THESIS

A STUDY OF THE TYPES OF BACTERIA SURVIVING A LONG-TERM PERIOD OF STORAGE

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INTRODUCTION

Storage and Storage Conditions:

The chief reason for storing butter in cold storage is to ensure a supply of good butter throughout the year. Most butter goes into storage in May, June and July, the season of the year which is best suited to cream production because of the freshening of the majority of the cows at this time, also the succulent condition of the pasture. Under normal conditions the bulk of butter in storage is taken out within nine months. Only in exceptional cases is butter held in storage over one year, and when this is done it is usually accompanied by a great sacrifice in quality and price.

In order to justify storage and to have the storing of butter fulfill the purpose for which it is intended, the butter must be protected against agents and conditions which cause it to deteriorate in quality. By deterioration in butter is meant any change in flavour that will tend to make it less valuable. The chief conditions injurious to the quality of butter in storage are air, light, heat and humidity.

Air and Light: It is a well known fact that prolonged exposure to air damages the flavour of butter and the changes brought about are believed to be due chiefly to oxidation. This oxidizing action is intensified in the presence of light. This fact can easily be proven by placing a sample of butter in direct sunlight and it will be found to rapidly acquire an oxidized or tallowy flavour and appearance. The tendency in storage is usually toward a tallowy character which begins

on the surface and progresses toward the centre of the package.

Heat intensifies every type of butter deterioration. It hastens oxidation and the action of bacteria and enzymes; it also favours mold development, and chemical activity. Butter that is intended for prolonged storage should be stored at a temperature of zero degrees Fahr, or lower. At higher temperatures its keeping quality is jeopardized and the poorer the quality the more rapid will be the deterioration with age. It not only assists in flavour deterioration, but the mold spots must be removed which means a lot of labour and loss of butter in the scraps. In commercial cold storages the air is dry, but when butter is removed from such a storage moisture frequently condenses on the surface. When such butter is held for a few days at temperature above freezing, mold may develop and cause serious loss. Thus storage at temperatures above freezing should always be in a dry atmosphere if possible.

Storage does not improve the quality of butter, rather the flavour tends to deteriorate with age. The deterioration is usually retarded and only very gradual at the low temperatures of commercial cold storage.

The exact changes responsible for the development of specific flavour defects have not been determined in the great majority of cases. It is assumed with reasonable certainty, however, that rancidity and tallowiness are due to the cleavage of the butterfat, rancidity to hydrolysis through bacterial or enzymatic action, or both, and tallowiness to oxidation through chemical means. Butterfat, on account of its relatively high percentage of the lower fatty acids, especially butyric, readily produces a strong odour characteristic of

these acids upon slight hydrolysis. Most other fats contain relatively small amounts of the lower fatty acids and a larger amount of the higher acids, such as palmitic and stearic which are less susceptible to hydrolysis. Fishiness in butter was first thought to be due to bacterial decomposition but now is supposed to be the result of the hydrolysis of lecethin, forming trimethylamine.

The work done for this thesis was to find out what types of bacteria had survived for six years, rather than to determine any specific defect that they might have caused. The sample of butter worked with had been kept in commercial cold storage for a period of six years and it was found that it had developed a tallowy appearance ~~(and flavor)~~ during this time.

Apparatus and Methods

Using a sterile trier a small plug was drawn from the sample of butter and placed in a sterile bottle which was held in a water bath at 45°C. until the butter was melted. After thoroughly mixing the sample three 1 c.c. portions and three .1 c.c. portions were plated directly on sterile petrie dishes using the following medium:

.5% beef extract

.5% yeast extract

.5% Peptone

.5% Glucose

1.5% Agar

2 c.c. skim-milk per 100 c.c. of medium

Distilled water.

All materials were warmed to 45°C. previous to use. One of the 1 c.c. portion plates and one of the .1 c.c. portion plates was incubated at 32° for a period of one week, a second 1 c.c. portion plate and .1 c.c. portion plate at room temperature for one week and the third set in the ice box for a period of four weeks.

At the termination of the incubation period well isolated colonies were picked off each of the plates onto agar slants of the same medium as used for plating. Twenty-eight colonies were picked from the plates incubated at 32°C., 22 colonies from the room temperature plates and 8 from the plates incubated in the ice box.

The cultures were next arranged in series. The 32° organisms were called series A and the cultures numbered from one to twenty-eight, the room temperature organisms series B and

numbered from one to twenty-two, the ice box organisms series C and numbered from one to eight. These slants were then incubated at their respective temperatures for the same period of time as the plates. A slide was then made of each culture, staining with methyline blue for thirty seconds and examining under the microscope.

Their action on gelatine was determined by preparing gelatine stabs and incubating for three weeks. From the agar slant cultures the gram reaction of the organisms was determined, using Hucker's modification of the gram staining technique. Also, from the agar slants their action on the following media was determined: litmus milk, nutrient broth, nitrate broth.

