Mold mycelia in cream and butter, with reference to the flavor score, age, acidity and butterfat content ... : and a correlation of the quality of cream churned and the grade of butter made in Montana
by James C Boyd

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry
Montana State University

Abstract:
One hundred seventeen cream samples, consisting of 63 samples of commercial churning cream, 18 indiscriminate mixtures and 36 mixed or blended samples were studied. The flavor score, age, acidity, butter-fat content and microscopic mold counts were determined and compared with the visual mold grade of the same cream.

Cream samples scoring 90 or above or less than, six days old or containing less than 0.51 percent acid were shown in all cases to he free of any appreciable mold development. Great samples scoring 89 or less or over six days old or containing over 0.51 percent acid, were subject to considerable mold development. Cream containing 40 to 50 percent butterfat apparently is not as subject to mold development as cream containing 20 to 30 percent butterfat. The microscopic mold count was found to be inaccurate in detecting moldy cream except in cream with a very high mold content. Flavor score,, acidity and microscopic mold counts were found not to be as good guides to the mold content of mixed or blended cream as they were to commercial Churning cream as received from the producer.

One hundred seventeen butter samples made from the 117 cream samples examined were studied. She relationship of the mold mycelia grade of this butter to the flavor score, age, acidity, butterfat content and microscopic mold count of the cream was very similar to the relationship of these factors to the visual mold grade of the cream. The visual mold grade was shown to be the most reliable method of detecting cream that will yield butter high in mold content. Cream grading fair, doubtful or excessive invariably yields butter with a high mold mycelia content and thus should be excluded from butter manufacturing, The following method of selecting cream is suggested. Examine it organoleptically. If it will make an 89 score butter or less then determine the acidity.

If it contains more than 0.51 percent acid, then determine the visual mold grade. If it grades fair to excessive, do not use it in butter manufacturing. As further precautions, advocate that the producer deliver the cream more frequently and increase the butterfat content.

Analysis of 83 butter samples sent in by the State Dairy Division inspectors and 205 monthly educational scoring butter samples showed that butter scoring 90 to 93 does not usually, but may occasionally, contain considerable numbers of mold mycelia.

MOLD MYCELIA IN CREAM AND BUTTER WITH REFERENCE TO

The Relationship of the Flavor Score, Age, Acidity and Butterfat Content of Cream to the Mold Mycelia Content and the Mold Mycelia Content of the Butter Made From it, and A Correlation of the Quality of Cream Churned and the Grade of Butter Made in Montana

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ABSTRACT

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INTRODUCTION

In the spontaneous souring of cream the acid-producing type of bacteria usually predominates until a pH of 4.8 to 5.0, or an acidity of approximately 0.8 to 0.9 percent, calculated as lactic acid, is produced. During this fermentation other types of bacteria are usually suppressed.

The thickened cream thus formed becomes a favorable medium for the growth of Oaspora lactis, the common milk mold. Mold growth converts the acid to carbon dioxide and water, thus making the product more alkaline. Putrefactive bacteria, which have remained inactive in the sour cream, now find conditions favorable for growth. The decomposition of the cream by this group of bacteria, together with the mold, results in a putrid flavor characteristic of old, sour and decomposed cream.

Due to distance of cream producers in Montana from creameries, cream is often held on the farm for several days before it is delivered to the butter manufacturing plant. During the summer months, the atmospheric temperature in parts of the state is sufficiently high to favor the undesirable fermentations. Thus farmers may offer for sale cream that is so decomposed that it would yield butter with a high mold mycelia count. Butter showing excessive mold mycelia is subject to seizure by the government.

This study was undertaken to determine the relationship of flavor score, age, acidity and butterfat content of cream to its mold content, and to the mold mycelia count of the resulting butter. This information,

when used as a guide, would be of material aid to creamery operators in selecting cream so that the resulting butter would be graded as satisfactory under the government standards for mold mycelia in butter.

LITERATURE REVIEW

According to Macy (8), mold was reported in butter by Trabut in Algiers as early as 1886. North and Reddish (10), in studying high grade experimental butter, found an apparent correlation between the presence of Oaspora lactis in appreciable numbers (500,000 or over per ml.) in butter serum and the use of old, stale cream in its manufacture. This was the first suggestion that the presence of mold in butter might indicate the use of decomposed and unfit cream in its manufacture.

Wildman (17) described a method whereby the relative abundance of mold mycelia in butter and cream might be estimated, while Clarke and others (4), using the Wildman method, found no more than a trace of mold mycelia in butter made from cream of good quality, but increasing amounts in butter made from decomposed cream.

