Factors Affecting the Development of Lipase Flavor in Butter

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FACTORS AFFECTING THE DEVELOPMENT OF LIPASE FLAVOR IN BUTTER

BY

LAWRENCE I. BELL

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science, Major in Dairy Science, South Dakota State University

1975

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FACTORS AFFECTING THE DEVELOPMENT OF LIPASE FLAVOR IN BUTTER

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Thesis Adviser

Date

Head, Dairy Science Department

Date
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>25</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>32</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>63</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>66</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>68</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Flow diagram of the treatments used to study the effects of various processing and storage factors on the development of lipase flavor in butter</td>
<td>27</td>
</tr>
<tr>
<td>2. Acid degree value of butter made from fresh and aged (24 h at 4.4 C) cream during storage at 4.4 C</td>
<td>36</td>
</tr>
<tr>
<td>3. Free fatty acid levels of butter made from fresh and aged (24 h at 4.4 C) cream during storage at 4.4 C</td>
<td>41</td>
</tr>
<tr>
<td>4. Flavor scores of butter made fresh (O – A – O –) and aged (O – A – O –) cream (24 h at 4.4 C), heat treated at 72 C (A), 85 C (B), and 93 C (C), during storage at 4.4 C</td>
<td>47</td>
</tr>
<tr>
<td>5. Flavor scores of butter made from fresh (O – A – O –) and aged (O – A – O –) cream (24 h at 4.4 C), heat treated at 72 C (A), 85 C (B), and 93 C (C), during storage at -28.9 C</td>
<td>49</td>
</tr>
<tr>
<td>6. A comparison of flavor scores of butter made from fresh (C, D) and aged (A, B) cream (24 h at 4.4 C) heat treated for 18 sec at 85 C (A, C) and 93 C (B, D) during storage at -28.9 C (O –) and 4.4 C (O –)</td>
<td>53</td>
</tr>
<tr>
<td>7. A comparison of flavor scores and acid degree values of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 85 C for 18 sec) during storage at -28.9 C and 4.4 C</td>
<td>56</td>
</tr>
<tr>
<td>8. A comparison of flavor scores and free fatty acids of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 85 C for 18 sec) during storage at -28.9 C and 4.4 C</td>
<td>59</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acid degree value of fresh and aged (24 h at 4.4 C) cream prepared in three replications at weekly intervals</td>
<td>34</td>
</tr>
<tr>
<td>2. Acid degree value of butter made from fresh and aged (24 h at 4.4 C) cream during storage at -28.9 C</td>
<td>38</td>
</tr>
<tr>
<td>3. Free fatty acid level of butter (µEquiv/g) made from fresh and aged (24 h at 4.4 C) cream during storage at -28.9 C</td>
<td>44</td>
</tr>
<tr>
<td>4. The effect of the storage temperature of butter on the free fatty acid level in butter</td>
<td>45</td>
</tr>
<tr>
<td>5. A comparison of the acid degree value, free fatty acid, and flavor score of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 65 C for 18 sec) during storage at -28.9 C</td>
<td>60</td>
</tr>
<tr>
<td>6. Range of acid degree values and free fatty acid levels in A, B, and C grade butter at three and four months of storage</td>
<td>61</td>
</tr>
<tr>
<td>7. Acid degree values, free fatty acids, and flavor grades of commercial butter samples</td>
<td>62</td>
</tr>
</tbody>
</table>
### APPENDIX TABLE

<table>
<thead>
<tr>
<th>Appendix Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Results of statistical analysis of treatment effects on FFA, ADV, and flavor of butter held in storage</td>
<td>67</td>
</tr>
</tbody>
</table>
ABSTRACT

Current trends in the dairy industry have resulted in an increase in the incidence of lipase flavored butter. Raw cream, fresh and aged 24 h, was divided into 3 fractions and heat treated at temperatures of 72 C, 85 C, and 93 C. Each fraction of cream was churned into butter, with the butter divided and stored at -28.9 C and 4.4 C. Lipase flavor development was measured organoleptically, by acid degree value (ADV), and free fatty acids (FFA) using a colorimetric procedure. Age of the cream had a significant effect (P<.01) on FFA and ADV levels, whereas heat treatment of the cream had a significant effect (P<.01) on flavor only. Storage time had a significant effect (P<.01) on FFA, ADV, and flavor and the temperature of storage had a significant effect (P<.01) on FFA and flavor. Correlation between FFA and ADV was 0.86. There was no correlation between flavor and either FFA or ADV.
INTRODUCTION

Since 1971, United States Department of Agriculture (USDA) butter graders have detected a significant increase in the amount of lipase flavored butter. Lipase flavor is the descriptive term used by butter graders for what has been referred to in earlier publications as hydrolytic rancidity. Longer storage and handling of raw cream contribute to lipase flavor development. Butter with a slight to definite lipase flavor is downgraded by USDA butter graders, and is sold at a greatly reduced price. About a half million pounds of butter is downgraded annually in the United States because of lipase flavor. In the North Central Region, which produces 50% of the butter in the USA, this amounts to a substantial economic loss.

Lipase flavor is most often described as being unclean, bitter, and soapy or rancid if the flavor is quite intense. Consumers will not continue to purchase a lipase flavored butter.

The 1974 per capita consumption of butter in the USA was 2 kg, down 1.1 kg from the 1964 level (60). By 1980 the per capita consumption of butter is projected to be about 1.7 kg (41). From 1960 thru 1973, per capita sales of butter declined by 18.6%. However, the total butter sales decreased by only 1.7% (41). The number of butter processing plants in the USA is decreasing with production per plant increasing (60). The North Central Region (Iowa, Minnesota, North Dakota, South Dakota, Wisconsin) produces about 30% of the nation's milk and over 50% of the butter (41). In the USA, 16.4% of the milk is used in the production of butter (41). Production of a high quality butter is of prime importance to the dairy industry in
the North Central Region. The recent increase in the price of vegetable-based spreads has placed butter in a competitive price position. The manufacture of high quality butter could possibly increase per capita consumption if the price of vegetable oils remains high.

Detecting lipase flavor in butter and identifying the causes have not been accomplished in past research as has been done for raw milk. A slight bitter or lipase flavor is difficult to detect in butter. As a result, experienced butter judges and USDA butter graders may often overlook this defect. A test which can accurately measure the lipase flavor below the flavor threshold is highly desirable. The acid degree value (ADV) test, which is the main chemical test used in industry for detecting lipase flavor, is not as accurate as desired in detecting lipase flavor at low levels. In butter, the ADV test does not seem to directly indicate the intensity of lipase flavor. A colorimetric test, which reportedly is sensitive enough to measure free fatty acids at very low levels in blood and biological fluids (37), could be a possible method for detecting the lipase flavor in butter at very low levels. Butter samples, which are normally analyzed and graded prior to going into storage, may show an increase in lipase flavor when taken out of long term storage. Little is known about the effects of storage of butter and processing of cream on butter quality.

The purpose of this study was to develop a better method for determining the extent of lipolysis in butter and to study the effects of processing of cream and butter on the development of lipase flavor.
Age of the cream, pasteurization temperature, storage temperature, and time of storage were the factors evaluated for their effect on the lipase flavor in butter.
LITERATURE REVIEW

Lipids often account for a major portion of both the desirable and undesirable flavors and aromas of foods (18). The flavor compounds formed from lipids can be soluble in either the water or the lipid phase with pH, temperature, and concentration having an influence on the degree of solubility in each phase. Mackay (40) and Forss (18) have shown that the flavor intensities of various compounds will vary greatly, depending upon whether they are in a lipid or a water phase. Since flavor compounds penetrate the taste receptors of the tongue more easily in a water than in a fat solution, they are detected at a lower level when in a water solution (18). For example, bitter flavor is very difficult to detect in lipids, but is easily detected in a water solution (40). Thus it could be concluded that the bitter or lipase flavor is in the water portion of foods (12).

The volatile compounds in lipids have a major influence on flavor and aroma, especially in dairy products. Over 100 volatile compounds have been identified as natural constituents of butter and milk (51). Those butter volatiles, present in concentrations greater than levels at which they can be detected organoleptically, would be expected to contribute to the flavor of sweet cream butter (51). Diacetyl, butyric and caproic acids, hexanol, acetaldehyde, dimethyl sulfide and delta-decalactone would be included in this group (51). Compounds that must be present in high concentrations to be detected organoleptically such as long-chain fatty acids would contribute little to the flavor of butter (51). The development of certain off-flavors in dairy products such as oxidized and rancid flavors have
been attributed to compounds from the lipid phase. A substantial increase of one or a group of flavor compounds can produce an objectionable off-flavor in butter (44).

Lipase or rancid flavor (hydrolytic rancidity) is one of the most common off-flavors in dairy products, and it is one which the consumer will not tolerate. Lipase flavor is due to the accumulation of short-chain free fatty acids, hydrolytically cleaved from milk fat under the catalytic influence of the lipase enzyme (58, 62). The sequence for lipase action (29) is:

\[
\text{triglyceride} \xrightarrow{H_2O} 1, 2 \text{diglyceride} + \text{FFA} \\
\text{2-monoglyceride} \xrightarrow{H_2O} 1-\text{monoglyceride}
\]

The rate of hydrolysis decreases as diglycerides and monoglycerides are formed, with very little if any glycerol being formed (29). The major volatiles in milk fat were identified in 1974 by Tamsma et al. (56) as fatty acids (caproic, caprylic, capric, and lauric), methyl ketones (nonanone, undecanone, tridecanone, and pentadecanone), and delta lactones (octa, deca, dodeca, and tetradeca). These compounds were identified by gas-liquid chromatography (GLC).

