EFFECT OF GROWTH OF PSEUDOMONAS PUTREFACIENS ON
DIACETYL AND FLAVOR OF BUTTER¹

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The cheesy and putrid defects² of butter are among the most serious manufacturing problems confronting the butter industry of Indiana. Numerous reports emphasize the significance of this problem elsewhere. Studies in this laboratory indicate that in the surrounding region the putrid defect and perhaps in most cases the pronounced cheesy defect are due to species of Pseudomonas putrefaciens. Certain of the less pronounced cheesy defects, however, appear to be due to other related water bacteria capable of some lipolytic and proteolytic action on milk and butter constituents.

During preliminary investigations on the nature and cause of these defects it was repeatedly noted in butter containing neither salt nor starter that the first apparent change produced by Ps. putrefaciens was loss of the typical full, clean odor. The butter at this stage appeared to have completely lost its normal odor and instead was characterized by a distinctly flat and in some cases by a slightly "oxidized-like" odor.

When butter was made with added diacetyl, the occurrence of cheesy or putrid defects was invariably preceded by almost complete destruction of all diacetyl aroma. The obvious conclusion was that Ps. putrefaciens and possibly related organisms destroyed the compounds essential to high aroma of butter. The ability of Ps. putrefaciens to destroy added diacetyl in milk and butter was therefore studied.

HISTORICAL

The subject of cheesy, also sometimes referred to as putrid, rabbito, surface taint, fetid, or limburger defect in butter has been reviewed (2, 3, 5, 6, 7, 14, 23). These papers, with many others, indicate the widespread occurrence of this general type of defect.

Considering the tremendous volume of research on production of aroma compounds in dairy products and other foods, comparatively few reports are available on the effect of microorganisms on diacetyl. Neuberg and Nord (13) and Nagelschmidt (12) demonstrated the ability of yeasts to reduce diacetyl to 2,3-butylene glycol. Michaelian and co-workers (10) noted a

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2 The defects referred to in this paper have, in every case, been produced by bacteria growing in the butter after its manufacture and should not be confused with the cheesy defect of cream which may be carried into butter.

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rapid decrease of diacetyl in a well-ripened butter culture when neutralized to a low acidity and held at a temperature favorable for development of citric acid fermenting streptococci. Subsequent studies (4) showed that *Streptococcus citrovorus* and *Streptococcus paracitrovorus* reduce diacetyl to acetylmethylcarbinol and 2,3-butylene glycol. Wiley *et al.* (21) reported that beta cocci produced diacetyl rapidly and then actively destroyed it. Virtanen and Kontio (20) investigated the action on diacetyl of various microorganisms isolated from butter. Diacetyl and acetylmethylcarbinol were added to sterile milk and respective lots inoculated and incubated at 19–21° C. for periods up to 160 hours. *B. punctatum* destroyed about 90 per cent of both compounds. *B. vulgatus* destroyed 30 to 50 per cent of the diacetyl but only 9 per cent of the carbinol. A mixture of yeasts destroyed 30 to 40 per cent of the diacetyl and as much as 30 per cent of the acetylmethylcarbinol. Certain strains of the colon-aerogenes group, green fluorescent bacteria and a number of species of aerobic spore formers are able to destroy acetylmethylcarbinol (22).

Slatter and Hammer (18) and Prill and Hammer (16) observed both increases and decreases in the diacetyl and acetylmethylcarbinol content of unsalted butters made from sweet cream with a butter culture. Salt appeared to prevent significant increase or decrease of diacetyl and acetylmethylcarbinol. However, in one lot of butter containing only 0.75 per cent salt there occurred a relatively rapid disappearance of these compounds. The increases and decreases were attributed to aroma bacteria from the starter. Toth (19) reported that in butter prepared from sour cream, diacetyl increased for some days during storage and later decreased. In butter from sweet cream a constant decrease took place during storage. According to Mohr *et al.* (11) the diacetyl content of butter during storage may remain constant or may decrease. In one trial they noted a pronounced decrease of diacetyl in unsalted but not in salted butter stored at 10° C. for 10 days. The diacetyl content of salted and unsalted butters decreased at about the same rate when the butters were stored at –20° C. Bungard (1) reported increases followed by decreases of diacetyl in butter.

