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Interrelationships of microorganisms in cream

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NOTE TO USERS

INTERRELATIONSHIPS OF MICROORGANISMS IN CREAM

by

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INTRODUCTION

Regulatory agencies and the dairy industry have sought for applicable chemical tests which accurately quantitatively measure the quality of dairy products. In recent years two methods have been developed by Federal Food and Drug Administration personnel. One of the methods is the water-insoluble acid (W.I.A.) determination developed by Hillig (58). This test indicates degree of fat hydrolysis by quantitatively measuring the amount of long-chain fatty acids present. The W.I.A. determination has been recognized as an official method in the seventh edition of "Official Methods of Analysis" of the Association of Official Agricultural Chemists (8). Standards have been established and regulatory agencies are using the method as a basis for seizure and condemnation. The other method was developed by Duggan (32) for the purpose of measuring protein hydrolysis by determining the free tryptophan content of milk, cream and butter. The tryptophan determination has not attained the status of an official method, but it has been used experimentally by the Federal Food and Drug Administration.

Interest in the W.I.A. and free tryptophan determinations and the need for information concerning the specific lipolytic, proteolytic and other deterioration values resulting from the growth of common contaminating organisms in cream, was

responsible for launching this study. A representative group, including a causative organism for each of the different types of fermentations most commonly occuring in dairy products, was selected. With sterile cream as a medium, each organism was grown alone and in combination with Streptococcus lactis at representative incubation temperatures.

STATEMENT OF PROBLEM

The following specific information was sought in these studies:

1. To determine the exact measurements of lipolysis, proteolysis, titratable acidity, organism populations, pH and score resulting from the growth of selected organisms in cream, individually and in combination with S. lactis.

2. To determine the influence of the interrelationship of the individual organisms with S. lactis upon the fermentations occurring in cream.

REVIEW OF LITERATURE

The Role of Organisms and Lipases in Producing Rancidity in Cream and Butter

Influence of lipases

The relationship of acid number variations in the fat to the quality and flavor defects of commercial butter was investigated by Fouts (39). In most samples of unsalted butter, the acid number of the fat increased during holding for 6 days at 21° C., and approximately 25 per cent of the samples became rancid. In the case of salted butter held under the same conditions, very few samples became rancid. No close correlation existed between the acid number of the fat and the quality of the butter. Some good butter samples had a relatively high acid number and some rancid butter samples had a relatively low acid number. In investigating some factors responsible for variations in the acid numbers of the fat in cream and commercial butter, Fouts (40) observed that the lipase of organisms was of greater significance than natural lipase in causing hydrolysis of raw cream. In raw cream in which both natural lipase and microorganisms were active, the lipolysis was greater at 5° C. than at 13 or 21° C. In cream containing formaldehyde, in which only natural lipase was active, hydrolysis increased as the holding temperature of the cream increased, within the range studied.

To determine if high acidities had any effect on fat hydrolysis in cream, Fouts (40) inoculated separate portions of sterilized cream with butter culture and Lactobacillus bulgaricus producing acidities of 0.89 per cent and 2.02 per cent. Sufficient lactic acid was added to a similar portion of cream to increase the acidity to 2.61 per cent. These samples were held 6 days at 21° C., at the conclusion of which time there was no change in the acid number of the fat.

Fouts (40) studied Oidium lactis (Geotrichum candidum), Mycotorula (Candida) lipolytica, Achromobacter lipolyticum, Pseudomonas fragi, Pseudomonas fluorescens and Alcaligenes lipolyticus and reported them all to be more actively lipolytic in cream than in butter. When the acidity of sour cream was reduced by neutralization, the acid number of the fat also was reduced, but not proportionately; hence it cannot always be assumed that butter with a low acid number was made from good quality cream.

Fouts (42) investigated the relation of volatile acidity of butterfat to rancidity. In samples of commercial unsalted butter showing widely varying degrees of rancidity the percentage of the total volatile acid which was found in the fat varied only slightly. There was no close correlation between the percentage of the total acids in the fat that was volatile and the degree of rancidity. Each of several

organisms studied exhibited a consistent tendency to produce volatile and non-volatile acids in the same ratio. This condition held true regardless of age of the culture used for inoculation, incubation temperature or degree of fat hydrolysis. Such consistency may indicate that certain organisms elaborate a lipase possessing a specificity or a preference for hydrolyzing certain triglycerides or portions thereof.

Specific quantities of volatile, solid and liquid groups of fatty acids, produced as a result of hydrolysis of butterfat by bacteria and molds, were determined by Richards and El Sadek (102). In every case, under identical conditions, they found the molds produced a greater total quantity of free fatty acids than did the bacteria. Bacteria produced less volatile acid and more solid acid than molds. This work fails to agree with the results obtained by Fouts (41), who found that several lipolytic bacteria produced a greater proportion of volatile acidity than did G. candidum.

Nashif and Nelson (86) studied the characteristics of the lipase of Ps. fragi and observed that the lipase was destroyed by mild precipitants such as ethanol and ether and was sensitive to pH changes. They were able to precipitate the lipase by salting out with $(NH_4)_2SO_4$ below 7° C. and then recovered most of the activity. The optimum pH for the lipase of Ps. fragi was found to be from 7.0 to 7.2 when

cocoanut oil was used as a substrate, whereas the optimum for the lipase of most organisms is 7.8. Appreciable activity remained after heating the enzyme preparation to 61.6 or 71.6° C. for 30 minutes and complete inactivation of the enzyme required heating for 30 minutes at 99° C. The lipase preparations were more stable when allowed to stand at 3 to 5° C. than at 15 or 36° C.

The factors affecting the lipase production of Ps. fragi were investigated by Nashif and Nelson (87). Maximum production of extra-cellular lipase in 3 days occurred at 15° C. or below, the optimum temperature depending on the organism strain. Little or no detectable lipase was produced at 30° C. or above. With longer incubation times the lower temperatures were increasingly more favorable for lipase production. Sodium chloride inhibited lipase production. Greater lipase production was associated with increased Ps. fragi populations but was not closely proportional. These investigators found lipase production in defined media containing citrate was increased materially by supplementation with L-leucine or a combination of L-leucine, DL-isoleucine and DL-valine. Lipase production in some protein digests and hydrolyzates, particularly peptone, was much higher than in the chemically defined media tested. Also addition of small amounts of tricaprylin, caprylic acid or caproic acid to vitamin-free casamino acids or peptone media caused a

pronounced increase in lipase production of Ps. fragi.

In studying the action of the lipase of Ps. fragi in cream and butter, Nashif and Nelson (88) found the enzyme to be active in cream between the pH of 4.9 and 8.2 with an optimum between 5.7 and 6.6, using incubation at 36° C. for 24 hours. Growth and lipase production of Ps. fragi in cream at 15° C. for 3 days was slow; however, enough lipase was produced to cause extensive fat breakdown in the cream and resulting butter. Over 50 per cent of the lipase activity was apparent after pasteurizing the cream at 71.5° C. for 30 minutes. These investigators found that butter containing residual lipase underwent considerable fat degradation during storage, even at -10° C., and pronounced rancidity occurred at -5° C. and higher. There was no measurable lipase activity below pH 4.9.