Vandaveer and Wildman (16), in studying the significance of mold mycelia in butter churned from cream of known history, concluded that flavors characteristic of decomposed cream were found to occur when cream was exposed to adverse conditions of time, temperature and sanitation and that the presence and growth of mold in cream was associated with the development of decomposed flavor. They also concluded that there was a correlation between mold mycelia content and the acidity of the cream churned and that a high mold mycelia count in butter showed conclusively

that decomposed and unfit cream was used in its manufacture.

Adams and Parfitt (1) concluded from a similar study that mold mycelia in butter were not a direct index to the organoleptic quality of the cream from which the butter was made. They state, however, that mold mycelia fragments in butter are an index to the age of cream, to the temperature of storage, and to the oxygen content of the cream from which the butter was made.

Clarke (3) points out that factory graders were able to distinguish unfit cream by taste and smell. Mold mycelia counts were highest, with but few exceptions, in cream graded second grade (No. 2) by cream graders.

Loy (7) asserts that in normal or standard manufacturing processes, there is no way of reducing mold mycelia counts, but that inefficient methods may increase the mold mycelia counts.

Elliker (5) states that, among other factors, a high fat content in the cream seems to result in less mold growth.

Parsons (11) describes certain modifications of the Wildman method of estimating mold mycelia in cream. These modifications were designed to provide more complete clumping of the mold which made for easier filtering. This modified method is known as the "Wildman M.B.B. Test", or visual mold test.

Morrison, Nelson and Martin (9), after studying the effect of holding cream in the buying station upon the mold content, and certain other quality factors, assert that the visual mold test does not reflect the changes which occur during holding in the cream station, but unquestion-

ably is of considerable value in grading cream as it comes from the farm.

Wildman (18) describes the method of estimating the mold mycelia in butter as used by the Food and Drug Administration. This method is known as the "Wildman Mold Mycelia Count".

Smith (19), as reported by Wildman, suggests the use of one or two drops of crystal violet to the butter-gum mixture used in the Wildman procedure to help discern the mold filaments, and Shadwick (13) suggests the use of light filters as an aid in discerning the mold filaments.

PURPOSES OF THE STUDY

The purposes of the study were to find the relationship of flavor score, age, acidity and butterfat content of cream to its mold mycelia content, and to correlate the flavor score, age, acidity, butterfat content and mold mycelia content of cream with the commercial grade and mold mycelia content of the butter made from it. Also to determine, as near as possible, from the above data, the quality of cream that should be excluded if the resulting butter is to be graded as satisfactory under the government standards for mold mycelia in the butter. In addition to devise a practical method of selecting cream to make butter that would grade satisfactory by the government mold standards, and to determine in a general way whether or not the grade of butter made by Montana plants is comparable to the quality of cream used in its manufacture.

PROCEDURE

Cream samples were secured from two creameries and two cream stations

in the vicinity of Bozeman during the months of September, October, November, and December, 1941. The majority of the samples were taken from door deliveries. Samples of sweet cream, for comparison and blending purposes, were skimmed from milk produced by the Montana State College dairy herd.

Some of the samples were stored at room temperature for a time to facilitate decomposition and mold growth, others were prepared by blending together different grades of cream, while others were used as they were when collected. The ages of this latter group were recorded when such information was available.

The original cream was scored by two experienced judges in accordance with the grade of butter that could be made from it under good factory conditions. The flavors allowed by the U.S.D.A. Marketing Service (15) for the various grades of butter were allowed in the cream. Half points were given in scoring when it was felt that the samples were between two scores. In compiling the data, however, those samples with half points were reduced to the lower score to conform with the policy of the U.S.D.A. Marketing Service of scoring only in full points.

After scoring, the mold mycelia content of the cream was determined by the Wildman visual mold test (11). Nine ml. of cream were mixed with 17.6 ml. of a methylene blue borax solution, agitated at 82.5°C. (180°F.) and filtered through a special cloth disc. The grade was determined by comparing the amount of agglutinated stained mold mycelia caught on a filter with a mold standard prepared by the American Butter Institute. Four grades are given in this standard, namely, "Good", "Fair", "Doubt-

ful", and "Excessive". However, preliminary comparisons of the visual mold test to the mold mycelia count on the resulting butter indicated that an additional grade between good and fair would be desirable in order to obtain better correlations in the higher grades of butter. This grade was established and called "Good minus".

The percentage of fat was determined by the Babcock method to determine whether the relative richness of the cream might be a factor in mold growth.

To determine whether a relationship exists between the visual mold test and the mold actually present, the number of mold per ml. of cream was determined by the direct microscopic count (12). One one-hundredth of a ml. of cream was spread over four square cm. on a clean microscopic slide. The fat was extracted with xylene, the preparation fixed with 95 percent alcohol, stained in alkaline methylene blue, dried and bleached in alcohol.