Lipase enzymes are very important to the dairy industry since they produce undesirable lipase (rancid) flavors in dairy products, and are essential for the development of desired flavors in certain cheeses (10). Lipases are enzymes that hydrolyze the esters from emulsified triglycerides at an oil-water interface (62). In milk and dairy products, the lipase enzymes hydrolyze the ester linkage on the triglyceride molecule to yield a FFA and a diglyceride. Diglycerides
can be broken down into monoglycerides but monoglycerides are not generally broken down into FFA and glycerol (29). The accumulation of FFA can be detected organoleptically if the concentration is high enough. The activity of the lipase enzyme is usually quite slow unless the milk is subject to some physical or thermal treatments which may speed up the reaction. From the characteristics of the lipases in milk, it is believed that milk contains a multiple lipase system (52). Early research suggested that there were different types of lipolysis or lipase enzyme action in milk. Milk that became rancid naturally, or without any activation treatment, was referred to as having undergone spontaneous lipolysis. Lipolysis that required activation was referred to as induced lipolysis. Tarassuk et al. (57), 1957, concluded that although milk contains more than one lipase enzyme, the enzymes can be grouped into two lipase systems (10, 29, 52). Tarassuk et al. (57) labeled the two lipase systems in milk as membrane lipase and plasma lipase. Membrane lipase was associated with what had been referred to as spontaneous lipolysis, and plasma lipase was associated with induced lipolysis. Milk that becomes rancid spontaneously contains a higher proportion of membrane lipase than normal (57). Downey (14) in 1969 was able to separate five lipases from milk. No attempt was made to differentiate between membrane and plasma lipases.

Membrane lipase is associated with the fat globule membrane and is adsorbed on the membrane upon cooling of freshly drawn raw milk (10, 29, 57). Increasing the rate and extent of cooling of raw milk will increase the rate of adsorption of membrane lipase onto the fat
globule membrane, or it alters the surface of the fat globule to make the fat more susceptible to lipase action (29). Herrington (25) observed that some cows produced milk that was naturally lipolytically active (10, 29, 57). Late lactation, dry feed or lack of green pasture, estrus, mastitis, and diseased ovaries have been suggested as being linked to lipolysis (62). Poor quality rations fed at reduced energy levels, feeding high carbohydrate diets and dry feed, have all been suggested as increasing the incidence of lipolysis (62). The general recommendation for cows producing milk that spontaneously goes rancid has been not to market that milk or mix it with other milk. Holding raw milk at 35°C for three hours prior to cooling has been shown to decrease spontaneous lipolysis (29, 57). Holding this milk at 37°C will almost entirely retard lipolysis (51).

Plasma lipase which causes induced lipolysis is part of the casein micelle of milk (10, 29, 62). To get lipolysis from the plasma lipase system the milk must undergo activation treatments such as temperature fluctuations, foaming, agitation, and homogenization of raw milk (24, 25, 29, 57, 62). Herrington (24) in 1954 noted that pipeline milking systems with risers were often associated with lipolysis (25, 62). In the construction of new dairy farm facilities, this is of primary importance, especially with the more highly automated systems. Jensen (29) in 1964 stated that the rapid lipolysis that occurs in raw homogenized milk may be attributed to the reaction of newly formed fat globules with casein, which contains lipase, resulting in an increase in substrate surface area. This would bring the lipase enzyme into more intimate contact with the fat. All forms of
agitation tend to increase the surface area of the fat (29). This is probably the main reason for an increase in lipase activity. According to Chandan and Shahani (10), in their review on milk lipases, agitation and temperature manipulation of milk aids in the release of the lipase enzyme from the casein micelle to which it is attached, and promotes adsorption of the enzyme onto the fat globule. These activation treatments also alter the fat globule membrane which may aid in adsorption of the enzyme onto the fat. However, they concluded that the lipase enzyme by itself is not affected by these activation treatments. Further support of the theory that lipase is associated with the casein is shown by precipitating and separating the casein by acidification and centrifugation. When added to a suitable substrate, the casein did exhibit some lipase activity (10).

The temperature of activation by agitation of milk can have a significant effect on the rate of lipolysis. In 1974 Fitz-Gerald (17) showed that milk exhibits maximum susceptibilities to lipase activity at 40-50°C and at 15°C. One would expect a high susceptibility at 40-50°C as the fat would be in the liquid state and would be easily broken up by agitation. The reason for the increase in lipase activity at 15°C is that the fat globule membrane is undergoing a transition at this temperature, because some of the solids within the membrane are melting, possibly making the membrane around the fat more susceptible to the enzyme (17). Milk samples that had been previously cooled had maximum susceptibilities to lipase by agitation at 40-50°C and 10°C. In addition to having a higher susceptibility at 10°C rather than 15°C, precooled milk exhibits a greater lipase
activity below 10 C than non-precooled milk. Thus, agitating raw milk even under normal refrigeration conditions can induce lipolysis. This is of particular importance on the dairy farm where milk is picked up for shipment on an every-other-day basis, and also in the milk plant where milk is stored in large silo tanks with continuous agitation. Lipase enzyme was not easily activated in raw skim milk at 40 C and no increase was noted when agitated at 15 C (17). This further supports the theory that it is the fat globule undergoing change at those temperatures and not the lipase enzyme itself.

The various constituents in milk have an effect on lipolysis. The rate of lipolysis is regulated to some extent by the inhibitory and stimulatory action of milk components upon the lipase enzyme (10, 52). By artificially changing the level of certain milk constituents, the rate of incidence of lipolysis can be altered. Shahani (53) has shown that in general, non-fat milk solids tend to inhibit the lipase enzyme. In addition, there was a direct correlation between the concentration of milk solids and the percent of enzyme inhibition (10, 53). Casein and all its fractions, except k-casein, were inhibitory to lipase activity (53). The various casein fractions, when present at low concentrations inhibited lipase activity from 35-90% (53). The k-casein fraction, however, stimulated lipase activity by as much as 38% when present at low concentrations (10, 53). Other proteins such as α-lactalbumin, β-lactoglobulin, serum albumin, and bovine plasma albumin also inhibited lipase activity (10, 53). At low concentrations, some of the albumins did show a slight stimulatory effect toward the lipase enzyme (10, 53).
Lactose did not significantly inhibit lipase activity, but various salts inhibited the enzyme activity by 3-50% (53).

In many research studies of lipase activity in dairy products, pancreatic lipase has been used. Hemingway et al. (23) observed that the addition of calcium to milk tended to accelerate the rate of lipolysis in fresh milk by pancreatic lipase. Other research has shown that calcium and magnesium salts tend to stimulate the activity of pancreatic lipase and tend to inhibit milk lipase (10, 53).

Many other chemicals have been investigated for their effect on the milk lipase system. Many of these inhibited lipase activity. Most of the compounds that stimulated lipase activity at low levels were inhibitory to lipase activity when present at higher concentrations (53).

The lipase enzyme is apparently sensitive to light, especially the shorter wave lengths. Frankel and Tarassuk (20) noted that exposing milk to artificial or natural light can reduce lipase activity up to 80%, with a greater degree of inhibition at the shorter wave lengths (10, 62). The light inhibitory effect on the enzyme could be reduced by the addition of H₂S or the removal of oxygen from the milk before exposure to sunlight (10, 62). Light treatment would not be practical for the normal control of lipase flavor in milk because light would promote the development of an oxidized flavor. Gamma irradiation has also been shown to substantially reduce lipase activity in other research studies (8, 62).

The optimum pH for the lipase enzyme will vary depending upon temperature, substrate, buffers, ionic strength, and the condition of
the interface where lipolysis must proceed (2, 10, 48, 52, 62). Most research indicates that milk lipase has an optimum pH in the range of 8-9 (2, 24, 29, 48, 52, 62). Richter and Randolph (48) in 1971 reported that milk lipase had an optimum activity at pH 9.2 and was only slightly active at pH 7.5. In some earlier research reported by Albrecht and Jaynes (2) in 1955 the lipase from raw skim milk had an optimum pH of 5.4 and 6.3. More recently, Chandan and Shahani (10) have shown milk lipase to be active between a pH range of 5.2 and 9.8 with the optima varying with substrate concentration. At a milk fat concentration of 16.5% in milk there were three different pH optima (8.5, 6.5 to 7.0, and 7.9), and at a milk fat concentration of <1% there was a broad optimum between pH 7.0 and 8.0, with an overall optimum at pH 8.5 (10). A weak lipase activity has been measured in raw milk at pH 4.1 and 5.7 (10, 62). The conflicting results for the pH optima of milk lipase may be partly due to not standardizing certain test conditions, or not working with the purified enzyme. Lipase has been shown to have pH optima of 9.5 at 10°C and 8.8 at 37°C when tributyrin was used as a substrate (24). Data showing that there are more than one pH optima for milk lipase gives support to the theory that there are more than one lipase in milk. Lipase separated from separator slime exhibited an optimum pH of 9.0-9.2 (9). Some of the lipases in dairy products may come from microorganisms. Most bacterial lipases have an optimum pH at neutrality or on the alkaline side (38). But the optimum pH of fungal and yeast lipases, which may be contaminants of cream and butter, is on the acid side (38).