Nashif and Nelson (89) studied the extra-cellular lipases of 12 cultures of Gram-negative, non-sporeforming, rod-shaped bacteria. All of the cultures examined produced lipases which were most active at pH 7.0 or slightly above. The optimum temperature for lipase production by the species of the genus Pseudomonas was at 21° C. or below, whereas the optimum for one culture of Ach. lipolyticum was 32° C. A culture of Ps. fluorescens and another culture which was not completely identifiable but resembled Ps. fluorescens produced considerable lipase in cream in 3 days at 21° C. These lipases remained at a high level of activity subsequent to

pasteurization at 71.6° C. for 30 minutes, indicating potential deterioration during holding of cream or the butter made therefrom. Lower levels of post-pasteurization lipase activity were found with several other cultures. Nonhomogeneity of the lipases produced by members of the genus Pseudomonas was demonstrated.

Nelson (90) investigated some characteristics of the lipase of G. candidum and found it to be inactive at pH 4.0 and completely destroyed at pH 3.0, even though the medium was immediately neutralized after being acidified. This is consistent with the knowledge that lipolysis of raw cream is retarded when a rapid natural lactic acid fermentation occurs. This investigator pointed out that a study of the substrate specificity showed that the extracellular lipolytic system of G. candidum contained a true lipase which was specific for triglycerides and exhibited maximum activity on naturally occurring triglycerides, this being the reason for the high W.I.A. values encountered in cream inoculated with G. candidum. This worker found the lipase in filtrates from cultures of G. candidum to be active over a pH range of 5.0 to 8.0 and a temperature range of 20 to 37° C. with an optimum at 30° C.

Nelson (91) also investigated the nutritional factors influencing growth and lipase production by G. candidum. The conditions which gave rise to rapid and extensive growth were associated with low lipase production, but were not

directly related. Rapid growth was produced by various carbohydrates, fats, organic acids, fatty acids and nitrogenous compounds. Water-insoluble vitamins had no effect. Maximum growth and lipase production in buffered carbohydrate-free media occurred at pH 5.35 to 5.85. Maximum lipase production in glycerol medium occurred at pH 6.70 to 6.85. This investigator also suggested that acid produced during carbohydrate utilization may have an influence on lipase inactivation as the pH declines.

Peterson and Johnson (94) studied the delayed hydrolysis of butterfat by certain lactobacilli and micrococci isolated from cheese. They found that 12 of 54 Lactobacillus cultures and four of eight Micrococcus cultures isolated from normal raw milk cheddar cheese, possessed intracellular lipases, active between pH 5.0 and 6.0, capable of considerable butterfat hydrolysis.

Roahen and Sommer (103) used sugar-saturated cream to inhibit organisms and studied lipolytic activity in milk and cream due to natural lipases. Gravity-separated cream showed much greater lipolytic activity than did centrifugally-separated cream of the same fat content. Incubation temperatures of 4, 27 and 37° C. were used, with lipolysis being greater at the higher temperature. The optimum pH for lipase activity at the lower temperature was found to be 8.4 to 8.6. These workers found natural lipolysis varied

considerably in milk from the same cow. A study of milks from 18 cows at known stages of lactation failed to indicate a relationship between lipolysis and stage of lactation. Also cream separated at 110° F. showed less lipolysis than cream separated at 75° F. Peterson et al. (95) found evidence of the presence of at least two natural lipases in milk, with the activity of each varying considerably from one cow to another. One lipase was more stable than the other.

Determination of fat hydrolysis in cream and butter

Water-Insoluble Acid Method. Of the various tests which have been developed for the quantitative measurement of fat hydrolysis in cream and butter, the water-insoluble acid (W.I.A.) method originally developed by Hillig (58) is receiving the most attention and is used most widely. The method is designed to determine all the water-insoluble free fatty acids in cream or butter whether present as acids or as alkaline salts. The mean molecular weight of the acids secured by this method indicates that they consist principally of oleic and palmitic acids produced by partial hydrolysis of milk fat. Analyses were performed on 15 samples of commercial churning cream and 12 samples of commercial butter. The values, calculated as milligrams of W.I.A. per 100 grams of fat, ranged from 100 to 2,022 for

the cream and from 51 to 1,278 for the butter. In this same work known quantities of oleic and palmitic acids were added to cream and butter with recovery analyses being performed as a check on the efficiency of the method. Recovery ranged from 93.7 to 105.9 per cent on 12 samples of cream, and from 93.2 to 105.9 per cent on 14 samples of butter. The average recovery was 96.3 per cent.

In an extensive study of 3 years duration, Hillig and Ahlmann (62) performed W.I.A. analyses on a great number of churnings of cream and the resulting butter, to determine the correlation between cream quality and W.I.A. value. The analyses were performed in 1944 with 43 churnings, in 1945 with 11 churnings and in 1946 with 46 churnings. Less W.I.A. was found in good quality cream and the resulting butter than in decomposed cream and the butter made therefrom. These workers found W.I.A. values for sweet cream butter ranging from 75 to 225 mg. per 100 g. of fat, whereas butter made from poor quality cream ranged from 300 to 700 mg. per 100 g. of fat. Samples of seven churnings were stored at 0 and 40° F. for periods extending to 22 months. With one exception, there was no significant change in W.I.A. content of the butter after 5 months, and in some cases even after 22 months at 0° F. At 40° F. mold appeared on some samples after 5 months and the W.I.A. content increased three-fold.

The correlation between progressing age and W.I.A. value was determined by Hillig *et al.* (65) in an experiment

in which quantities of cream were subdivided and held for varying periods of time. A typical example including analyses on the cream, resulting butter and buttermilk follows:

Age of cream in days	1	4	8	11
W.I.A. in the cream	234	657	934	1,052
W.I.A. in butter from the cream	225	659	952	1,084
W.I.A. in the resulting buttermilk	14	29	32	27

In some cases abnormally high W.I.A. values were encountered in sweet cream. A study of separation methods indicated water separators to be a contributing factor in early cream deterioration. These investigators performed W.I.A. analyses on many cans of cream actually used in commercial churnings and found values ranging from 50 to 60,000 mg. per 100 g. of fat. They also found values on butter ranging from 21 to 3,299. As a result of this investigation, these workers recommended that the maximum permissible W.I.A. content of legal cream and butter be established at 400 mg. per 100 g. of fat. This recommendation has achieved legal status with the Federal Food and Drug Administration.

The possibility of saponified fat being discarded in the ether-extract phase of the W.I.A. determination was investigated by Hillig (59). The ether-extract phase reacted negatively to a test which was able to detect as little as

1 mg. of W.I.A. derived from saponified fat. Hillig (59) has emphasized the necessity for using a minimum of 0.2 to 0.5 ml. of 1 N NaOH in excess of the amount required to neutralize the sample to the phenolphthalein end point. Addition of an excess of 2 ml. of 1 N NaOH did not cause any increase in the quantity of W.I.A., but low values were obtained if insufficient alkali was used. The presence of excess alkali suppresses the tendency of the soaps to hydrolyze in a water solution and facilitates extraction from the ether phase into the water phase.