The microscopic slides were examined under the oil immersion objective of a microscope having a field diameter of 0.146 mm. The numbers of mold were estimated by counting 50 fields and calculating the average number per field. The average number of mold per field multiplied by four, which was the number of square centimeters in the preparation, and then by 600,000, the microscope factor, gave the estimated number of mold per ml.

The acidity of the cream was determined by the method recommended by Sommer (14). Nine grams of cream plus nine ml. of distilled water were titrated with N/10 NaOH using three to five drops of a one percent alcoholic solution of phenolphthalein as the indicator. The results were cal-

culated as lactic acid.

The amount of N/1 NaOH necessary to reduce the acidity of the cream to 0.20 percent acid was calculated and added. Precautions were taken to insure that the cream and alkaline solution were not above room temperature when mixed (6). The cream was then pasteurized in half gallon glass Mason jars by heating to 65.5°C. (150°F.), holding for 30 minutes and then cooling to 7°C. (45°F.). The cream was held over night at 2° to 4.5°C. (36° to 40°F.) and churned the next day in the same jars with a small experimental churn. After the butter granules were about the size of a large pea the buttermilk was drained and the butter washed twice with tap water at 5.5°C. (42°F.). Sufficient salt was added so that the resulting butter contained approximately two percent salt. After working the butter was held at 2° to 4.5°C. (36° to 40°F.), usually over night, or until it could be scored and the mold mycelia count determined.

In order to remove the possibility of surface contamination at least 1/8 inch of the surface of the butter was scraped off and discarded, after which a one-gram sample was taken from the exposed surface and examined for mold mycelia count by the Wildman method (18). Two drops of crystal violet and seven ml. of hot gum tragacanth solution were added to the butter and the mixture stirred until the fat globules were approximately 0.1 to 0.2 mm. in diameter. A portion of the mixture was mounted on a Howard mold counting slide and examined under the low power of a microscope having a standardized field, 1.5 square mm. in area. The percentage of mold mycelia was determined by examining 50 fields for the pres-

ence of mold mycelia and recording the results as positive fields. The number of positive fields were multiplied by two to give the percentage.

In accordance with the method of grouping mold mycelia counts followed by the American Butter Institute (2), the following grades were assigned to the butter; satisfactory, if it contained 30 percent or less mold mycelia; fair, if it contained 31 to 59 percent; unsatisfactory, from 60 to 80 percent; and very unsatisfactory, from 81 to 100 percent.

In an attempt to determine what effect various percentages of a poor quality cream, high in mold mycelia content, added to a good quality cream, would have on the mold content of the mixed cream and the resulting butter, a series of mixed or blended samples were prepared in the following manner. Cream was held at room temperature until it decomposed and showed a grade of doubtful to the visual mold test. Three, five, ten, 15, 20 and 25 percent, respectively, of this decomposed cream was mixed with a very good quality sweet cream. These samples were then divided into from four to seven portions and each portion treated as previously outlined.

Through the cooperation of the State Dairy Division inspectors, butter samples were picked up throughout the state and sent to the laboratory for scoring and mold mycelia counts. Data concerning whether or not a cream grading program was being used by the plants manufacturing this butter were recorded and sent along with the butter samples.

The mold mycelia counts and flavor scores of each sample of butter sent to the Dairy Industry Department by the various manufacturing plants in the state for the monthly educational scoring were recorded. The

acidity of the cream used in the manufacture of the butter was obtained from churn reports filled in by the operators and sent along with the butter sample.

Questionnaires designed to furnish specific data concerning (1) the age and acidity by seasons of the cream when received, (2) the grading program followed, (3) the holding time and temperature before pasteurizing, (4) the acidity of the churnings before neutralizing, and (5) general information concerning the manufacturing methods, sanitary condition of equipment and building and can washing methods were filled out and sent in by the plant operators who had expressed a desire to cooperate in this mold mycelia study.

RESULTS

A total of 117 cream samples were studied, 63 of which were commercial churning cream obtained from the creameries and cream stations in the vicinity of Bozeman. Eighteen of the 117 samples were indiscriminate mixtures of decomposed cream and fresh sweet cream. These samples were used as a preliminary step to the preparation of 36 blended samples consisting of from three to 25 percent decomposed cream mixed with 97 to 75 percent high-grade, fresh, sweet cream.

The entire 117 cream samples were studied organoleptically, by the visual mold test, the direct microscopic mold count, and the acidity test. The butterfat content was determined for 62 and the age was recorded for 40 of the 63 commercial samples used.