Most research indicates that milk lipase exhibits its optimum
activity at about 37°C (2, 9, 10, 29, 38, 48, 52, 62). Chandan (8) has shown that purified milk lipase liberated a higher concentration of FFA at 37°C than at 20, 30, or 45°C in both cream and tributyrin. Keeping milk cold has been the generally accepted procedure for limiting lipase activity in raw milk. Microbial lipases are also most active within a temperature range of 30-40°C (38). A few strains of bacteria produce a lipase that is optimally active at 45 and 25°C (38). At 30 and 45°C, milk lipase exhibited only 22 and 27% of the activity it had at 37°C (48). Fitz-Gerald (17) has shown that the temperature of activation by agitation of whole and skim milk has a dramatic effect on lipolysis. The comparison of fluctuating temperature (±3 and ±5°C) and constant temperature has an influence on the activity of the lipase enzyme (11). Chang (11), 1974, demonstrated that at a constant temperature, lipase has an optimum activity at 35°C in both tributyrin and olive oil but the optimum was 4-5°C lower in samples where the temperature fluctuated. Below 35°C, the fatty acid yield was significantly lower for samples held at a constant temperature than in samples where the temperature fluctuated (11). Above 44°C the FFA concentration in the constant temperature samples was greater (11).

Although milk lipase exhibits its optimum activity at 37°C, a longer time at this temperature can substantially reduce the activity of the enzyme (57, 62). Milk held at 37 or 35°C for three hours prior to cooling exhibited less lipase activity than samples cooled immediately (57). This was partly due to the change in the rate of adsorption of membrane lipase onto the fat globule membrane. However,
this may be partly due to the instability of the lipase enzyme at this temperature. When held at 37 C the lipase enzyme retained 54% of its original activity after one hour, 40% after two hours, and almost completely lost all activity in five hours (48). The enzyme also was unstable or lost activity when held at 23 and 4 C, but to a lesser extent than at 37 C (48). Samples stored at 4 C retained 77% of their activity after 48 hours and at 45 C all lipase activity was lost after one hour (46).

The earlier research of Chandan and Shahani (9), 1963, also has shown that the lipase enzyme is very unstable, particularly at higher temperatures. The lipase enzyme is quite stable when held at -4 C. When held at this temperature for up to five months the enzyme lost only 30% of its original activity (9). However, the enzyme lost 13.6% of its activity in 48 hours at 4 C, and completely lost its activity in 24 hours at 37 C and one hour at 45 C (9). Despite a considerable inactivation of the enzyme at 37 C, maximum lipolysis occurred at this temperature (9). Other research has suggested that the lipase enzyme should be assayed at a temperature lower than 37 C, possibly 15 C, because the enzyme is much more stable at these lower temperatures (62).

The most effective means of stopping, preventing, or retarding lipolysis has been through the use of heat treatment or pasteurization. It has been assumed that pasteurization temperatures inactivate the lipase enzyme (5, 10, 20, 25). Other work has indicated that higher than normal pasteurization temperatures are needed to inactivate the lipase enzyme (61, 62). Milk heated to 90 C for 3.3 seconds has been
shown to have an increase in lipase activity after 48 hours of storage (10). Dairy products which have received a high heat treatment have shown some lipase activity during storage (52). Although pasteurization temperature may almost totally inactivate the enzyme, products held in storage may exhibit enough lipase activity to decrease the quality of the product. The lipase from many microorganisms in milk are heat resistant (38). Although the majority of bacteria in milk are destroyed by pasteurization, some psychrophilic, lipolytic bacteria have been isolated from pasteurized milk (38). Short-chain fatty acids are the flavor producing fatty acids, however the unsaturated long-chain FFA are readily oxidized to highly flavored products that may directly influence the quality of butter (38).

Milk lipases have been shown to work in a wide variety of fats and oils in addition to milk fat (10, 52). The rate of hydrolysis appears to be inversely related to the chain length of the fatty acid on the triglyceride (2, 10, 52). Purified lipoprotein lipase is active against all three general classes of substrates, including water soluble, water insoluble, and emulsified substrates (16). Egelrud (15) suggested that all the lipase in milk exists as a lipoprotein lipase, and that other researchers were dealing with several different conditions for its assay. Unless research is being done from a purified source of the enzyme results may vary more than would be anticipated due to impurities carried along with the enzyme. Lipase shows activity against a wide variety of substrates in the absence of activators that had previously been considered necessary
for lipase activity (16). However, the lipase activity was only 50% as high in tributyrinate samples that did not contain activators, compared to samples that contained activators (16). Downey and Andrews (14) in 1969 separated five enzymes from milk by gel-filtration. These appeared to be lipases in that they all hydrolyzed triglycerides. All five enzymes had a greater activity in the hydrolysis of emulsified triglycerides than in the hydrolysis of triacetin (14). In 1971 Richter and Randolph (48) demonstrated that milk lipase hydrolyzed tributyrinate more actively than triolein or tripalmitin, suggesting that lipase is more selective toward short-chain triglycerides. However, tripalmitin was hydrolyzed much more slowly than triolein (48). Among the longer chain fatty acids, chain length may not be as important as some other factors in the rate of hydrolysis, such as the degree of saturation. Milk lipase hydrolyzed butter oil, safflower oil, and corn oil at about the same rate, but all at a faster rate than olive oil (48). Chandan and Shahani (9) found the rate of hydrolysis with the lipase enzyme was in the following decreasing order: tributyrinate, milk fat, olive oil, half and half, milk, and whipping cream. The slower rate of lipolysis of milk fat in its natural form (milk and cream) suggests that milk constituents have an inhibitory effect upon the enzyme (9). Larger fat globules have also been associated with a slower rate of lipolysis, thus whipping cream, which is not homogenized, has a slower rate of lipolysis than milk and half and half (9). In synthetic emulsions (tributyrinate, milk fat, olive oil) the fat was surrounded by a carbohydrate emulsifier membrane, whereas in the
natural system the fat globule is surrounded by a complex membrane containing lecithin and other lipoproteins, and phospholipids (9). The natural fat membrane appeared to offer more protection to the fat globule from the lipase enzyme. Thus, the nature of the fat globule membrane may have had an influence on the susceptibility of the fat to lipolysis.

When mixed triglycerides were hydrolyzed, there was an obvious preference by the lipase enzyme for fats containing short-chain acids (butyric thru lauric) (13, 29, 49). Since butyrate is on the primary position of the triglyceride, there is often a preferential release of butyrate (29). Hemingway et al. (23) found that individual fatty acids were hydrolyzed from the triglyceride at different rates by pancreatic lipase. This would suggest that pancreatic lipase has some preference for certain fatty acids or groups of fatty acids. During the early stages of lipolysis there was a preferential release of short-chain fatty acids, with most of these passing into the aqueous phase (23). Some of the volatile short-chain acids exhibit their flavor characteristics to a higher degree in the aqueous phase than in the fat phase. A high pH increased the amount of short-chain fatty acids in the aqueous phase but decreased the total amount of short-chain acids hydrolyzed (23). There was an increase in the proportion of palmitic, myristic, and lauric (saturated fatty acids) hydrolyzed as lipolysis progressed (23). During the early stages of lipolysis of a substrate the rate of release of fatty acids was slow, then accelerated logarithmically and later declined (23). A reduction in substrate availability and inhibition caused by product
accumulation may be responsible for a decline in the rate of hydrolysis (23). In milk fat, butyric and caproic acids are found almost exclusively in combination with medium and long-chain fatty acids (8). About 97% of the triglycerides in milk fat contains one mole of butyric or caproic acids in combination with two long-chain acids per molecule (8). This in itself may explain the sequence of release of fatty acids during lipolysis.

In 1973 research by Nilsson-Ehle et al. (45) indicated that the lipoprotein lipase of milk had a specificity for the 1 or 3 position of acylglycerols. It has also been shown that lipase hydrolyzes triglycerides at a faster rate than diglycerides and diglycerides at a faster rate than monoglycerides, with a preferential release of fatty acids from the primary positions, 1 or 3 (10, 52). The rate of hydrolysis at the 2 position was relatively insignificant (10).