The effect of holding temperature of cream on the W.I.A. content of butter was studied by Babel (9) who observed that there was no significant difference in the W.I.A. of butters made from cream held at 55° F. and 75° F. This investigator observed that when cream sours rapidly, the lipases are inhibited because of the unfavorable pH. Cream having a clean, high-acid flavor produced butter with a relatively low W.I.A. content, whereas cream with low acidity but evidence of putrefactive types of organisms usually produced butter with a high W.I.A. value. The W.I.A. content of the butters did not increase materially with an increase in age of the cream at either temperature of holding.

Armstrong et al. (7) studied the correlation of W.I.A. value and numerical score of commercial butter using the method of Hillig (58) except that fritted glass filters

were used, rather than gooch crucibles packed with asbestos. These workers found that 92 score butter uniformly contained less than 150 mg. of W.I.A. per 100 g. of fat. Butter scoring 90 had a W.I.A. range of from 137 to 303, 89 score butter varied from 174 to 409 and 88 score butter ranged from 183 to 612 mg. of W.I.A. per 100 g. of fat. Oleic and palmitic acids were added and recoveries by the W.I.A. method were found to range from 90 to 105 per cent.

In determining the effectiveness of the Four-day Grading Plan as a device for segregating cream with high W.I.A. values, Freeman and Barkman (47) performed W.I.A. analyses on cream secured from 15 buying stations in Kentucky during four seasons of the year. The cream also was examined for flavor, odor, acidity, fat content and sediment. The cream was churned under commercial conditions and included 27 churnings from 2,654 individual deliveries of cream. These investigators found a general relationship between W.I.A. content and age of cream. However, there were definite variations and age could not be used as a basis for predicting W.I.A. content. Winter cream had the highest W.I.A. content and fall cream the lowest. These workers particularly noted that organoleptic grade was not a satisfactory guide to W.I.A. content and they found no relationship between W.I.A. and other grading factors. Also noted was a wide variation in the proportion of the W.I.A. in the cream that was carried

over into the butter manufactured therefrom, which is contrary to the findings of Hillig et al. (65) previously mentioned.

Cream may be held under satisfactory refrigeration by the producer and then be subjected to atmospheric temperature for 1 or 2 days while passing through a buying station enroute to a churning plant. Hillig and North (67) simulated the effect of such treatment by holding cream separated from fresh milk, at 4° C. for 6 to 8 days and then removing the cream to 25° C. incubation. No marked deterioration occurred while the cream was held at 4° C.; however, when removed to 25° C., W.I.A. values increased as much as three-fold within 48 hours. This was especially true when lipolytic counts attained high levels at the termination of the 4° C. storage period.

The effect of the neutralization process on the W.I.A. and butyric acid values of cream was studied by Hillig et al. (64). These workers analyzed each of 36 churnings of cream before and after neutralization with regular commercial soda and lime neutralizers. They found that neutralization does not cause an increase in W.I.A. or butyric acid in cream or in the butter churned therefrom. They also reported that the W.I.A. content of butter usually will be less than the W.I.A. content of the cream from which it was churned. A survey of their data indicates that the values for butter are usually about 15 per cent less (with variations) than

the values for the corresponding cream.

Forster (37) observed that vacuum pasteurization devices do not remove any W.I.A. because the free fatty acids are not volatile under pasteurization conditions. Martin et al. (84) observed that there is a possibility of some fat hydrolysis occurring with steam injection type pasteurizers, thereby increasing W.I.A. values.

The effect of the continuous process of butter making on the W.I.A. and butyric acid values of the finished butter was studied by Hillig and Ahlmann (63). Some W.I.A. was removed in the centrifuging operation of the continuous system because the W.I.A. content of the oil immediately prior to vacreation was uniformly less than the value of the corresponding cream prior to separation. Characteristic decreases in W.I.A. randomly selected from their data were from 424 to 346 and from 277 to 228. Neutralization of the oil had no effect. The churning operation had no effect on W.I.A. content when the barrel churn was compared to the texturator. These workers found that mold mycelia counts of butter made by the continuous process were much lower than of butter made in a barrel churn from the same cream. Butter made by the continuous process and stored for 4 months at sub-zero temperatures did not show any change in W.I.A. value.

A portion of the U. S. D. A. Bureau of Dairy Industry

dairy herd at Beltsville, Maryland, was used by Hillig and Palmer (68) to determine if alteration of feeding regimes exhibited any influence on the W.I.A. content of cream. There was no change in W.I.A. when cows were removed from pasture and put on dry feed. Several different types of hay, grain and silage were fed with no indication that types of feed had any influence on W.I.A. content of cream separated from the milk produced by these cows.

Kester et al. (72) sought to determine whether defect-producing bacteria were responsible for increases in W.I.A. and butyric acid values in butter during storage, transportation and merchandising. Pure cultures of organisms were inoculated into cream which was promptly churned into butter, using sterile equipment and wash water. Ps. fragi produced little increase in W.I.A. in salted butter but considerable increase in sweet butter and Ps. fluorescens produced some increase in both salted and unsalted butter. Ach. lipolyticum caused considerable increase in W.I.A. and butyric acid in both salted and unsalted butter. Pseudomonas putrefaciens caused no increase in W.I.A. or butyric acid. These workers reported W.I.A.-to-butyric acid ratios ranging from 300 on a control down to 9 on an extremely rancid sample. The ratio of completely hydrolyzed triglycerides of normal butterfat would be about 26 and these investigators observed that the excess butyric acid may have come from lactose fermentation, a probability which previously has been

demonstrated and explained by Hillig and Montgomery (66).

Many samples of cream several days old were observed by Kester (71) to have low acidity and high W.I.A. values. This worker found that lack of extensive acid development favored the production and activity of the Ps. fragi lipase and also the activity of natural milk lipase.

The strong lipolytic ability of the molds is commonly recognized. Purko et al. (99) studied the rate at which G. candidum produced W.I.A. in sterile cream incubated at 4, 10, 16, 29 and 37° C. for 9 day periods. The cream was sampled at 3 day intervals. W.I.A. values as high as 30,000 mg. per 100 g. of fat were encountered in samples held at 37° C. for 9 days. These investigators used nine cultures of G. candidum and found that eight of these cultures produced rapid and extensive fat hydrolysis in cream.

Working with raw cream at incubation temperatures ranging from 38 to 85° F., Peters et al. (93) found that W.I.A. values tended to decrease as incubation temperature increased and that high W.I.A. values were associated with cream in which the pH did not drop rapidly. High W.I.A. values frequently occur in low-acid cream marketed in late winter or early spring. These investigators inactivated organisms in cream using one part of 36 per cent formaldehyde to 500 parts of cream and found that the native milk lipase con-

tinued to produce W.I.A. but pointed out that native lipase is not a significant factor in producing high W.I.A. values in the presence of developed acidity, for the enzyme is not active at low pH. These workers further noted that in several samples of cream to which no formaldehyde was added, the W.I.A. values declined during holding and they suggested that decreases may be due to utilization of a portion of the acids by microorganisms.

