

The raw milk used in the experiments was obtained from the Holstein herd at the University of Manitoba. The milk was thoroughly mixed and then divided into two lots. One lot was pasteurized in a High Temperature Short Time unit at 161° F. for 16 seconds and the other lot was pasteurized in a vat at 143° F. for 30 minutes. Sixty samples were taken from the processing line after they had been processed in the regular manner followed by the dairy. The first and last bottles off the line were not used. The samples were then stored at 35° F., 40° F. and 50° F. A parchment paper was fastened over the top of each bottle to prevent contamination from the air since preliminary trials indicated that contamination with yeast could occur around non-hooded milk bottle caps.

## Analysis of the Samples.

The samples were analyzed at three day intervals for a period of 18 days. The following determinations were made: Bacterial Counts: The milk samples were plated in two series of duplicate plates in two dilutions with tryptone glucose yeast agar (4). One series was incubated at 32° C. for 48 hours and the other series at 25° C. for 72 hours. The average count used in the data was determined according to Standard Methods for the Examination of Dairy Products (4).

Coliform Counts: Counts were made by plating 1 ml of the sample or a dilution of the sample in violet red bile agar. If necessary brilliant green lactose bile broth or eosin methylene blue agar was used to confirm the test (4).

Acidity and pH: The acidity was determined by titration with 0.1N NaOH (the result was expressed as percent lactic acid). The pH values were determined with a Beckman glass electrode pH meter model H2 (28). The pH data were statistically analyzed using direct pH values according to the method of Shiue (55).

Flavor Score: The flavor was judged according to the score card approved by the American Dairy Science Association. Each flavor was classified as slight, distinct or strong according to its intensity. The points deducted for the off flavors and intensities were as suggested by G. M. Trout and associates (47). The maximum score alloted was 41 out of a possible 45, and the minimum score was 0. A score of 0 indicates that the quality of the milk was so low that it would not be suitable for fluid consumption.

The data obtained in the above trials was then analyzed statistically.

# RESULTS

The bacterial counts, flavor scores, pHs and acidities of the samples will be found in Tables 1 - 16 in the Appendix. (see page 39). The analyses of variance of the above characteristics will be found in Tables I - IV below.

Table I. The Analysis of Variance of the Bacterial Counts.

| Variation | df | SS | MS | F |
|---|---|---|---|---|
| Samples | 7 | 304.323 | 43.475 | 19.99** |
| Methods | 1 | 112.718 | 112.718 | 51.85** |
| Storage temperatures | 2 | 373.192 | 186.596 | 85.83** |
| Temp. x method | 2 | 4.627 | 2.314 | 1.06 |
| Error (a) | 35 | 81.110 | 2.174 | |
| Total (a) | 47 | 875.970 | | |
| Storage days | 6 | 443.873 | 73.978 | 4.20** |
| Days x methods | 6 | 44.646 | 7.441 | 5.75** |
| Days x temp. | 12 | 121.895 | 10.158 | 7.85** |
| Day x temp. x method | 12 | 15.530 | 1.294 | 2.41** |
| Error (b) | 252 | 134.985 | 0.536 | |
| Total (b) | 335 | 1636.899 | | |

Least significant difference for storage temperature is 0.399.
L.S.D. for days in storage is 0.293.

** indicates that the F value is significant at the 1% level.
* indicates that the F value is significant at the 5% level.

Table II. The Analysis of Variance of the Flavor Scores.

| Variation | df | SS | MS | F |
|---|---|---|---|---|
| Samples | 7 | 2652.461 | 378.923 | 3.04* |
| Methods | 1 | 943.361 | 943.361 | 7.59** |
| Storage temp. | 2 | 4344.906 | 2172.453 | 17.48** |
| Temp. x method | 2 | 2110.580 | 1055.290 | 8.49** |
| Error (a) | 35 | 4349.019 | 124.258 | |
| Total (a) | 47 | 14400.327 | | |
| Storage days | 6 | 9386.909 | 1564.484 | 2.60* |
| Days x methods | 6 | 634.065 | 105.677 | < 1 |
| Days x temp. | 12 | 5996.731 | 499.728 | 3.94* |
| Day x temp. x method | 12 | 1518.885 | 126.573 | 3.23** |
| Error (b) | 252 | 9862.603 | 39.173 | |
| Total (b) | 335 | 41799.520 | | |

L.S.D. for storage temperature is 3.02.
L.S.D. for days in storage is 2.50.

Table III. The Analysis of Variance of the pH Values.

| Variation | df | SS | MS | F |
|---|---|---|---|---|
| Samples | 7 | 6.369 | 0.9098 | 2.96* |
| Methods | 1 | 2.220 | 2.220 | 7.23* |
| Storage temp. | 2 | 19.789 | 9.8949 | 32.24** |
| Temp. x method | 2 | 4.167 | 2.0835 | 6.78** |
| Error (a) | 35 | 10.744 | 0.3069 | |
| Total (a) | 47 | 43.249 | | |
| Storage days | 6 | 8.117 | 1.3528 | 2.87* |
| Days x method | 6 | 1.500 | 0.2500 | 1.10 |
| Days x temp. | 12 | 14.653 | 1.2211 | 5.50** |
| Days x temp. x method | 12 | 2.666 | 0.2222 | 3.10** |
| Error (b) | 252 | 18.043 | 0.0716 | |
| Total (b) | 335 | 88.228 | | |

L.S.D. for storage temperature is 0.150.
L.S.D. for days in storage is 0.107.