In the early research on lipolysis the lipase enzyme was shown to have an effect on FFA concentrations in raw milk. This was reported in the review by Herrington (24) in 1954 and others (25, 58). Since the FFA concentrations in dairy products has a dramatic effect on flavor and consumer acceptability, limiting the lipase action is important to the industry from an economic viewpoint. Adding FFA to milk has been one method used to measure the effect of each fatty acid and combinations of FFA on milk flavor. Al-Shabibi et al. (3) have shown that caproic, caprylic, capric, and lauric acids impart an off-flavor to milk that is descriptive of the lipase flavor, but that capric and lauric made the most substantial contribution to this flavor. Much of the early research on rancidity assumed that butyric
acid was primarily responsible for the off-flavor because of its strong and objectionable taste and odor. Al-Shabibi (3) found that when butyric acid alone was added to milk, it did not impart a rancid-like off-flavor. In fact, the off-flavor produced by adding butyric acid diminished during two days of cold storage which is not typical of the lipase (rancid) flavor (3). Scanlan et al. (30) in 1965, and Bills et al. (7) in 1969, concluded from flavor panel analysis that the short-chain even numbered fatty acids in milk, butyric through lauric, are almost entirely responsible for the rancid flavor, with the longer chain fatty acids, myristic through oleic, being of little importance. However, no single short-chain acid had a predominating influence (50). The flavor of the individual acids seems to interact to some degree in producing the typical flavor of blue cheese, and probably also holds true for this flavor in other dairy products. Lipolysis takes place at the fat-water interface, with the FFA partitioning into the two phases (7). Butyric and caproic acids could be detected at a lower level in an oil medium than in water, whereas caprylic, capric, and lauric acid could be detected at a lower level in water. Most of the FFA in milk according to Kinter et al. (32), were in the fat (71-73%) with a smaller amount (21-25%) in the fat globule membrane and an even smaller amount (10%) in the serum.

Baldwin (4) in 1973 concluded from his studies that the aroma of short-chain fatty acids, butyric through capric, plays a more important role in detecting the off-flavor than does taste alone. The contribution of fatty acids to the flavor of foods increases as the pH
decreases (4). At a reduced pH, the lipase (rancid) flavor can be detected at a lower FFA concentration (4). At low concentrations, the FFAs probably contribute to the desirable flavors of dairy products, but become objectionable as the concentration increases. Thus, by controlling the pH of a food product, the influence of fatty acids on flavor can be controlled to some extent.

The concentration at which FFA can be detected organoleptically in butter shows a pattern related to chain length (42). From butyric through caprylic acid an increased concentration of the acid is necessary to be detected organoleptically, and this then decreases with an increase in chain length from caprylic through lauric acid (42). McDaniel et al. (42) concluded that typical butter flavor was due to an interaction of several flavor components at levels below which they can be detected individually. In taste panel studies there was a decrease in the preference of butter when FFAs were present in concentrations well above the minimum levels at which they could be detected organoleptically.

Factors on the farm that contribute to lipolysis were well enumerated in 1954 by Herrington (24). Excessive agitation and foaming of milk, often caused by pumps running continuously, pipe-lines with risers, and leaky fittings, are the major causes of lipase flavor in milk on the farm (5). Vigorous agitation of warm milk may rupture the fat globule, allowing the lipase enzyme to come into direct contact with the fat, thereby speeding up the rate of lipolysis. In pipe-line milking systems, the agitation warm milk receives can be greatly reduced by the installation of a receiver
jar for milk, with appropriate controls to allow the pump to operate only when there is enough milk in the system to prevent the pump from being starved. Rewarming and cooling of milk in bulk tanks where the capacity of the refrigeration unit is not sufficient to keep the milk cold when fresh warm milk is added to cold milk will also increase the lipase flavor.

In the dairy plant lipolysis of milk may be increased by the excessive pumping of milk without regard to keeping the milk cold (5). Moving milk through starved centrifugal pumps or obstructed pipelines will greatly increase the agitation the milk receives. When warm raw milk is separated or clarified and not pasteurized immediately there will be a rapid increase in lipase flavor (5). In addition, homogenizing milk without pasteurization will result in a very rapid development of lipase flavor (5). Mixing raw milk with pasteurized homogenized milk will also result in a lipase flavored product (5).

Lipolysis in milk, in addition to producing an off-flavor, also has a tendency to decrease the surface tension in milk, inhibit growth of bacteria, and possibly affects coagulating properties of rennet in milk (29, 62). The most noticeable effect is the objectionable flavor and odor produced by the accumulation of short-chain free fatty acids (29, 62). Lipolysis may also have a tendency to decrease the fat test slightly (29, 62). Mercuric chloride, which is used to preserve composite milk samples, appeared to enhance lipolysis which in turn appeared to be responsible for reductions in fat tests in composite milk samples. Measuring the increase in FFA concentrations
has been the main method used for measuring lipolysis in milk.

Flavor is the basic quality factor in grading butter and is determined organoleptically by taste and smell (63). The U. S. grade of butter is determined on the basis of classifying first the flavor characteristics, and then the characteristics of body, color, and salt (63). The U. S. grades of butter from highest to poorest qualities are Grade AA (93 score), Grade A (92 score), Grade B (90 score), and Grade C (89 score) (63). Butter which fails to meet the requirements for U. S. Grade C will not be given a U. S. grade (63). A U. S. Grade AA must possess a fine and highly pleasing butter flavor, but may possess a slight feed and a definite cooked flavor (63). A U. S. Grade A butter must have a desirable butter flavor, but may possess the following flavors to a slight degree: aged, bitter, coarse-acid, flat, smothered, and storage, and a definite degree of feed and cooked (63).

The acid degree value test (ADV) of Thomas et al. (58), 1955, has been the accepted chemical procedure for measuring lipase flavor in the dairy industry. The principle of the ADV test is to titrate the free acids in fat to a phenolphthalein end point using a weak base. Various modifications of the procedure of Thomas et al. have been proposed (27). The correlation between ADV and flavor analysis appears to be fairly good in fluid milk.

Frankel and Tarassuk (19) in 1955 proposed a procedure for extracting the fat from milk using ethanol, petroleum ether, and ethyl ether, with subsequent titration to a phenolphthalein end point. For calculation purposes, the percent fat must also be
determined when using this procedure. By this procedure 95-100% of the long-chain and 52-58% of the short-chain acids (butyric, caproic, and caprylic) were recovered.

Some of the other early research suggests that since lipolysis affects the surface tension of milk, this parameter could be used to measure lipolysis (62). Attempts were made to correlate a decrease in surface tension to the degree of lipolysis, however, other factors in milk also have an effect on the surface tension (62). More recently, 1966, a pH-stat method has been proposed by Farry et al. (47) for measuring the rate of lipolysis. This method has found use in research laboratories for measuring lipase activity, but does not appear to be adaptable for routine work in the dairy plant. In the pH-stat method the desired amount of base is added to a sample to maintain a constant pH over time (47). One of the advantages of the pH-stat method is that it allows for the measurement of the velocity of the reaction and the effect of certain treatments of the milk on the velocity of lipolysis.

Several methods have been employed for separating and measuring the FFA content in dairy products. The procedure of Bills et al. (6) has been one of the more widely used procedures for the analysis of individual FFA. An ion exchange resin was used with this procedure to separate the FFA from other lipids, with the free acids being removed from the resin via methylation. After concentrating the methyl esters of the FFA they were analyzed by GLC. Lyophilization of milk and extraction of the fatty acid salts (32), silicic acid (22, 28), sephadex (1), ion exchange (31), and Soxhlet extraction
have all been used for separating FFA for GLC analysis. The measurement of FFA by GLC has not been widely used for measuring lipolysis in dairy products, especially in industry. The high volatility and water solubility of short-chain fatty acids makes GLC analysis of all the FFA in milk difficult.

The Rhodamine B method of Nakai et al. (43) in 1970 and the Rhodamine 6G method of Kason et al. (30) in 1972 for measuring lipolysis in milk are based on the principle of extracting the fat from milk and mixing with Rhodamine B or 6G and measuring the intensity of color development. The correlation between ADV and Rhodamine B was 0.924 (43) and 0.835 (30) for ADV and Rhodamine 6G.

Lauwerys (37) in 1969 used a colorimetric procedure to measure FFA concentrations in albumin solutions. Others have used the colorimetric procedure for measuring FFA concentrations in biological fluids (33, 34). The principle of the colorimetric test is to form copper soaps of the fatty acids in chloroform. The intensity of color development, upon adding a color reagent, can be quantitatively measured by a spectrophotometer and correlated to the FFA concentration (33, 37, 62). The colorimetric test can accurately measure concentrations of FFA as low as 0.01 µeq. per ml (33, 37). The recovery of palmitic acid in an albumin solution was not less than 95% by the colorimetric procedure (37). In blood, as little as a 0.2 ml sample can be used to accurately measure the FFA level (33). Novak (46) developed a similar procedure, but he formed cobalt soaps of the fatty acids instead of copper soaps (46). The cobalt procedure can be run on a smaller sample volume but appears to be
more complex to run than Lauwerys' procedure. An automated procedure for measuring FFA, using the Technicon Autoanalyzer has been developed based on the principle of the colorimetric test (39). The colorimetric test has not been applied to the dairy industry but has been used extensively in the medical field for measuring FFA in blood plasma. Each of the procedures commonly used in the dairy industry for measuring lipase flavor and/or FFA have some drawbacks. This may be due to incomplete recovery of all the free fatty acids or the procedure may be too involved for routine laboratory analysis. In some procedures, as is the case with organoleptic analysis, repeatability of the results and agreement between technicians may not be as high as desired. Consequently, there still is a need for an improved method for measuring lipolysis in dairy products.
MATERIALS AND METHODS

Fresh raw cream, 35% fat, which had been separated from raw milk was obtained from local dairies and divided into two separate lots of 273 kg each. One lot of cream to be called fresh cream was divided into three fractions of about 90 kg each, and each fraction was immediately heat treated for 18 seconds at one of three temperatures, 72 C, 85 C, or 93 C in a high temperature short time (HTST) pasteurizer* (Fig. 1). The other lot of cream to be called aged cream was stored in milk cans for 24 hours at 4.4 C to allow some rancidity to develop and the ADV to increase before being divided into three fractions and heat treated as above. The cream was not homogenized. After heat treatment, all the cream was held in milk cans for 24 hours at 4.4 C before churning into butter. The cream was processed and butter was manufactured in the dairy products laboratory at South Dakota State University.