The W.I.A. determination is time-consuming, requiring from 10 to 12 hours (excluding reagent preparation) to properly analyze eight samples. This time requirement minimizes the commercial acceptability of the method. A "sorting method" which slightly reduced the time required per sample and reduces the completion time on a group of samples by about 1.5 hours has been developed by Hillig (60). The final drying and weighing is eliminated. The revision makes use of the fact that the molecular weights of the water-insoluble acids of cream and butter lie within a restricted range. This investigator found that in 71 of 73 samples of butter examined the mean molecular weights of the W.I.A.'s were between 260 and 280, with the average being 269 and, for simplicity of calculation, an average of 270 was assumed. The dried acids were dissolved in neutral alcohol or benzene, titrated with standard alkali, and the mean molecular weight of 270 used in quantitatively deter-

mining the W.I.A. The author reported that these results compared favorably with the gravimetric determination.

A rapid method, requiring only about 15 minutes, of estimating the W.I.A. in butter has been developed by Hillig (61). The method is applicable to butter only. If it is desired to determine the W.I.A. content of cream, the sample must be churned. The fat is thoroughly washed several times with ice cold water to remove the water-insoluble acids, then the fat is dissolved in ether and titrated with 0.05 N sodium ethylate, using phenolphthalein as indicator. The quantity of W.I.A. can be rapidly and accurately determined by calculation, using the titration value and the average molecular weight of the free fatty acids previously determined by Hillig (60) to be 270. The author reports the complete analysis on butter may be performed in 15 minutes, with a little longer required for cream. The titration method was compared with the original method of Hillig (58), which has become the official method of A.O.A.C. (8), using a great number of samples ranging from 170 to 2,200 mg. of W.I.A. per 100 g. of fat, and was found to yield results within 5 per cent of the original method. Reeder and Shadwick (100) have developed a field kit for use by plants in detecting objectionable cream by the rapid titration determination of W.I.A.

Use of basic dyes for detection of fat hydrolysis. The detection of fat hydrolysis by the use of several basic dyes was reported by Knaysi (73, 74). The dyes were nile blue, methylene blue, spirit blue and neutral red. They were studied with respect to preparation, solubility, stability and color contrast in the measurement of the degree of fat hydrolysis. Nile blue was found to be highly unstable and therefore not suitable for general use in fat analysis. The base of neutral red was recommended as being most suitable for general use for determining the quality of edible fats.

Knaysi and Guthrie (75) developed a method in which the base of neutral red dye may be used in estimating butter quality by measuring the degree of hydrolysis of the butterfat. One ml. of the melted fat is dissolved in pure xylol which is saturated with the base of neutral red and the resulting color compared with similarly prepared standards containing known quantities of oleic acid. Use of the base of neutral red dye to estimate the quality of milk fat has been reported by Krukovsky and Knaysi (77). The procedure was the same as used for butter. In neutral fat and in xylol the dye base gave an orange yellow solution. Free fatty acids formed red soaps with the dye base. The degree of intensity of the red color was proportional to the concentration of soap and therefore proportional to the concen-

tration of the free fatty acids. Greenberg et al. (52) modified the Knaysi and Guthrie (75) method for the estimation of free fatty acids in butterfat, to facilitate the use of basic neutral red dye in the field testing of cream for the purpose of estimating the W.I.A. content.

Armstrong et al. (7) have criticized the neutral red method because of the narrow color range, the instability and the difficulty of maintenance of standard solutions.

A method of detecting fat hydrolysis which depends upon the action of free fatty acids in the presence of an alcoholic solution of alpha-naphtholphthalein (ANP) has been developed by Roberts et al. (104). The dye solution is prepared by adding 0.1 g. of the dye to 150 ml. of 95 per cent ethyl alcohol and 100 ml. of distilled water. The dye is neutralized to blue-green color, using 0.1 N NaOH. Butter is melted in a water bath at 140° F. and 12 drops of the butterfat are measured with a standard medicine dropper into a test tube containing 5 ml. of the blue-green ANP indicator solution. The test tube is shaken vigorously five times and the color observed in 5 minutes. With increasing concentration of acid the color changes from blue-green or dark green to medium green, light green, orange, yellow and finally white. The orange color is considered to differentiate between positive and negative fat hydrolysis.

Armstrong and Harper (6) and Armstrong et al. (7) have also worked with the ANP colorimetric method for the measurement of fat hydrolysis in butter. They report the method will give a rapid roughly quantitative measure of free fatty acids within an accuracy of 100 mg. of W.I.A., below a concentration of 400 mg. of W.I.A. per 100 g. of fat. These workers state that by exercising extra precautions greater accuracy may be achieved because it is possible to detect color changes with variations of 25 mg. of W.I.A. at concentrations of less than 400 mg. of W.I.A. per 100 g. of fat. These investigators further found that the ANP method is not affected by the presence of butyric and lactic acids, but it does measure caproic and any longer chain fatty acids.

An adaptation of the ANP method for the determination of fat hydrolysis in cream, using ethyl alcohol and petroleum ether for extracting the fat, has been developed by Harper and Armstrong (57). For rapid field work in screening the quality of cream samples, these workers advise the direct use of the solvent extract, but for more reliable laboratory work, evaporation is accomplished and analysis performed on the solvent-free fat. These investigators used 30 samples of cream in comparing the Hillig (58) and ANP methods. They found that W.I.A. values of less than 300 mg. per 100 g. of fat gave a green ANP test, whereas W.I.A. concentrations above 300 mg. per 100 g. of fat gave an orange or yellow

color. By using the midpoint of each color range in comparing the ANP method to the Hillig method, they found an average difference of 36 mg. of W.I.A. when working with samples containing less than 400 mg. of W.I.A. per 100 g. of fat. They do not indicate whether the results by the ANP method were consistently higher or lower than the results by the Hillig method. However, they do point out that additions of known quantities of W.I.A. gave appropriate response in the ANP method of analysis.

Determination of free fatty acids by titration. Breazeale and Bird (19) developed a method of separating free fatty acids from fat and quantitatively determined the amount of free fatty acids by titrating with alcoholic KOH. These investigators reported that their method recovered from 94.6 to 99.4 per cent of added fatty acids and checked satisfactorily with the methods of Clarke et al. (22) and the A.O.A.C. (8). In the A.O.A.C. procedure the fat is weighed into neutralized alcohol and titrated with 0.1 N NaOH.

The Role of Various Organisms and Proteases in Protein Hydrolysis in Milk, Cream and Butter

Proteolysis by the lactic acid bacteria

One of the earliest studies of proteolytic activity of lactic acid bacteria was carried out by Peterson et al. (98),

using 22 strains of streptococci and lactobacilli. Non-protein nitrogen, amino nitrogen and ammonia nitrogen formation were used as measures of proteolysis. Non-protein nitrogen was the most abundant form produced. In some cases more amino and ammonia nitrogen was consumed than was produced, indicating nutritional utilization by the organism. There was no significant difference between streptococci and lactobacilli in proteolytic activity. In the case of both species, proteolysis continued long after sugar destruction had ceased.

Anderegg and Hammer (4) observed proteolysis by a large number of strains of S. lactis isolated from dairy products. Some, including all strains used in butter cultures, produced an increase in soluble nitrogen when grown in skim milk culture medium. Addition of calcium carbonate to neutralize acidity increased the proteolysis. These workers observed that organisms causing proteolysis coagulated milk quickly and that proteolysis was not due to the acidity developed. An increase in soluble nitrogen usually was accompanied by an increase in amino nitrogen. These investigators also noted that Streptococcus citrovorous (Leuconostoc citrovorum) and Streptococcus paracitrovorous (Leuconostoc dextranicum) did not cause proteolysis. Frazier and Rupp (46) noted that S. lactis, Lactobacillus casei and L. bulgaricus were able to decompose albumen in milk serum.