Some investigators (8, 9, 17, 23) have indicated that diacetyl has an inhibitory effect on bacteria. The inhibiting effect of diacetyl on growth of various organisms in sterile skim milk has been observed by us. However, the inhibiting concentrations were higher than would occur in average butter.

**EXPERIMENTAL**

The apparent ability of putrid butter organisms to destroy diacetyl was noted both in commercial salted butter and in unsalted laboratory samples. Since in laboratory churnings the usual percentage of salt appeared to inhibit development of *Ps. putrefaciens*, no salt was added to the experimental butter. Under commercial conditions the salt sometimes provides
less assurance of complete inhibition of this organism than under laboratory
conditions.
Sterilized cream in 2000-ml. quantities was churned at about 7.2° C. (45° F.) in sterile “Dazey” churns. The butter was inoculated with *Ps. putrefaciens* by adding a sufficient quantity of a 24-hour beef infusion broth culture to provide about 1,000,000 cells per ml. of wash water. After one
washing with either sterile or contaminated water, the butter was worked
in the churns with large, sterile, metal spoons. A volume of water equal
to the original volume of cream was used. This appeared to adequately
wash the butter granules and the large number of organisms in the water
provided sufficient inoculation of the butter.
Diacetyl in the form of starter distillate was added to the butter after
washing and partial working. Five per cent of starter was added a short
time before churning to some lots of cream. The starters used did not
appear to have a high aroma, but the butters prepared from them always
had a desirable aroma. After thorough working, the butter was placed in

![Figure 1](image.png)

**Fig. 1.** Effect of growth of *Pseudomonas putrefaciens* on diacetyl added in the form
of starter distillate to butter.

approximately 100-gram amounts in sterile 4-ounce sample jars and stored
at 21.1° C. (70° F.). Samples were examined for odor and flavor, daily,
and for diacetyl content on the day of preparation and again after 4 and 7
days of storage. The method for determining diacetyl was essentially that
of Prill and Hammer (15).

Figures 1 and 2 present a summary of the results of several experiments.
The analyses substantiated earlier observations on loss of aroma in butter
due to growth of *Ps. putrefaciens*. Within 7 days at 21.1° C. the diacetyl
content was reduced from 0.109 mgm. to 0.034 mgm. per 100 gm. In the
sterile control only a slight drop occurred during the 7-day period.
The diacetyl contents of the starter butters were comparatively low. One
evident reason for this was the fact that the starter culture employed had a low diacetyl content. Also the fact that the starter was added only shortly before churning undoubtedly resulted in the butter carrying over less diacetyl from the starter than would have been true if starter and cream had been in contact for a period of several hours.

The starter butter containing no *Ps. putrefaciens* showed a slight increase in diacetyl. Such increases were noted in other experiments. In some samples containing starter plus added starter distillate, marked decreases occurred. This might be expected in the light of other reported investigations. When *Ps. putrefaciens* was present, the diacetyl in starter butter decreased slightly. The main purpose of adding starter in this study was to determine the effect of *Ps. putrefaciens* on diacetyl in butter when its activity was inhibited by starter bacteria. Cultures of *Streptococcus lactis* added in the same manner as starter cultures also effectively inhibited development of *Ps. putrefaciens*.

The aroma and clean, full flavor of both starter and uninoculated starter distillate samples were maintained throughout the storage period. Samples of inoculated butter containing starter distillate became flat and lost all aroma in 24 to 48 hours and within several hours thereafter became definitely putrid.

![Fig. 2](image_url)

**Fig. 2.** Effect of *Pseudomonas putrefaciens* on diacetyl in butter made with starter.

Results of other experiments showed comparatively rapid destruction of diacetyl at refrigeration temperatures when active development of *Ps. putrefaciens* occurred in the butter. In one such trial the diacetyl content fell from 0.080 mgm. per 100 grams of butter to 0.040 mgm. in 4 days at 7.2°C. and in the corresponding sample at 21.1°C, it fell to 0.025 mgm. in 2 days.

**DISCUSSION**

In general the results indicated relatively rapid destruction of diacetyl in butter under conditions favorable to the growth of *Ps. putrefaciens*. The rapid disappearance at 7.2°C. (45°F.) coincided with observations on commercial samples that developed a cheesy or putrid defect under normal
storage and retail conditions. It is probable that Ps. putrefaciens rapidly destroyed the diacetyl in the aqueous phase. Diacetyl in the fat phase may have been sufficiently protected so that little was affected by the organisms. This explanation might account for the complete lack of aroma following growth while one-third to one-fourth of the original diacetyl still remained intact in the butter.