Samples of cream of approximately 90 kg each were added individually to a 302 l drum churn** and made into butter. The fresh cream was churned into butter one day with the aged cream processed one day later. A sample of cream was taken from the churn and analyzed for the percent fat by the Babcock procedure. The amount of fat was determined by multiplying the fat percent times the weight of the cream. This data was used to determine the amounts of butter color and salt to add during the churning process. Butter color was


** Vane Churn, General Dairy Equipment Company, Minneapolis, Minnesota.
FIG. 1 Flow diagram of the treatments used to study the effects of various processing and storage factors on the development of lipase flavor in butter.
CREAM 35%

FRESH

AGED 24hr

HEAT TREATMENT

72°C  85°C  93°C

CHURNED

-28.9°C
STORAGE

4.4°C
STORAGE

Figure 1.
added to the cream in the churn at the rate of 15 ml per 45.4 kg of fat present. Cream was churned into salted butter using the procedure of Wilster (63). Salt was added to the fat at the rate of two percent by weight. The fat and moisture in the butter were determined by Kohman analysis. Both the fresh and aged creams were prepared in three replications at weekly intervals and made into butter.

Butter samples were hand packed in 454 g capacity round, white, plastic containers with clear plastic tops. Each container was coded to identify the type of cream, the heat treatment the cream received, and the temperature at which the butter was to be stored. The butter samples from each churning were divided and stored at -28.9 C and 4.4 C. Butter samples stored at -28.9 C were analyzed for lipase flavor monthly by organoleptic evaluation and total free fatty acids (FFA) by the ADV (58) and by the colorimetric (37) test. Samples stored at 4.4 C were analyzed organoleptically monthly and twice monthly for FFA by ADV and by the colorimetric test. Samples were analyzed over a six month period, with a separate container being selected for each sampling. Commercial butter samples, supplied by commercial dairies, were also examined organoleptically and analyzed for FFA and ADV.

The acid degree values (ADV) for butter were determined by a modification of the procedure of Thomas et al. (58). Butter samples were melted in a beaker placed in a 39 C incubator for about two hours. The lipid portion of the butter, 9 ml, was pipetted into a Babcock cream test bottle, to which 5 ml of BDI reagent was added. The bottles were placed in a boiling water bath for ten minutes with agitation at five minute intervals. Hot water was added to the
bottle to bring the level of the fat up to the base of the neck and the bottle was centrifuged for one minute in a heated Babcock centrifuge. After centrifugation, the samples were placed in a boiling water bath for five minutes, after which 50% aqueous methyl alcohol was added to raise the level of the fat well up into the neck of the bottle. Bottles were again centrifuged for one minute and placed in a 66°C water bath for tempering the fat column. A 2 ml tuberculin syringe was used to transfer exactly 1 ml of the tempered fat and 5 ml of the fat solvent (petroleum ether:N-propanol, 4:1, v/v) to a 100 ml beaker. A few drops of phenolphthalein in methanol was added to the fat-solvent mixture and titrated to the first definite color change using 0.02 N KOH in methanol. The ADV of each sample for each sampling period was determined in triplicate with the average of the three used as the recorded value. Results were expressed in terms of ADV, which was the ml of 1N base required to titrate 100 g of fat.

The FFA concentration in butter was determined by a modification of Lauwerys' Colorimetric Procedure for determining the FFA concentration in biological fluids (37). Butter samples were tempered to 4.4°C prior to analysis. Approximately one gram of the butter was transferred to a 16 x 125 mm screw top test tube with a miniature butter trier, 10 x 130 mm, made to fit into the test tubes. The exact weight of the butter transferred was determined using an analytical balance, and recorded. The test tubes containing the butter were placed in a forced air oven at 43°C for about five minutes to liquify the fat. Hexane, 5 ml, and 0.01 N H₂SO₄, 5 ml,
were added to the test tube, shaken vigorously, and centrifuged for 30 seconds in a clinical centrifuge at 1550 rpm to separate the fat-hexane phase from the acid-water phase. The non-lipid portion of the butter partitioned into the acid-water phase. A 1 ml aliquot of the upper fat-hexane phase was transferred to another test tube containing 3 ml chloroform, 2 ml hexane, and 3 ml of copper reagent (37). Test tubes were capped and shaken vigorously for about one minute and centrifuged for one minute as above. During shaking, copper soaps of the FFA formed. During centrifugation, the copper soaps of the FFA partitioned into the upper chloroform-hexane phase, with the excess copper reagent forming a layer below the chloroform-hexane. A 1 ml aliquot of the upper chloroform-hexane phase was transferred to another test tube containing 0.5 ml of 0.1% sodium diethyldithiocarbamate in butanol. At room temperature the color development was rapid and stable. Chloroform-hexane (1:1, v/v), 9 ml, was added to the test tube and thoroughly mixed. The intensity of the color development was measured using a Bausch & Lomb Spectronic 20 with a wave length of 440 nm. The concentration of FFA in the sample was determined by using a regression equation derived from the analysis of a series of standard concentrations of palmitic acid, 1 to 14 μeq, made up in chloroform-hexane (1:1, v/v).

Butter samples were evaluated at monthly intervals organoleptically by three experienced judges, one of which is a USDA butter grader. Samples were coded so that the identity or previous history of the butter was unknown to the judges. Samples were judged with the same criteria and standards used for commercial butter samples.
Butter samples that were in storage at -28.9 C were placed in a 4.4 C refrigerator for 24 hours prior to testing. All samples were set out at room temperature for 30 minutes prior to organoleptic evaluations. A numerical value was applied to the standard butter grades so the results could be analyzed by a computer (93 score (AA) = 0, 92 score (A) = 1, 90 score (B) = 2, and 89 score (C) = 3). Statistical analysis (Appendix Table 1) was by least squares analysis of variance for a five factor (cream age, heat treatment, time, storage temperature, and replication) design experiment (55). The different test for measuring lipase flavor were compared by linear correlation (55).
RESULTS AND DISCUSSION

All butter samples that are given a USDA butter grade are graded by organoleptic evaluation. Organoleptic evaluation is an excellent method for determining the consumer acceptability of butter; but is not accurate in detecting some off-flavors, such as lipase, at very low levels. At low levels, the lipase flavor exhibits a slight bitter characteristic, which may not be detected by many experienced judges. In the past few years, there has been an increased incidence of lipase flavored butter (59). Some of the more modern methods of handling cream have contributed to the increased lipase flavor problem, such as longer storage and handling of cream, shipping cream over longer distances for manufacturing, and increased agitation of the cream via pumping.

The ADV test, which is the major chemical test for measuring lipolysis in dairy products, was developed by Thomas et al., 1954, (58) to determine the extent of lipolysis in milk and cream. This test has been modified and is used in industry for analyzing butter for lipase flavor. In some instances, butter with a high intensity of lipase flavor does not always exhibit a higher ADV. This would seem to indicate that the ADV may not be as adaptable to analysis of butter as it is for milk and cream. Several other tests have been proposed for detecting and measuring lipolysis in milk and cream (30, 43, 47, 62), but there had not been a method proposed for butter analysis.

A colorimetric test (37), which has been used for measuring FFA in biological fluids, was modified in this study to measure the FFA
concentration in butter. This test is sensitive to small changes in FFA and can be used with a small sample volume. The colorimetric test was shown to be accurate in measuring the FFA concentration in butter over a wide range. A series of standards of palmitic acid were evaluated to determine the repeatability of the results at the various concentrations and to derive the regression equation. The analysis of the standards at each concentration was run in triplicate, with the points falling along a straight line. Using the average value from each triplicate analysis, the regression equation was derived by the method outlined by Steel and Torrie (55) for linear regression. The regression equation derived from the values for the standard solutions was: μEquiv. of FFA = 0.09691 + 28.17139 (OD), where the OD represents the optical density at 440 nm. For the analysis of butter samples, values are expressed as μEquiv. of FFA/g of butter.

In industry, the presence of lipase flavor in butter is often attributed to the age and quality of the cream. For this study, fresh and aged (24 h at 4.4 °C) cream were used to evaluate the effect of age of cream on the lipase flavor and FFA level in butter as measured by the ADV and the colorimetric test. The data, as presented in Table 1, show that the ADV of the aged cream was higher than the fresh cream. The lipase flavor can usually be detected in cream at an ADV of 0.80 or higher (35, 54). Freshly drawn raw milk normally has an ADV of about 0.4 (35, 54). When the ADV increases to 1.2 to 1.3, the lipase flavor can be detected organoleptically. The higher concentration of the fat in the cream accounts for the lower ADV at
which the lipase flavor can be detected in cream (35, 54). Storage of cream, even at normal refrigeration temperatures, will not totally arrest lipase enzyme activity.

TABLE 1. Acid degree value of fresh and aged (24 h at 4.4 C) cream prepared in three replications at weekly intervals.