Hammer and Patil (55) investigated proteolysis by S. lactis with special reference to butter cultures and butter, using about 120 strains isolated from milk, cream and butter cultures. Proteolysis was measured by determining soluble and amino nitrogen in milk cultures. Appreciable proteolysis occurred in as little as 36 hours and the more rapid acid producers generally gave the largest amount of proteolysis, with proteolysis becoming evident shortly after coagulation occurred. In general S. lactis cultures fell into two groups, one of which coagulated milk quickly and caused proteolysis, the other being slow and variable in rate of coagulation and non-proteolytic.

Collins and Nelson (26) investigated the effect of S. lactis on the soluble nitrogen in milk, using four strains of organism. These investigators found a rapid increase in trichloroacetic acid-soluble nitrogen during the first day or two, followed by a gradual but smaller increase for a 15 day period, until approximately 15 per cent of the total nitrogen was in soluble form. Extent of increase varied with different strains. The results indicate that the proteolytic enzymes of S. lactis are not entirely endocellular, because proteolysis obviously was taking place before autolysis of the S. lactis cells occurred.

Morgan and Nelson (85) studied the distribution of ten amino acids in soluble fractions of milk cultures of S. lactis, by obtaining tungstic and lactic acid filtrates of

milk after incubation with S. lactis for 15 days at 21° C. Microbiological assays for free valine, leucine, isoleucine, threonine, arginine, methionine, histidine, tryptophan, tyrosine and phenylalanine revealed marked increases for the filtrates of inoculated milk as compared to the uninoculated controls. Active strains of S. lactis were able to effect a marked increase in leucine and phenylalanine during the first three days of incubation, with a slower increase extending through the fourteenth day.

Amundstad (3) reported that cell-free extracts of two strains of S. lactis grown in broth showed optimum proteolytic activity in peptone and caseinate solutions at pH 6.6 and 6.8, as determined by acid-soluble nitrogen and amino nitrogen. A cell-free extract of Streptococcus cremoris showed optimum activity at pH 6.3 to 6.5 and was greater in activity than the extract from S. lactis.

Baribo and Foster (12), in studying the intracellular proteinase of certain organisms isolated from cheese worked with a strain of S. lactis which was grown in carrot, liver extract broth. Proteolysis was determined by Kjeldahl analysis of nitrogen soluble in trichloroacetic acid. Sodium caseinate was used as a substrate. The cell-free extract was most proteolytic at 40 to 42° C. and at a pH of near neutral, with a second optimum at pH 5.0 to 5.5. There was some relatively stable proteolytic activity over a pH range of 3.4 to 8.6. The extract was activated by reducing agents.

Added metallic ions either had no effect or were inhibitory. All of these characteristics were verified in subsequent work by van der Zant (113).

Zimmerman (117) investigated the enzymes of ripening cheddar cheese and reported a cell-free extract of S. lactis did not show proteolytic activity when using either casein or hemoglobin as a substrate. The same extract caused hydrolysis of glycyl-L-leucine and DL-alanylglycine, with the optimum pH for hydrolysis of the former being 7.6 to 7.8, and 8.0 for the latter. Added manganous ions increased rate of hydrolysis of glycyl-L-leucine. No significant difference in the rate of ripening was found when the extract was added to cheddar cheese.

In studying proteolysis in milk by S. lactis, van der Zant and Nelson (114) observed that S. lactis organisms caused a rapid increase in both soluble nitrogen and tyrosine and tryptophan during the first 24 hours, followed by a smaller but gradual increase during the remainder of the experimental period of 72 to 90 hours. These workers found marked increases in tyrosine and tryptophan even when the production of soluble nitrogen, as determined by the Kjeldahl procedure, was negligible. Considerably more soluble nitrogen and tyrosine and tryptophan were produced when the reaction was controlled at pH 6.0 to 7.5, than in comparable samples without controlled pH. The cell-free culture medium did not cause proteolysis.

van der Zant and Nelson (115) studied the characteristics of an endocellular proteolytic enzyme system of S. lactis and found cell-free extracts, prepared from S. lactis grown in either milk or vitamin test casein medium and disintegrated sonically, showed the presence of a heat-labile proteolytic enzyme system with optimum activity against milk, casein and lactalbumen at pH values near neutrality. Presence of casein and nicotinic acid in the broth growth medium increased the proteolytic activity of the cell-free extract. The minimum level of proteolytic activity was at pH 5.0, which was low enough to limit the activity and significance of proteolysis from this source in most common types of cheese and acid dairy products.

van der Zant (113) also observed proteolysis by two strains of S. lactis grown in heated skim milk, with and without calcium carbonate added (10 g./100 ml. of milk), for 36 hours with the milk under agitation to prevent settling of the $CaCO_3$. With $CaCO_3$ present, the pH did not drop below 5.0, whereas it dropped to 4.1 without $CaCO_3$. Proteolysis was 2.5 times greater with the $CaCO_3$ present. Sterile lactic acid added to heated skim milk to give titratable acidities ranging from 0.2 to 1.0 per cent, followed by incubation at 32° C. for 24 hours failed to show any increase in soluble nitrogen or tyrosine and tryptophan, indicating protein breakdown was not due to lactic acid formation by S. lactis. This same conclusion

was reached by Anderegg and Hammer (4) and by Hammer and Patil (55).

The effect of heat on the proteolytic enzyme activity of cell-free extracts of S. lactis was determined by van der Zant (113). The optimum for heat stability was at pH 7.0, where proteinase activity was retained after heating to 50° C. for 30 minutes but was almost completely destroyed at 55° C. for 15 minutes. In another instance one peptidase was inactivated at 61.7° C. for 2 minutes at pH 7.0, whereas another retained activity under the same conditions, proving two peptidases of varying heat resistance to be present. At pH 5.0 and 9.0 the inactivation was quite rapid at even lower temperatures. This investigator checked the stability of cell-free extracts when stored at pH values ranging from 5.0 to 9.0 at 2° C. and 32° C. The extract was stable at 2° C. over the above pH range after 4 days storage, but at 32° C. considerable destruction occurred at pH 5.0 and 9.0, with less destruction at pH 6.0 and 8.0. When the extract was stored at pH 4.0 for 12 hours, the peptidase activity was completely destroyed.

Knowles (76) studied acid production and protein degradation of a number of different streptococci and micrococci in proteolyzing milk and found Streptococcus liquefaciens to be the most active proteolyzing organism of the group, with the most proteolysis occurring during the first 7 days.

Long and Hammer (80) worked with a number of cultures of acidoproteolytic streptococci and found they coagulated milk by enzyme action rather than acid formation. Titratable acidity of 15 of these cultures at time of coagulation averaged 0.27 per cent and the pH averaged 5.9. With this group of organisms, proteolysis was largely complete after a comparatively short incubation period of 2 to 5 days, with some continued increase up to 28 days, when incubated at 21 and 37° C., with temperature making little difference.