Table IV. The Analysis of Variance of the Acidities.

| Variation | df | SS | MS | F |
|---|---|---|---|---|
| Samples | 7 | 0.3235 | 0.0462 | 1.66 |
| Methods | 1 | 0.1869 | 0.1869 | 6.72* |
| Storage temp. | 2 | 1.4437 | 0.7218 | 25.96** |
| Temp. x method | 2 | 0.4337 | 0.2169 | 7.80** |
| Error (a) | 35 | 0.9731 | 0.0278 | |
| Total (a) | 47 | 3.3609 | | |
| Storage days | 6 | 0.5911 | 0.0985 | <1 |
| Days x method | 6 | 0.1270 | 0.0212 | 1.13 |
| Days x temp. | 12 | 1.0354 | 0.0863 | 4.61** |
| Days x temp. x method | 12 | 0.2241 | 0.0187 | 3.87** |
| Error (b) | 252 | 1.2157 | 0.0048 | |
| Total (b) | 335 | 6.5542 | | |

L.S.D. for storage temperature is 0.045.

The H.T.S.T. method of pasteurization on the average gave lower bacterial counts, higher flavor scores, higher pH values and lower acidities than the vat method. The mean of the logarithm of the bacterial count of the H.T.S.T. pasteurized milk was 4.25 and for the vat pasteurized milk it was 5.45. The mean of the flavor scores for H.T.S.T. pasteurized milk was 31.4 and for vat pasteurized milk it was 28.1. The analyses of variance (Tables I and II) show that the differences between the two methods on the basis of bacterial count and flavor are significant at the 1% level. The mean of the pH values for the H.T.S.T. pasteurized milk was 6.58 and for the vat pasteurized milk it was 6.42.

The mean of the acidity for the H.T.S.T. pasteurized milk was 0.19% and for the vat pasteurized milk it was 0.23%. The analyses of variance (Tables III and IV) show that the differences between the methods on the basis of pH and acidity are significant at the 5% level.

The bacterial counts, flavor scores, pH values and acidities vary with changes in storage temperatures. Generally, lower storage temperatures gave lower bacterial counts, higher flavor scores, higher pH values and lower acidities. The differences in these values, as affected by the storage temperature, are significant at the 1% level. (Tables I, II, III and IV). The greatest difference occurred between 40° F. and 50° F. The samples stored at 35° F. and 40° F. were almost the same. These points are illustrated in Figures 1, 2, 3 and 4.

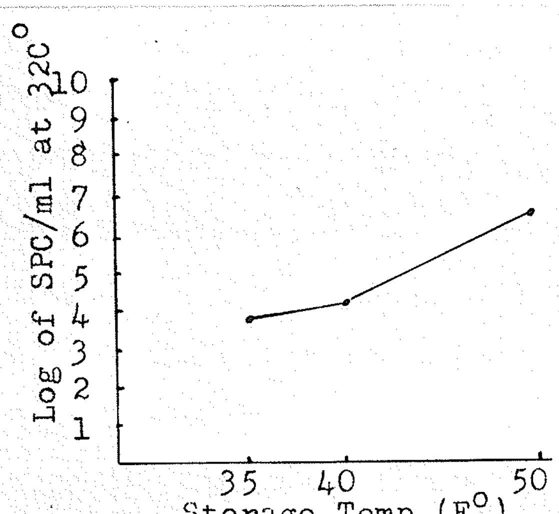


Fig.1 The effect of storage temp. on bacterial count.

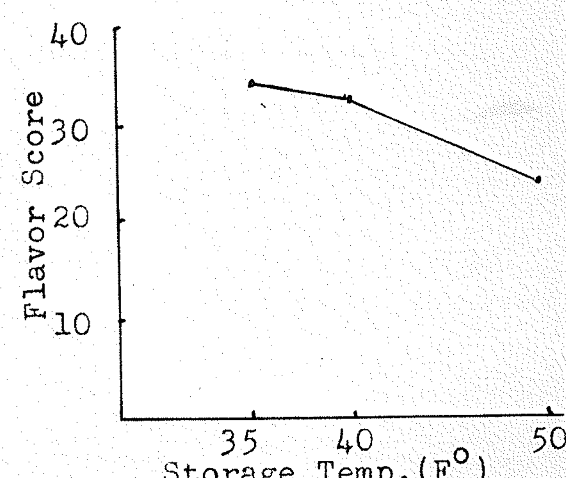


Fig.2 The effect of storage temp. on flavor.