<table>
<thead>
<tr>
<th></th>
<th>Replication 1</th>
<th>Replication 2</th>
<th>Replication 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh cream</td>
<td>.80</td>
<td>.61</td>
<td>.81</td>
</tr>
<tr>
<td>Aged cream</td>
<td>--</td>
<td>.97</td>
<td>1.08</td>
</tr>
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Fig. 2 shows the comparison of ADV of butter from fresh and aged cream (24 h at 4.4 C) heat treated at 72 C, 85 C, and 93 C for 18 seconds and stored at 4.4 C for up to six months. These samples were analyzed for ADV twice monthly. Each point on the graph represents the average value of triplicate analyses of three different samples, or nine different analyses. In all samples, the butter made from aged cream had a much higher ADV throughout storage than did the butter from fresh cream. Statistical analysis indicated that the age of the cream had a highly significant (P<.01) effect on the ADV of the butter. This would appear to be directly correlated to the ADV of the cream from which the butter was made. The ADV of the aged cream was about 0.3 higher than the ADV of the fresh cream. The ADV of the aged cream butter was about 0.35 higher than the ADV of the fresh cream butter. This is in agreement with previous research (17) which states that lipolysis takes place in dairy products even at
FIG. 2 Acid degree value of butter made from fresh and aged (24 h at 4.4 C) cream during storage at 4.4 C.
refrigerated temperatures. A higher ADV would be expected in both cream and butter with a longer storage of cream in the low state. This would suggest that prompt pasteurization of cream would be important in reducing the rate of lipolysis in cream that is to be made into butter. The rate of lipolysis in the fresh and aged cream butter was about the same throughout storage, as can be seen in Fig. 2. For about the first two and one-half to three months of storage there was a general increase in the ADV of all samples, but after three months storage, the ADV for all samples tended to decrease. At the end of six months storage, many of the ADV values had returned to about their initial levels, with some values being lower than in the butter before storage. Other researchers have noted this same trend for some cheese samples held in storage (21).

The ADV of duplicate samples of fresh and aged cream butter samples held in storage for six months at -28.9 C is presented in Table 2. Statistical analysis shows that the length of storage of the butter had a highly significant (P<.01) effect on the ADV of the butter. However, the values in Fig. 2 and Table 2 suggest that the level of FFA, as measured by ADV, decreased during the latter months of storage. The decline in the ADV during the latter months of storage was probably due to an inability of this test to measure all of the FFA in solution. Since the butter would contain an increased level of sodium ions (Na+) which were added in the form of salt (NaCl) during butter manufacture, it is possible that some of the FFA in the butter formed fatty acid salts. The principle of the ADV test
TABLE 2. Acid degree value of butter made from fresh and aged (24 h at 4.4 °C) cream during storage at -28.9°C.

<table>
<thead>
<tr>
<th>Months</th>
<th>Fresh Cream</th>
<th>Aged Cream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72 °C</td>
<td>85 °C</td>
</tr>
<tr>
<td>0</td>
<td>1.06</td>
<td>1.05</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.02</td>
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<tr>
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<td>1.01</td>
</tr>
<tr>
<td>6</td>
<td>1.03</td>
<td>1.03</td>
</tr>
</tbody>
</table>

is to titrate the free acids in the fat with KOH to a phenolphthalein end point. If some of the free acids are in form of salts, less KOH would be needed for the titration, as the acidifying effect of the fatty acids would already have been neutralized. However, since the fatty acids and their salts are responsible for the lipase flavor (62), the formation of fatty acid salts would be expected to increase the lipase flavor.

The difference in ADV values in butter made from cream that had received different heat treatments was not great. In the fresh cream butter samples held at 4.4 °C, only the butter from cream heat treated at 72 °C had a net increase in the ADV at the end of six months storage. This agrees with previous research which has indicated that the lipase enzyme is not totally inactivated by pasteurization temperatures (61). There was no clear trend for one set of samples
to develop an increase in ADV at a faster rate than other samples. In the early months of storage, butter from fresh cream heat treated at 85 C had the most rapid increase in ADV, but then declined rapidly after the three month period. In the aged cream butter samples, the ADV remained close throughout the storage period for all samples. Heat treatment did not have a significant influence (P<.05) on the ADV of butter held in storage. This suggests that increasing the pasteurization temperature of the cream will not necessarily be followed by a lower ADV in butter held in storage.

The temperature at which butter was held in storage did not have a significant influence (P<.05) on the ADV, indicating that the shelf-life of butter in terms of ADV would not be increased by storing at temperatures below normal refrigeration temperatures. Butter samples held at -28.9 C (Table 2) showed the same general trends as samples held at 4.4 C, but did not show as wide a variation in ADV during the six months of storage. The inability of the ADV test to detect any significant differences in the rates of lipolysis in butter stored at -28.9 C and 4.4 C is likely associated with an inability of the test to give an accurate indication of an increase in lipolysis throughout the storage of the butter.

The FFA level of butter held in storage at 4.4 C increased in all samples as shown in Fig. 3. Samples were analyzed at the same frequency as with the ADV test. The colorimetric test appeared to be more sensitive to small changes in FFA and more accurate than the ADV test. As in the ADV test, the FFA as measured by the colorimetric test, was significantly higher (P<.01) in the aged cream butter than
FIG. 3 Free fatty acid levels of butter made from fresh and aged (24 h at 4.4 °C) cream during storage at 4.4 °C.
Figure 3.
in the fresh cream butter. This again points out the importance of limiting the time that milk and cream is held in storage before pasteurization. With the trend to few butter manufacturing plants with larger capacity per plant, milk and cream is often held for longer periods of time before being made into butter, possibly up to several days. This cream may also receive more agitation via pumping and trucking which can also increase the rate of lipolysis. Holding cream for longer than 24 h would probably result in a higher FFA level over the fresh cream butter than was noted in this study. Throughout the six months of storage, the FFA level in aged cream butter remained significantly above the fresh cream butter. The heat treatment the cream received did not have a significant effect (P<.01) on the FFA values, according to statistical analysis. However, in both the fresh and aged cream the FFA concentration from cream heat treated at 72 C increased faster and at a more consistent rate than in butter from cream heat treated at higher temperatures. Butter from the cream heat treated at the highest temperature had the lowest net increase in FFA levels. However, these differences were minimal. The higher heat treatment seemed to have a greater degree of lipase inactivation but this was not significant (P<.05) for the range of heat treatments used. Using a wider range of heat treatments would probably have given some significantly different results in relation to the rate of lipolysis. Heat treatment, even at 93 C for 18 seconds, did not totally inactivate the lipase enzyme or prevent lipolysis. This agrees with much of the previous research which indicated that the lipase enzyme is not totally inactivated by
pasteurization or even higher temperature (10, 52, 61, 62). No previous data could be found where the heat treatment of the cream was studied in relation to inhibiting lipolysis in butter. Pasteurization of the cream does, however, greatly reduce the rate of lipolysis from what it would be in raw cream. Increasing the heat treatment of the cream above 93°C probably would not have a substantial influence on decreasing lipolysis in butter, but butter from cream heat-treated at temperatures below 72°C would probably exhibit an increased rate of lipolysis.

Although there was an increase and then a decrease in FFA levels between sampling periods in some samples, the overall net effect of storage was to increase the FFA levels in all samples. The fairly steady increase in the FFA level as measured by the colorimetric test, indicated a consistent rate of lipolysis throughout storage. This does not compare favorably with the ADV test which showed a decrease in values during the latter months of storage. This may partly be explained in that the ADV test would not be expected to measure fatty acid salts whereas the colorimetric test would do so. The acidification of the sample being analyzed by the colorimetric test would remove the ions from the fatty acids by converting it from a salt to a free fatty acid which could be measured by the colorimetric procedure. The length of time butter is held in storage had a highly significant (P<.01) effect on the FFA level. The fairly steady rate of lipolysis suggests that it is just a matter of time until the lipase flavor reaches the intensity where it will become objectionable. Although this test measures all FFA, it is the increase in the
short-chain fatty acids, butyric through lauric, which are responsible for the lipase flavor (3, 7, 42, 50).

The effect of storage on the FFA level (μEq/g) in butter from fresh and aged cream heat treated at 72 C, 85 C, and 93 C is presented in Table 3. Table 4 shows the comparison of the FFA levels of butter from fresh and aged cream heat treated at 85 C and held at -28.9 C and at 4.4 C. Butter samples held at -28.9 C showed the same general trends as samples held at 4.4 C, but the increase in FFA was less when butter was held at the lower temperature (Table 4). This agrees with other research which showed that reducing the temperature will reduce the rate of lipolysis (10, 16). In the fresh cream butter samples held at -28.9 C, as shown in Table 3, the butter from fresh cream

<table>
<thead>
<tr>
<th>Months</th>
<th>Fresh cream butter</th>
<th>Aged cream butter</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>72 C</td>
<td>85 C</td>
</tr>
<tr>
<td>0</td>
<td>6.73</td>
<td>6.74</td>
</tr>
<tr>
<td>1</td>
<td>6.87</td>
<td>7.10</td>
</tr>
<tr>
<td>2</td>
<td>7.60</td>
<td>7.69</td>
</tr>
<tr>
<td>3</td>
<td>7.43</td>
<td>7.96</td>
</tr>
<tr>
<td>4</td>
<td>7.35</td>
<td>7.61</td>
</tr>
<tr>
<td>5</td>
<td>7.35</td>
<td>7.60</td>
</tr>
<tr>
<td>6</td>
<td>8.16</td>
<td>8.36</td>
</tr>
</tbody>
</table>
heat-treated at the higher temperature had an overall greater increase in FFA than when the cream was heat treated at the lower temperatures. In the aged cream butter, the opposite effect was true. Since the overall differences between heat treatments were small, there was no apparent trend for an increase in the rate of lipolysis dependent upon heat treatment as there was for samples held at 4.4 C.