Dudani (31) studied the characteristics of peptidases present in a cell-free extract of S. liquefaciens and observed two optima at pH's 5.0 and 8.0, for peptidase activity against glycyl-L-leucine. Two pH optima, at 6.9 and 8.2, also were found against DL-alanylglycine and at the higher pH hydrolysis was activated by manganous, cobalt and manganese ions. A pH of 7.4 was optimum for digestion of both casein and lactalbumen.

Braz and Allen (18) studied protein metabolism and acid production by the lactic acid bacteria and observed that most species of streptococci and lactobacilli, when grown in milk at 20° C., produced an increase in protein after several weeks incubation. These investigators state that this increased protein is derived mainly from the proteose-peptone fraction, which shows a corresponding decrease. It was observed that occasional species showed an exception and

caused protein degradation. Also mixed cultures of either streptococci or lactobacilli brought about marked proteolysis after 4 weeks. Addition of chalk increased proteolysis. Growth of these organisms in milk resulted in concomitant synthesis and degradation of proteins and their split products, the latter being inhibited by high acidity. Neutralization with $CaCO_3$ removed the inhibitory effect and resulted in a preponderance of protein degradation. These workers were unable to associate proteolytic ability to acid-producing ability of lactic acid organisms. However, Hammer and Patil (55) observed that the more rapid acid producers were more proteolytic.

Proteolysis by miscellaneous organisms

Frazier and Rupp (43) isolated and classified 229 cultures of various species of organisms proteolytic in milk. Tryptophan determinations were performed using the bromine test and amino nitrogen was measured by the formol titration. Cocci generally produced only a moderate increase in amino nitrogen in milk. Bacilli varied from weakly to actively proteolytic. These workers also noted that autoclaved milk favored action of the more actively proteolytic organisms, but had no advantage over steamed milk in the case of the weakly proteolytic organisms.

Frazier and Rupp (44) studied the action on casein and gelatin of the same 229 organisms proteolytic to milk. Only

four cocci and four rods were found which produced no increase in amino nitrogen on casein and gelatin. Several organisms were observed to split calcium caseinate more readily than sodium caseinate.

Frazier and Rupp (45) inoculated the 229 organisms into synthetic media which contained various simple nitrogenous compounds as sole source of nitrogen and observed for population increase, increase in ammonia and change in pH. Organisms which used ammonia as a sole source of nitrogen apparently were able to use any of the simpler amino acids if the medium contained a fermentable sugar as a source of carbon. Some organisms which used urea as a sole source of nitrogen caused an alkaline reaction due to liberation of ammonia, whereas others caused an acid reaction and liberated no free ammonia.

Frazier and Rupp (46) incubated cultures of proteolytic bacteria in milk for 10 days at 30° C. and analyzed for total, protein, non-protein, amino and ammonia nitrogen. Most organisms which decomposed casein also broke down albumen, with some cocci decomposing a greater proportion of the casein than of the albumen.

Dernby and Blanc (30) studied the optimum and limiting pH of the anaerobes Clostridium sporogenes, C. histolyticum, C. canadiense, C. putrificum and C. perfringens. The range in which they all lived was from pH 5.0 to 9.0. The optimum

for all seemed to be about 7.0. Gelatin and peptone were proteolyzed in the pH range of 4.0 to 8.0. The optimum for both of these proteolytic activities seemed to be about 6.0.

Lisk (79) studied the decomposition products of spore-bearing bacteria in heated milk, using the following organisms: Bacillus cereus, B. albolactis (cereus), B. mesentericus-vulgatus (subtilis), B. mesentericus-fuscus (subtilis), B. megatherium, B. simplex (cereus), B. subtilis-viscosus[1] and B. brevis. The organisms were inoculated into sterile milk and changes observed in ammonia content, amino nitrogen, lactose, pH and volatile acid. All the above organisms showed a progressive increase in ammonia nitrogen. Decomposition of milk protein proceeded beyond amino acids to form indol. Hydrogen sulfide formation was noted in some cases. Decrease in pH accompanied an increase in titratable acidity in all cultures except B. albolactis.

Parfitt and Spitzer (92) investigated the action of certain organisms such as B. mesentericus, B. albolactis and B. mycoides (cereus) upon the proteins of milk and gelatin. They found greater hydrolysis in gelatin than in milk, with the greatest hydrolysis on milk occurring

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[1]U.S.D.A. Agr. Monograph 16 (109) places B. subtilis var. viscosus of Chester as only B. subtilis of a probably smooth form.

in neutral solutions. They considered that the acidity produced from the lactose retarded the action of the proteases.

Knowles (76) in studying organisms proteolytic in milk, observed that yellow hemolytic staphylococci produced more proteolysis (about 13 per cent of the total protein) after holding for 7 days or more, than other staphylococci. This worker also observed proteolysis in milk by Micrococcus freudenreichii and noted that Micrococcus caseolyticus proteolyzed casein, producing only water-soluble intermediate products.

Berger et al. (13) studied the peptidases from autolyzed cell preparations from Leuconostoc mesenteroides. He observed that there were at least two dipeptidase and two tripeptidase enzymes present, each of which had two pH optima for activity, one between 5.0 and 6.0 and the other between 7.0 and 8.0. Activity of these enzymes was stimulated by several metallic ions.

Long and Hammer (82) found Ps. putrefaciens had low acid resistance and was destroyed at pH values of 5.3 and lower. Cultures of Ps. putrefaciens proteolyzed litmus milk, with complete digestion occurring in 2 to 4 weeks, with the final pH after 32 days ranging from 5.7 to 7.0. The growth temperature range was found to be from 3 to 30° C.

Albert et al. (1) found Bacterium linens in 2 per cent peptone grew at a pH range of 6.0 to 9.8, with no growth at

pH 5.0. Cultures of this organism produced an alkaline reaction and then conspicuous proteolysis. During extended incubation soluble nitrogen greatly increased and there was some increase in amino nitrogen. The different strains varied in the extent of proteolysis produced. This organism liquefied gelatin in 15 days or more and had a significant characteristic of growing in 15 per cent NaCl in broth or skim milk.

Hucker (69) studied some of the acid-proteolytic cocci when grown in milk and observed that the micrococcus types produced from 8.6 to 58.9 per cent soluble nitrogen in excess of controls. Peptonization was increased when $CaCO_3$ was added to reduce acidity. Several species of micrococci were observed to liquefy gelatin and split casein.

Berger et al. (14) obtained cell-free extracts by freezing and thawing from cultures of Escherichia coli. B. megatherium, Proteus vulgaris, Ps. fluorescens, B. mesentericus, B. subtilis, C. butylicum, C. sporogenes, C. acetobutylicum, Lactobacillus pentosus, Propioniobacterium pentosaceum and Phytomonas tumefaciens (Agrobacterium tumefaciens). The peptidase activity of these extracts was determined and the optimum for peptide hydrolysis was from pH 8.0 to 9.0 for all except L. pentosus and P. pentosaceum, which contained acidopeptidases active at pH 5.5 to 6.0.