Early in the study it was evident that cream samples which scored 90 to 93 and had acidities of 0.10 to 0.40 percent, and were less than

six days old, contained no evidence of any appreciable amount of mold development. Thus only a few of these samples were studied for comparison purposes. Most emphasis was placed on those cream samples that did show evidence of considerable mold development.

The organoleptic flavor scores of the 63 commercial cream samples were compared with the visual mold grades of the same samples. The results are presented in Table I.

The flavor scores of the samples ranged from 93 to 87; the majority of the samples (88.8 percent) scored 89 or less. The visual mold grades ranged from good to doubtful; 45 (71.4 percent) of the samples graded good, five good minus, 12 (19.0 percent) fair and one doubtful.

Two cream samples scored 93, one 92, one 91 and three 90, all of which graded as good. Seventeen cream samples scored 89, of which 13 (76.4 percent) graded good, two good minus, and two fair. Twenty-seven cream samples scored 88, of which 17 (62.9 percent) graded good, two good minus and eight (29.6 percent) fair. Of the 12 cream samples which scored 87, eight (66.6 percent) graded good, one good minus, two fair and one doubtful.

The ages and visual mold grade of 40 of the 63 commercial cream samples were compared and the results presented in Table II. The ages of the cream varied from three to eight days; the majority of the samples (60.0 percent) were seven days old or older. The visual mold grade varied from good to fair; 33 (82.5 percent) of the 40 samples graded good, two good minus and five fair.

Four cream samples were three days old, and six were four days old,

TABLE I. RELATIONSHIP OF FLAVOR SCORE OF CREAM TO THE VISUAL MOLD GRADE AND THE MOLD MYCELIA GRADE OF THE RESULTING BUTTER

Flavor Score	No. of Samples	Visual Mold Grade of Cream					Mold Mycelia Grade of Butter			
		Good	Good Minus	Fair	Doubtful	Excessive	Satisfactory 0 to 30 percent	Fair 31 to 59 percent	Unsatisfactory 60 to 80 percent	Very unsatisfactory 81 to 100 percent
93	2	2	0	0	0	0	2	0	0	0
92	1	1	0	0	0	0	1	0	0	0
91	1	1	0	0	0	0	1	0	0	0
90	3	3	0	0	0	0	3	0	0	0
89	17	13	2	2	0	0	12	3	1	1
88	27	17	2	8	0	0	17	3	2	5
87	12	8	1	2	1	0	9	1	0	2
Total	63	45	5	12	1	0	45	7	3	8

TABLE II. RELATIONSHIP OF AGE OF CREAM TO THE VISUAL MOLD GRADE AND THE MOLD MYCELIA GRADE OF THE RESULTING BUTTER

Age in Days	No. of Samples	Visual Mold Grade of Cream					Mold Mycelia Grade of Butter			
		Good	Good Minus	Fair	Doubtful	Excessive	Satisfactory 0 to 30 percent	Fair 31 to 59 percent	Unsatisfactory 60 to 80 percent	Very unsatisfactory 81 to 100 percent
3	4	4	0	0	0	0	4	0	0	0
4	6	6	0	0	0	0	6	0	0	0
5	4	3	1	0	0	0	3	1	0	0
6	2	1	0	1	0	0	1	0	0	1
7	12	10	1	1	0	0	10	1	1	0
8	12	9	0	3	0	0	9	0	0	3
Total	40	33	2	5	0	0	33	2	1	4

all of which graded good. Four cream samples were five days old, of which three graded good and one good minus. Two cream samples were six days old, of which one graded good and one fair. Twelve cream samples were seven days old, of which ten (83.3 percent) graded good, one good minus and one fair. Twelve cream samples were eight days old, of which nine (75.0 percent) graded good and three fair.

The visual mold grades of the same 63 commercial cream samples compared in Table I were compared with the acidities of the same samples and the results are in Table III.

The acidities of the cream samples varied from 0.12 percent to 0.87 percent; the majority of the samples ranged from 0.31 to 0.80 percent. The visual mold grade varied from good to doubtful; 45 (71.4 percent) of the 63 samples graded good, five good minus, 12 (19.0 percent) fair and one doubtful.

Four cream samples had acidities ranging from 0.10 to 0.20 percent and two cream samples had acidities ranging from 0.21 to 0.30 percent, all of which graded good. Seven cream samples had acidities ranging from 0.31 to 0.40 percent, of which six (85.7 percent) graded good and one fair. Eleven cream samples had acidities ranging from 0.41 to 0.50 percent, of which ten (90.0 percent) graded good and one good minus. Twenty-three samples of cream had acidities ranging from 0.51 to 0.60 percent, of which 17 (73.9 percent) graded good, four good minus and two fair. Eight cream samples had acidities ranging from 0.61 to 0.70 percent, of which three graded good and five fair. Seven cream samples had acidities