TABLE 4. The effect of the storage temperature of butter on the free fatty acid level in butter.

<table>
<thead>
<tr>
<th>Months</th>
<th>Fresh cream butter</th>
<th>Aged cream butter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-28.9 C</td>
<td>4.4 C</td>
</tr>
<tr>
<td>0</td>
<td>6.74</td>
<td>6.74</td>
</tr>
<tr>
<td>1</td>
<td>7.10</td>
<td>7.00</td>
</tr>
<tr>
<td>2</td>
<td>7.69</td>
<td>7.72</td>
</tr>
<tr>
<td>3</td>
<td>7.96</td>
<td>7.56</td>
</tr>
<tr>
<td>4</td>
<td>7.61</td>
<td>7.66</td>
</tr>
<tr>
<td>5</td>
<td>7.60</td>
<td>8.05</td>
</tr>
<tr>
<td>6</td>
<td>8.36</td>
<td>9.02</td>
</tr>
</tbody>
</table>

The age of the cream from which the butter was made did not have a significant (P<.05) effect on the flavor of butter as shown in Fig. 4 and 5. Although the butter from aged cream had a higher intensity of lipase flavor than the butter from fresh cream, these differences were not great.

The major criteria to be measured was the development of lipase flavor during storage. In all samples evaluated organoleptically,
FIG. 4 Flavor scores at butter made from fresh (○ - △ - □ - ) and aged (● - △ - ■ - ) cream (24 h at 4.4 °C), heat treated at 72 °C (A), 85 °C (B), and 93 °C (C), during storage at 4.4 °C.
Figure 4.
FIG. 5 Flavor scores of butter made from fresh (–○–△–□–) and aged (–○–△–□–) cream (24 h at 4.4 C) heat treated at 72 C (A), 85 C (B), and 93 C (C) during storage at -28.9 C.
The only off-flavor that developed to any significant degree was the lipase flavor. The fresh cream butter samples tended to increase in lipase flavor at a faster rate than the aged cream butter, even though initially the fresh cream butter had a less intense lipase flavor. However, this was not consistent for all samples. Had the cream been stored for a longer period of time before heat treatment and subjected to activation treatments such as agitation via pumping which would be common in industry, the extent of lipase flavor would have been much greater in the aged cream butter and probably would have been of significant importance. In Fig. 4, (butter held at 4.4°C), the intensity of lipase flavor increased very rapidly in all samples after two to three months of storage. Storage of the butter had a highly significant (P<.01) effect on flavor scores, as it did on ADV and FFA values by the colorimetric test (Fig. 2 and 3, and Tables 2, 3, and 4).

The lipase flavor did not level off at the C grade (No. 3 flavor score) in Fig. 4 but it did become unacceptable. Measuring the lipase flavor intensity beyond the C grade is of no real importance to the industry, as the butter is unacceptable to the consumer at this point. The development of lipase flavor was less in butter from cream heat treated at 85°C than butter from cream heat treated at 72°C and 93°C. Thus, it would appear that the normal pasteurization of cream is the best temperature to use in heat treating cream to limit the lipolysis in butter.

Statistical analysis indicated that the heat treatment of the cream had a highly significant (P<.01) effect on the development of
lipase flavor. However, as is shown in Fig. 4 and 5, increasing the heat treatment of the cream above its normal pasteurization temperature (85 C) will not result in a lower rate of lipolysis during storage. This again supports other research (10, 52, 61) which has shown that pasteurization temperatures do not totally inactivate the lipase enzyme.

Fig. 6 shows the flavor scores of butter in storage at -28.9 C and 4.4 C for six months. In all cases, butter held at the higher temperature had a higher rate of lipolysis than that stored at the colder temperature. These differences usually became apparent at three to four months of storage. When butter was held at -28.9 C, less than one-third of the samples were graded C after six months storage, whereas about five out of six of the butter samples held at 4.4 C were graded C after six months storage. Except for a few samples from cream heat treated at 85 C, all butter samples stored at 4.4 C were given a C grade (No. 3 score) after five months storage. The storage temperature of the butter had a highly significant effect (P<.01) on lipase flavor. This appears to agree with previous research that has shown that decreasing the temperature of milk below 37 C is associated with a decreased rate of lipolysis (8, 17, 48).

Storage of butter in frozen storage is absolutely necessary to minimize the development of lipase flavor if the product is not going to be consumed shortly. Storing butter at normal refrigeration temperature (4.4 C) for more than a short period of time would likely result in a product with a strong lipase flavor. In instances where butter must move through many marketing channels before it reaches
FIG. 6 A comparison of flavor scores of butter made from fresh (C, D) and aged (A, B) cream (24 h at 4.4 C), heat-treated for 18 sec at 85 C (A, C) and 93 C (B, D), during storage at -28.9 C (○) and 4.4 C (●).
the consumer, temperature regulation is very important. Butter that is poorly handled through marketing channels may develop enough lipase flavor to render the product unacceptable to the consumer.

Different sample replications had a significant ($P < .01$) effect on ADV, FFA by the colorimetric test, and flavor scores. The three sample replications were prepared at weekly intervals. Since the quality of cream varies from day to day and the milk will vary in its susceptibility to lipolysis, the influence of the supply of cream would be expected to affect the degree of lipolysis. The season of the year would also be expected to have a significant effect of the lipase flavor in butter. However, this parameter was not measured in this study.

The comparison of flavor and ADV of butter held in storage at 4.4 C is shown in Fig. 7. The ADV does not show the same general trends as the flavor scores, although the ADV is supposed to be an indicator of lipase flavor. While there was a highly significant ($P < .01$) increase in lipase flavor of butter held in storage, the ADV test did not indicate a net deterioration in the quality of the butter after six months. The ADV test appears to be an acceptable procedure for measuring lipolysis in milk and cream, but the conclusion from this study would be that the ADV test is not an accurate indicator of the intensity of lipase flavor in butter. The ADV test should not be used to predict the quality of butter or of deterioration in butter quality. Statistical analysis ($P < .01$) showed no correlation in butter between ADV and flavor scores. During storage, the lipase flavor in samples held at 4.4 C increased at a
FIG. 7 A comparison of flavor scores and acid degree values of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 85 C for 18 sec) during storage at -28.9 C and 4.4 C.
faster rate than samples held at -28.9 C, but the ADV test was unable to detect any differences in the rate of lipolysis. This further supports the conclusion that the ADV test is unable to detect any differences in the rates of lipolysis in butter samples.

Fig. 8, shows the comparison of FFA (colorimetric test) to flavor scores. The FFA increased throughout storage as did the lipase flavor during storage of the butter. The FFA level in the butter remained higher in the aged cream butter than in fresh cream butter throughout storage but if flavor scores are used as parameters, lipolysis was much greater in samples held at 4.4 C than -28.9 C, with the age of cream not having any influence on lipase flavor. There was no correlation between flavor scores and FFA values as measured by the colorimetric test. Thus it can be concluded that the total FFA level is not an indication of the intensity of lipase flavor in butter. This is in agreement with other research which showed that specific fatty acids are responsible for the flavor and the total concentration would not necessarily be an indication of the lipase flavor intensity (7, 50). The colorimetric test could be used to indicate a decrease in the quality of butter held in storage, but these values cannot be correlated to flavor.

Table 5 shows a comparison of ADV, FFA, and flavor scores of butter held in storage at -28.9 C for six months. The same general trends hold true as when samples were held at 4.4 C. The ADV showed very little change, FFA as measured by the colorimetric test showed a slight increase, but flavor scores exhibited the greatest increase. These data also show that the ADV is not an indicator of lipase
FIG. 8 A comparison of flavor scores and free fatty acids of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 85 C for 18 sec) during storage at -28.9 and 4.4 C.
flavor. The FFA (colorimetric test) tended to increase as the lipase flavor increased but there was no correlation between absolute values. It could be concluded from this that neither the ADV test nor the colorimetric test may be used to predict the lipase flavor in butter.

TABLE 5. A comparison of the acid degree value, free fatty acid, and flavor score of butter made from fresh and aged (24 h at 4.4 C) cream (heat treated at 85 C for 18 sec) during storage at -28.9 C.