Studies on the activity of proteinases

Ferris (34) studied the proteolysis of cream and butter, determining amino nitrogen and nitrogen not precipitated by phosphotungstic acid. Proteolysis in cream began as soon as the acidity reached 0.2 to 0.3 per cent. The resulting butter held in storage showed only a slight increase in soluble nitrogen compounds when the butter was made from sweet cream, while the butter made from neutralized cream developed a much greater per cent of soluble nitrogen. Representative samples of cream and butter secured from 13 creameries were examined by Ferris (35). "Second grade" cream showed values of 8.1 to 12.6 per cent of the total nitrogen present as amino nitrogen, whereas the values on "first grade" cream ranged from 1.6 to 9.6 per cent. The amino nitrogen values on the "second grade" butter ranged from 3.4 to 8.0 per cent and the values on the "first grade" butter ranged from 0.8 to 6.3 per cent. The score designations of the grades were not indicated. Presumably the "first grade" referred to 90 score or grade B, and the "second grade" referred to 89 score or grade C.

Talce (111) found that proteinase activity of several microorganisms occurred at varying pH levels in butter. Considerable reduction in butter quality due to proteinase activity was observed at widely differing pH levels.

The increases of free tryptophan and tyrosine, as a measure of the activity of pure trypsin and pepsin upon milk

variously treated, were observed by Lembke et al. (78). Examination by chromatography showed that trypsin liberated tryptophan only, whereas pepsin liberated proline, hydroxyproline and tyrosine from raw milk. Boiled skim milk was degraded more readily by trypsin than was raw skim milk, whereas the opposite was true with pepsin. Irradiated skim milk retarded the action of both enzymes.

Peterson et al. (96) found the optimum activity of cheese proteinase to be at pH 5.0, whereas the endocellular proteinase of S. lactis showed optimum activity against casein at pH 7.0, suggesting that the proteinase derived from cheese did not come from S. lactis.

Peterson et al. (97) found most of the proteinase activity in cheese to be of bacterial origin and only a small amount due to rennin and milk proteinase. These workers observed a greater quantity of cysteine-activated proteases in raw milk cheese and considered these proteinases to be especially desirable in cheese ripening, for they appear to be at least in part responsible for the more rapid flavor development in raw milk cheese. These investigators suggested the desirability of isolating the raw milk organisms which are responsible for cysteine-activated proteinases and adding them to cheese starter.

Several staphylococci and micrococci were observed by Knowles (76) to produce significant degradation in the protein

of milk at pH values found in ripening cheese. These organisms might have possible value as ripening agents but are considered undesirable because of their possible effect on flavor.

Storrs (110) found that if pancreatic enzymes used for producing soft-curd milk were allowed to act in milk sufficiently long before pasteurization, pronounced hydrolysis occurred. He observed that free tyrosine increased with the degree of enzyme addition and estimated that in properly treated samples, protein hydrolysis amounted to approximately 1 per cent of the amount theoretically possible.

Methods of measuring proteolysis of cream and butter

Several methods of measuring proteolysis in dairy products have been used. The Kjeldahl method for nitrogen determination in the various fractions of protein breakdown products has been used most extensively. The Van Slyke (116) method of quantitative determination of amino acids also has been commonly used as a measure of the rate and extent of protein degradation.

In measuring the extent of proteolysis in milk, Frazier and Rupp (43) used a bromine test for determination of free tryptophan in milk. They considered this method to be only approximate, as it depended upon comparison with a standard of limited stability prepared by adding bromine to a previously acidulated control.

Folin and Ciocalteau (36) developed a method involving a phenol reagent for colorimetric determination of tyrosine and tryptophan in a trichloroacetic acid filtrate of the substrate. Anson (5) made use of the Folin-Ciocalteau reagent to determine the effect of pepsin, trypsin, papain and cathepsin, using a denatured hemoglobin solution as a substrate. Hull (70) used the phenol reagent prepared according to Folin and Ciocalteau on a 0.72 N trichloroacetic acid filtrate of precipitated milk protein to determine protein hydrolysis by quantitatively measuring free tyrosine and tryptophan. Rhodes (101) used this method in following protein degradation in cottage cheese. The method has been demonstrated to be sensitive and accurate.

Measurement of proteolysis by sensitive methods depending upon the ability of native protein to bind dyes have been reported by Grief (53) and Carroll (21). Using anionic dyes, Carroll was able to measure the effect of as little as 1 part per billion of pepsin on bovine albumen. These dyes impart pigment to the intact proteins, such as albumen, but the hydrolyzed protein fraction does not react with the dye.

Duggan (32) developed a sensitive colorimetric method of determining the free tryptophan in milk, cream and butter and performed numerous analyses on these products. Ninety per cent acetone was used as the solvent to extract free tryptophan. Purified para-dimethylaminobenzaldehyde was

added in the presence of an oxidizing agent to develop a blue color in the protein-free filtrate containing tryptophan. This investigator analyzed numerous samples and reported free tryptophan values as low as 0.6 p.p.m. for high quality milk and the maximum value was 32.6 p.p.m. for a sample of cream which had been incubated 240 hours at 37° C. Recoveries generally ranged between 90 and 110 per cent. This worker reported the following conclusions concerning the application of the method:

1. Negligible quantities of free tryptophan are present in normal sweet milk and cream.
2. The amount of free tryptophan in milk and cream increases with age if the products are held under conditions conducive to bacterial and enzymatic activity.
3. The amount of free tryptophan in butter depends upon the free tryptophan content of the original cream.

Erekson (33) used the Duggan Method as a device to follow the ripening progress in cheese. Mild flavored cheese contained from 15 to 91 gamma of free tryptophan per gram, while cheese "high" in flavor contained up to 889 gamma per gram. Experimental raw milk cheese cured 9 months at 55 to 60° F. showed an average of 296.3 gamma per gram, compared to 117.2 gamma per gram for pasteurized milk cheese cured under the same conditions, with the raw milk cheese being much "higher" in flavor.

Miscellaneous Quality Control Tests for Cream and Butter

Several miscellaneous quality control tests for cream and butter have been developed in recent years. Claydon (23) developed a colorimetric method for estimating the quality of cream. Blue color was developed by adding crystal violet solution to a cream sample previously treated with a fixed quantity of NaOH. The intensity of the color varied with the quality of the cream. The sample was evaluated by being compared with a previously prepared permanent standard. The color value was primarily related to the acidity and physical condition of the cream.

A fluorescent method for assessing the keeping quality of butter has been developed by Grant and White (51). These investigators observed fluorescence to be a characteristic of the salt extract of the defatted material. The method reflected deteriorative changes in the non-fat component. There was a close correlation between fluorescence measurement and flavor score, but the method did not detect undesirable flavor due to absorbed materials. The method also is used to detect deterioration in egg powder.