The diacetyl contents of starter butters, both control and inoculated, were low. Nevertheless the butter had a definite, pleasant aroma. It is possible that the aroma in the starter butter was enhanced or maintained by continual slow production of diacetyl during storage of the butter not only in the control sample but also in that where Ps. putrefaciens was slightly active. In the non-starter butter containing Ps. putrefaciens the opposite condition may have been true, namely, the organisms destroyed all diacetyl that might have been able to contribute to the aroma of the butter.

It has been suggested that diacetyl may be the component that enables butter starters to inhibit development of Ps. putrefaciens in butter (23). The low diacetyl contents in the starter butter, however, suggest that it is the activity of the lactic acid bacteria (probably lactic acid production) rather than presence of diacetyl that inhibits action of Ps. putrefaciens in butter made with starter. The ability of Streptococcus lactis alone to inhibit Ps. putrefaciens in butter further substantiates this conclusion.

One fact that should be borne in mind is that commercial butters present a somewhat different problem than laboratory churned samples. For example neither salt nor starter definitely insures the inhibition of Ps. putrefaciens in commercial butter. Various factors such as methods of working, printing, storage and distribution influence the activity of the microorganisms involved. However, the close correlation between changes occurring in commercial and laboratory samples definitely indicates a similarity in the sequence of changes taking place under the two different conditions.

One of the most troublesome angles in this problem, particularly to the creameryman who has been without the technical advice of a control laboratory, has been the failure to recognize the nature of the defect caused by Ps. putrefaciens and related bacteria. The cheesy and putrid defects sometimes have not been recognized by the butter manufacturer or handler except where they have progressed to an advanced stage. Possibly the term surface taint would be more suggestive for this defect; however, the so-called cheesy or putrid defect is usually not confined to the surface. Many commercial samples received in this laboratory or picked up in retail outlets appear to run through the whole series of changes to the final cheesy or putrid state only after periods considerably longer than they ordinarily would be stored in the household refrigerator. This means that such samples, when in the hands of the creameryman, wholesaler and retailer, and often the final purchaser, show only a flat, disagreeable odor and flavor difficult to identify. Many creamerymen are aware that their butter is off
in flavor but recognize it only as flat. Part of a recent large shipment was returned to one manufacturer with the complaint that it was "flat and metallic." This butter had a pleasing, full flavor with high aroma at the time of shipment from the creamery. The cause of this "flat" flavor was _Ps. putrefaciens_. This fact was indicated by bacteriological examination and also because the "flat" flavor was followed by the development of a typical putrid flavor. This type of flavor defect seems more common in commercial butter than is generally realized. Results to be published later indicate that species other than _Ps. putrefaciens_ and the aroma bacteria may destroy diacetyl in butter.

The "metallic" flavor mentioned above may have been confused with the tallowy flavor reported by Toth (19) and others to accompany reduction of diacetyl in butter. In such occurrences the fat apparently is oxidized. When the masking effect of a strong aroma has been removed by the action of microorganisms or other factors, a tallowy or oxidized flavor might become more apparent. On the other hand our studies and those of others (23) indicate that an oxidation of certain compounds in the butter may possibly be due to _Ps. putrefaciens_. The flavor developed would undoubtedly be masked by the pronounced cheesy or putrid flavor following active growth of the organism.

**SUMMARY**

Observations on both commercial and laboratory samples of butter in which _Ps. putrefaciens_ developed showed that the first apparent stage of decomposition by this organism was a complete loss of typical butter aroma. The butter became flat in odor and flavor. This stage was then followed by development of typical cheesy or putrid flavors.

Chemical analyses indicated that during a 7-day storage period at 21.1°C, the diacetyl content of the sterile control butter samples remained fairly constant. More than half the diacetyl was destroyed under the same conditions in similar lots of butter contaminated with _Ps. putrefaciens_.

The diacetyl content of butter prepared with starter and contaminated with _Ps. putrefaciens_ remained relatively stable over a 7-day storage period at 21.1°C, although some destruction of diacetyl occurred.

Results and observations indicate that the ability of _Ps. putrefaciens_ and other organisms to produce a flat-flavored butter is perhaps more prevalent than is commonly realized.

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