During the time the work was being done the cultures were transferred every month to ensure vigorous growth. Precautions were taken to be certain that all test media were inoculated with vigorously growing organisms.

Experimental Results.

The first grouping of these organisms was made on the basis of the results of the microscopic examination. They were all found to be rods varying in size. In series A, 26 were spore formers with 2 non-spore formers, and in series B 18 were spore formers with 4 non-spore formers. Series C were all non spore formers. In size series A and B fell into three distinct groups, viz.; short thick rods, medium-sized rods and long narrow rods. Series C were all medium-sized rods.

TABLE I

SERIES A

Description	Colony
Short thick rods	1:2:22:23:25:28
Medium sized rods	3:4:7:8:9:10:11:13:14:15:16:17: 19:20:20:24:26:27
Long narrow rods	5:6:12:18:21

SERIES B

Description	Colony
Short thick rods	3:4:7:21:22
Medium sized rods	1:2:5:8:10:11:13:14:15:16:17:18:20
Long narrow rods	6:9:12:19

SERIES C

Description	Colony
Medium sized rods	1:2:3:4:5:6:7:8

The remainder of the groupings are based upon Bergey's classification. The activity of these organixms on the different media stated above were used in the determination.

In series A, of the short thick rods two cultures were found to resemble Bacillus amarus. One showed no activity on any of the media and was probably dead. The medium sized rods were found to fall into four groups. Eight cultures resembled Bacillus sphaericus, six resembled Bacillus agri and one Bacillus pabuli. The remaining three resembled Bacillus coagulans except for their activity on nutrient broth. Three of the long narrow rod cultures were found to resemble Bacillus

sphaericus and were similar to those in the medium sized rods group which resembled Bacillus sphaericus except they were somewhat longer.

In series B, three of the short thick rod cultures were similar to the group in series A that resembled Bacillus amarus. The remaining two cultures were similar to Bergey's description of Bacillus hesii. The medium sized rods in this series fell into five groups, four of them corresponding to the four groups found for the medium sized rods in series A. Four cultures were found to resemble Bacillus agri, two to resemble Bacillus sphaericus, two to resemble Bacillus pabuli and two to resemble Bacillus coagulans, except for their activity on nutrient broth. The remaining three cultures were found to answer to Bergey's description of Bacillus albus. Of the long narrow rods in this group, three were non spore formers and their activity was similar to that given by Bergey for Achromobacter healii. These cultures were similar to those in series A listed under Achromobacter healii except that they were much longer. The other cultures in this group answered to Bergey's description of Bacillus brevis except for the gram stain reaction,where it was found to be negative.

In series C,the ice box organisms, three cultures were found to resemble Bergey's description of Bacillus freudenreichii, and three Bacillus lactis. The reactions of the remaining two corresponded to Bergey's description of Bacillus albolactis except for their action on nutrient broth, where no pellicle was formed as in Bergey's description.

The physiological properties of the different groups of organisms studied are listed in tabular form in the appendix.

Discussion

In this study a number of colonies of bacteria were isolated from storage butter and traced down to see what types would survive this period of storage. Fifty-eight colonies were isolated and found to fall into thirteen different groups according to Bergey's classification.

These groupings cannot be taken as final as their activity on only a limited number of media was determined and,if more were used,the classification might have been changed somewhat. Some were found that did not definitely resemble a species listed by Bergey but differed in one respect. These were taken as varieties of the species they most closely resembled. There was considerable variation in number of cultures falling into each of the thirteen groups. The largest group, Bacillus sphaericus, contained thirteen cultures, while the two groups, Bacillus robus and Bacillus brevis, contained but one culture each. Considerable correlation as to groups was found to exist between series A and series B.

Lockhead and Jones found that micrococci and non-sporulating rod shaped organisms frequently survived in frozen vegetables. The absence of micrococci from this butter and the relative scarcity of non-spore forming organisms may be due to the fact that the butter was made from pasteurized cream.

Conclusion

An examination of butter which had been in cold storage for six years showed that at least thirteen distinct types of organisms had survived. Eleven of the thirteen cultures isolated were spore formers and two were non spore formers. All of the cultures isolated were rod shaped and none were chromogenic. Time did not permit a complete classification of the organisms found.

It cannot definitely be stated that all of these types would have a detrimental effect upon the butter, and no deterioration was determined due to their presence. Although storage of butter under favourable conditions may not reduce the numbers and types of bacteria present, it certainly will have a retarding effect upon their reaction, particularly those connected with butter deterioration.

TABLE 2

Some physiological properties of cultures found resembling Achromobacter healii

Incubation temperature.....32°

Action on gelatine agar.....stratigorm to crateriform liquifaction

Action on litmus milk.....milk completely reduced, coagulated, peptonized and turned acid.

Action on nutrient broth.....pellicle and some sediment formed.