<table>
<thead>
<tr>
<th>Months</th>
<th>Butter from fresh cream</th>
<th></th>
<th>Butter from aged cream</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADV</td>
<td>FFA</td>
<td>FlavorX</td>
<td>ADV</td>
</tr>
<tr>
<td>0</td>
<td>1.05</td>
<td>6.74</td>
<td>1.0</td>
<td>1.37</td>
</tr>
<tr>
<td>1</td>
<td>1.02</td>
<td>7.10</td>
<td>1.2</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>1.06</td>
<td>7.69</td>
<td>1.0</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>7.96</td>
<td>1.0</td>
<td>1.45</td>
</tr>
<tr>
<td>4</td>
<td>1.02</td>
<td>7.61</td>
<td>1.8</td>
<td>1.38</td>
</tr>
<tr>
<td>5</td>
<td>1.01</td>
<td>7.60</td>
<td>1.7</td>
<td>1.33</td>
</tr>
<tr>
<td>6</td>
<td>1.03</td>
<td>8.36</td>
<td>2.1</td>
<td>1.42</td>
</tr>
</tbody>
</table>

FlavorX - 1.0 = A grade, 2.0 = B grade, 3.0 = C grade.

The fact that the ADV and FFA level does not reflect the grade of butter (A, B, or C) is shown in Table 6. The range in ADV and FFA values was about the same for the three grades of butter. The reason for the lack of correlation between FFA tests and flavor scores is most likely closely related to fatty acid chain length. In flavor analysis, the judge is measuring only the short-chain free fatty acids (butyric through lauric) and their salts (62, whereas the colorimetric and ADV tests measure the total FFA. The correlation
between these two tests was 0.86, indicating that the agreement between the two methods of measuring total FFA was good. The more rapid increase in the lipase flavor (Fig. 7 and 8, and Table 5) would seem to suggest that the fatty acids causing lipase flavor were being hydrolytically cleaved from the triglyceride at a faster rate than the longer chain acids. This is in agreement with previous research (13, 29, 49) which has shown that the lipase enzyme has a preference for short-chain fatty acids, which are the flavor producers (3, 7, 50). During the early stages of lipolysis the lipase enzyme had a stronger preference for the short-chain acids than later in the stages of lipolysis (23). This in itself may explain why the lipase flavor of butter held in storage increased very rapidly, whereas the total FFA increased at a more steady rate.

**TABLE 6. Range of acid degree values and free fatty acid levels in A, B, and C grade butter at three and four months of storage.**

<table>
<thead>
<tr>
<th>Flavor</th>
<th>Range of ADV</th>
<th>Range of FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>.96 - 1.68</td>
<td>6.10 - 12.18</td>
</tr>
<tr>
<td>B</td>
<td>.93 - 1.68</td>
<td>6.10 - 12.06</td>
</tr>
<tr>
<td>C</td>
<td>.81 - 1.60</td>
<td>5.35 - 13.14</td>
</tr>
</tbody>
</table>

The values for ADV, FFA, and flavor grades of commercial butter supplied from commercial sources are presented in Table 7. Samples were graded for flavor by USDA butter graders prior to chemical analysis. The range in values was similar to the butter samples prepared for this study. As was the case with the prepared samples,
the ADV and FFA values do not seem to be an accurate indicator of flavor grades. This can be easily observed by noting that samples 6 and 8, both B grade butter, had a higher ADV and FFA level than samples 4 and 5 which are C grade. Sample 4, a C grade butter, had only a slightly higher ADV and FFA level than sample 2, a AA grade butter. This agrees with the results from butter samples prepared in the laboratory, showing that ADV and FFA cannot be correlated to flavor scores.

TABLE 7. Acid degree values, free fatty acids, and flavor grades of commercial butter samples.

<table>
<thead>
<tr>
<th></th>
<th>ADV(^{a}) (µeq/gm)</th>
<th>FFA(^{b}) (µeq/gm)</th>
<th>Flavor USDA grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.72 ± 0.08(^{c})</td>
<td>4.52 ± 0.39(^{c})</td>
<td>AA</td>
</tr>
<tr>
<td>2</td>
<td>0.76 ± 0.01</td>
<td>5.40 ± 0.38</td>
<td>AA</td>
</tr>
<tr>
<td>3</td>
<td>0.85 ± 0.11</td>
<td>5.20 ± 0.25</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>0.85 ± 0.09</td>
<td>5.92 ± 0.33</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>0.90 ± 0.10</td>
<td>5.86 ± 0.45</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>1.10 ± 0.11</td>
<td>7.89 ± 0.49</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>1.10 ± 0.12</td>
<td>8.75 ± 0.30</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>1.27 ± 0.15</td>
<td>9.36 ± 0.20</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>1.51 ± 0.12</td>
<td>11.13 ± 0.15</td>
<td>C</td>
</tr>
<tr>
<td>10</td>
<td>2.62 ± 0.15</td>
<td>19.98 ± 0.76</td>
<td>C</td>
</tr>
</tbody>
</table>

\(^{a}\) Acid degree value
\(^{b}\) Free fatty acids
\(^{c}\) Standard deviation of the mean.
SUMMARY

The lipase flavor (hydrolytic rancidity), which is the most prevalent off flavor in butter, has been detected in butter with an increasing frequency in recent years. Butter is normally graded organoleptically prior to going into storage. In some instances, butter taken out of long term cold storage may exhibit an increased level of lipase flavor. The ADV test measures free fatty acids, but is not as accurate as desired for measuring the lipase flavor in butter, therefore, a more sensitive test is needed. A colorimetric test was modified to determine the FFA concentration in butter. The age of cream at the time of pasteurization, length of time butter was held in storage, storage temperature, and sample replications were factors studied to determine their effects on the development of lipase flavor in butter. All samples were examined by organoleptic evaluation and analyzed by ADV test, and FFA by the colorimetric test.

The age of cream at the time of pasteurization had a highly significant effect on ADV and FFA concentration of butter, but did not have a significant effect on flavor scores. This would seem to indicate that the aging of cream at refrigeration temperatures would have a more dramatic influence on the total FFA as measured by the two chemical tests than on the short-chain flavor producing fatty acids, which are measured organoleptically.

The degree of heat treatment of cream (72 C, 85 C, and 93 C for 18 sec.) had a significant (P<.01) influence on flavor scores, but did not have a significant influence on ADV and FFA. However, higher heat treatments could not be directly correlated to greater
destruction of the lipase enzyme. Butter samples from cream heat treated at 72°C and 93°C had a greater increase in lipase flavor than butter from cream heat treated at 85°C. The temperature at which butter was held in storage (−28.9°C and 4.4°C) had a highly significant influence (F<.01) on FFA and flavor values but did not have a significant influence on ADV. In all cases, butter held at the warmer temperature had a higher rate of lipolysis. The length of time butter was held in storage had a highly significant (F<.01) influence on ADV, FFA, and flavor scores. During six months of storage, the increase in the intensity of the lipase flavor was more rapid than the changes in ADV and FFA. The FFA levels increased at a fairly steady rate throughout the storage time. The ADV increased during the first two and one-half to three months of storage and then declined through the six months of storage to about the initial level prior to storage. This would suggest that the short-chain flavor producing fatty acids, butyric through lauric, are hydrolytically cleaved from the triglyceride at a faster rate in butter than the longer-chain fatty acids. The inability of the ADV test to detect a net increase in the lipase flavor of butter held in storage is probably associated with an inability of this test to measure all the free fatty acids. This could be associated with the formation of fatty acid salts of the FFA in butter. Sample replication had a highly significant (F<.01) influence on ADV, FFA, and flavor values, suggesting that the day-to-day variation in the cream supply will have an influence on the lipase flavor in butter.

Statistical analysis indicated there was no correlation between
ADV and flavor scores, and FFA and flavor scores. The correlation between ADV and FFA (colorimetric test) was 0.86. Thus, it can be concluded that the total FFA level is not an indicator of the intensity of lipase flavor in butter. The ADV test appears to be a good test for measuring lipolysis in milk and cream, but should not be used for butter. The ADV test does not give an indication of the intensity of lipase flavor and is unable to detect a deterioration in butter during storage. The colorimetric test for measuring FFA in butter does not give results that can be correlated to flavor and should not be used for that purpose. The colorimetric test was, however, able to detect an increase in lipase flavor in butter during storage. The colorimetric test was more sensitive to small changes in FFA concentration than was the ADV test. At present organoleptic evaluation appears to be the best procedure for measuring lipase flavor development in butter.
APPENDIX
### APPENDIX TABLE 1. Results of statistical analysis of treatment effects on FFA, ADV, and flavor of butter held in storage.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FFA</th>
<th>ADV</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Cream age</td>
<td>***</td>
<td>***</td>
<td>N.S.</td>
</tr>
<tr>
<td>(E) Heat treatment</td>
<td>N.S.</td>
<td>N.S.</td>
<td>***</td>
</tr>
<tr>
<td>(R) Replication</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>(T) Storage times (months)</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>(S) Storage temperature</td>
<td>***</td>
<td>N.S.</td>
<td>***</td>
</tr>
<tr>
<td>A x H</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>A x R</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>H x R</td>
<td>***</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>A x T</td>
<td>***</td>
<td>N.S.</td>
<td>***</td>
</tr>
<tr>
<td>A x S</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>H x S</td>
<td>***</td>
<td>***</td>
<td>N.S.</td>
</tr>
<tr>
<td>R x S</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>T x S</td>
<td>***</td>
<td>N.S.</td>
<td>***</td>
</tr>
</tbody>
</table>

* = Significant P .05.
** = Significant P .025.
*** = Significant P .01.

Correlation between FFA and ADV was 0.86.
There was no correlation between flavor and FFA, nor between flavor and ADV.