Reaction to gram stain.....negative

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....Series A - two

Series B - three

TABLE 3

Some physiological properties of cultures found resembling Bacillus amarus

Incubation temperature.....Series A 32°

Series B - room temperature

Action on gelatine agar.....no liquifaction

Action on litmus milk.....slight reduction. No coagulation

Action on nutrient broth.....broth turbid with some sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....Series A - three

Series B - three

TABLE 4

Some physiological properties of cultures found resembling Bacillus sphaericus

Incubation temperature.....Series A - 32°

Series B - room temperature

Action on gelatine agar .....no liquifaction

Action on litmus milk.....unchanged

Action on nutrient broth.....broth turbid with slight sediment

Reaction to gram stain .....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this

group.....Series A - eleven

Series B - two

TABLE 5

Some physiological properties of cultures found resembling Bacillus coagulans

Incubation temperature.....Series A - 32°

Series B - room temperature

Action on gelatine agar.....no liquifaction

Action on litmus milk.....milk completely reduced, coagulated and turned acid.

Action on nutrient broth.....broth turbid with slight sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this

group.....Series A - three; Series B - two

TABLE 6

Some physiological properties of cultures found resembling Baccilus agri

Incubation temperature.....Series A - 32°

Series B - room temperature

Action on gelatine agar.....liquifaction infundibuliform

Action on litmus milk.....milk completely reduced, coagulated, peptonized, acid.

Action on nutrient broth.....turbid with slight sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....Series A - six; Series B - four

TABLE 7

Some physiological properties of cultures found resembling Bacillus pabuli

Incubation temperature.....Series A - 32°

Series B - room temperature

Action on gelatine agar.....Liquifaction crateriform

Action on litmus milk.....unchanged

Action on nutrient broth.....broth turbid with slight sediment

Reaction to gram stain.....positive

Reduction on nitrates.....nitrates not reduced

Number of cultures in this group.....Series A - one; Series B - two

TABLE 8

Some physiological properties of cultures found resembling Baccilus robur

Incubation temperature.....32°

Action on gelatine agar.....liquifaction stratiform

Action on litmus milk.....unchanged

Action on nutrient broth.....turbid with slight sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....one

TABLE 9

Some physiological properties of cultures found resembling Bacillus hesii

Incubation temperature.....room temperature

Action on gelatin agar.....liquifaction crateriform

Action on litmus milk.....completely reduced; coagulated, turned acid.

Action on nutrient broth.....turbid with slight sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....two

TABLE 10

Some physiological properties of cultures found resembling Bacillus albus

Incubation temperature.....Room temperature

Action on gelatine agar.....liquifaction crateriform

Action on litmus milk.....milk reduced; no coagulation acid

Action on nutrient broth.....turbid with pellicle and slight sediment

Reaction to gram stain.....negative

Reduction of nitrates.....nitrates reduced to nitrites

Number of cultures in this group.....three

TABLE 11

Some physiological properties of cultures found resembling Bacillus brevis

Incubation temperature.....Room temperature

Action on gelatine agar.....liquifaction crateriform

Action on litmus milk.....complete reduction, no coagulation acid

Action on nutrient broth.....turbid, pellicle with sediment

Reaction to gram stain.....negative

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....one

TABLE 12

Some physiological properties of cultures found resembling Bacillus freudenreichii

Incubation temperature.....Ice box

Action on gelatine agar.....slight liquifaction

Action on litmus milk.....complete reduction, no coagulation, turned alkaline

Action onnutrient broth.....turbid

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....three

TABLE 13

Some physiological properties of cultures found resembling Bacillus lactis

Incubation temperature.....Ice box

Action on gelatine agar.....liquifaction crateriform

Action on litmus milk.....complete reduction; no coagulation, turned acid

Action on nutrient broth.....turbid with some sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates not reduced

Number of cultures in this group.....three

TABLE 14

Some physiological properties of cultures found resembling Bacillus albolactis

Incubation temperature.....ice box

Action on gelatin agar.....liquifaction crateriform

Action on litmus milk.....complete reduction; coagulation, turned acid

Action on nutrient broth.....turbid with slight sediment

Reaction to gram stain.....positive

Reduction of nitrates.....nitrates reduced to nitrites

Number of cultures in this group.....two

REFERENCES

1. Stark, C. N., Scheib., - "A study of fat splitting and casein digesting bacteria isolated from butter". Journal of Dairy Science, Vol. XIX, No. 3 (1936).

2. Associates of Rogers - "Fundamentals of Dairy Science", New York (1929)

3. Lecture Notes - Dairy 12 (1937-38)

4. Hunziker, O. F. - "The Butter Industry", La Grange, Illinois (1927)

5. Bergey, D. H. - "Bergey's Manual of Determinative Bacteriology), Baltimore (1934)

6. A. G. Lockhead and A. H. Jones - "Studies of Numbers and Types of Microorganisms in Frozen Vegetables and Fruits", Food Research, 1: 1: 29-39 (1936)