Butyric acid values in cream have been considered to be a measure of the degree of fat hydrolysis. However, research by Hillig and Montgomery (66) has demonstrated that considerable quantities of butyric acid may be formed by lactose degradation. In establishing the source of butyric acid in

deteriorating milk and cream, these workers held raw cream 2 to 3 weeks at 20, 25 and 32° C., withdrawing samples at frequent intervals and analyzing for lactose and lactic acid. Throughout the incubation period lactose decreased progressively and during the first few days lactic acid increased. Later in the incubation period lactic acid decreased and volatile acid, principally butyric, determined chromatographically, increased rapidly. Butyric acid appeared to be arising out of lactose degradation, but fat hydrolysis was investigated as a possible source. The Reichert-Meissl number on the fat extract and the distillation constant on the acids remained unchanged throughout the interval. Inasmuch as the butyric fatty acid contributes to the Reichert-Meissl number, the source of increase could not have been fat hydrolysis. To strengthen this conclusion these investigators incubated, sampled and analyzed skim milk in the same manner as the cream samples. As much as 0.57 per cent butyric acid developed in the skim milk, whereas they calculated a maximum of only 0.003 per cent would have been possible if fat degradation was the sole source of butyric acid. Formulae were presented showing how lactose may decompose to butyric acid. The sum of the breakdown products formed added up to the original lactose equivalent. These investigators analyzed a number of samples of cream offered for commercial churning and found from 0 to 300 mg. of butyric acid per 100 g. of fat. Analyses on 321 samples

of commercial butter showed a range of from 0 to 38 mg. of butyric acid per 100 g. of fat. No determinable butyric acid was found in any of 23 samples of sweet cream butter. These investigators concluded that when butyric acid was present in cream, some was usually carried over into the butter made therefrom, the proportion being unimportant, for a detectable amount indicated decomposed cream was used. Results of this study led to the recommendation that a maximum legal tolerance of 2 mg. of butyric acid per 100 g. of fat be established for cream and butter. This standard has official status with the Federal Food and Drug Administration but is used less commonly than the W.I.A. standard.

The effect of neutralization on the butyric acid content of cream and the resulting butter was investigated by Hillig et al. (64). After determining the butyric acid content of the cream and the resulting butter for 36 commercial churnings, these workers concluded that neutralization had no effect on the butyric acid content of the cream or of the butter made therefrom.

Hillig and Ahlmann (63) observed that in the continuous process of buttermaking, about one-half of the butyric acid present in the cream was retained in the oil, with none being lost as a result of vacreation, neutralization or churning of the oil.

Cream was held by Hillig and North (67) at 4° C. for 6 to 8 days and then removed to 25° C. for 2 days in order to determine the effect of adverse exposure subsequent to proper refrigeration. Similar circumstances are frequently encountered in cream properly protected by the producer, but abused en route to a churning plant. These workers observed as much as three-fold increases in lactic and butyric acids within 48 hours after exposure to the higher temperature. In most cases the butyric acid did not appear until the cream deteriorated to "class II", which apparently corresponded to 90 score or grade B.

Kester et al. (72) observed that Ach. lipolyticum inoculated into sterilized cream caused considerable increase in butyric acid in salted and unsalted butter. Ps. putrefaciens caused no increase in butyric acid. These investigators concluded that butter made from satisfactory cream may become unsatisfactory from the standpoint of W.I.A. and butyric acid values if heavily contaminated with lipolytic organisms.

Peters et al. (93) found high butyric acid values in cream samples held 7 days at 85° F., in which the W.I.A. values had barely changed. These results suggested that butyric acid was arising from some source other than fat hydrolysis, presumably lactose degradation. In one sample examined by these workers, 1,406 mg. of butyric acid per 100 g. of fat was produced, which would indicate hydrolysis of

half of the fat present. This degree of hydrolysis was sufficiently improbable to justify the assumption that butyric acid was formed from other sources, such as lactose.

Interrelationship and Activity of Organisms Grown as Mixed Cultures in Milk and Cream

Only a few reports have been published concerning the interrelationship of microorganism activity in milk and cream.

Lactic streptococci and lactobacilli

Rogers (105) observed that when equal inoculations of S. lactis and L. bulgaricus were introduced into sterilized milk the S. lactis was dominant in the inoculum, and a typical S. lactis fermentation ensued. Incubation was at 30° C., which was definitely partial to S. lactis development and domination.

Hansen (56) prepared extracts of autolyzed cells of several strains of S. lactis and S. cremoris which, when added to milk cultures of L. casei, raised the end point of fermentation. This worker believed that an effect of this nature may be of significance in explaining the predominance of L. casei in cheddar cheese, a medium in which lactic streptococci develop abundantly and then die and disintegrate.

A different opinion concerning the relationship between

S. lactis and L. casei is reported by Baribo and Foster (11). These workers used nine strains of S. lactis and S. cremoris and three commercial starter cultures. All of the foregoing cultures produced a heat-stable substance which inhibited L. casei. The material also slightly inhibited certain other strains of lactic streptococci. The lactic streptococci varied considerably in their ability to produce the inhibitor. Strains of L. casei differed markedly in their susceptibility to the substance. When given sufficient time (several days) the lactobacilli overcame the effect of the inhibitor. These investigators noted that most of the inhibitor was formed by the lactic streptococci during the first 24 hours of their growth in broth, although the concentration continued to increase slowly for as long as 60 hours. Whey and curd obtained during cheddar cheese manufacture were inhibitory to the only strain of L. casei tested in this manner. Baribo and Foster (11) suggested that the inhibitory substance, which seemed to arise out of normal starter cultures, was responsible for the early domination of S. lactis in cheddar cheese ripening, but as ripening progressed the few resistant strains of L. casei gradually achieved numerical supremacy and dominated the cheddar cheese flora.

The characteristics of the growth inhibitor for L. casei produced by lactic streptococci were investigated by Baribo and Foster (10). The inhibitory substance produced by one

strain of S. lactis was associated with the cells that produced it, but was liberated from the cells by acid development. Boiling or steaming a culture of S. lactis for 10 minutes at pH 4.8 had little effect on its inhibitory activity, but heating at pH 7.4 rapidly destroyed about half the inhibitor in the culture. At acid reactions the substance is relatively stable to heat. These investigators found that the inhibitory material was more active at acid reactions, with its ability to inhibit a test culture of L. casei being markedly increased when the reaction of the culture medium was below pH 5.4.

Effect of acid on Lactobacillus acidophilus and S. lactis cultures

Black and Harris(15) considered that acidity materially affected the activity of L. acidophilus cultures and suggested that in order to derive maximum benefit from a culture the acidity at which the maximum number of organisms is secured should be determined for each strain. Most strains of L. acidophilus gave maximum populations at acidities of 1.0 per cent, with some individual variations. In all but one strain a decrease in population occurred when the acidity reached 1.2 per cent and much greater destruction was evident at acidities of 1.5 to 1.8 per cent. Storage at 9° C. resulted in less loss of population than storage at 20 to 24° C.

Sherman and Hodge (107) separated fast-growing from slow-growing strains of S. lactis and found slow growth was associated with greater acid tolerance.

Effect of free fatty acids on certain organisms

Tarassuk and Smith (112) observed that rancid milk had an inhibitory effect on the growth and acid fermentation of S. lactis. This inhibitory effect was shown to result from the low surface tension of rancid milk and appreciable growth of S. lactis in rancid milk was shown to increase the surface tension. Under optimum conditions of growth in respect to temperature of incubation and initial number of organisms, the increase in the surface tension of rancid milk due to S. lactis activity yielded a final surface tension value approaching that of normal milk. The change in surface tension apparently resulted from the utilization of surface tension-lowering fatty acids by S. lactis.

The inhibitory effect of rancid milk on certain bacteria was studied by Costilow and Speck (28). Milk which had undergone lipolysis to the extent that the surface tension was reduced about 10 dynes per centimeter, was found to have a definite inhibitory effect on S. lactis, Streptococcus zymogenes and L. casei. The inhibition of Streptococcus bovis and E. coli, though detectable, was definitely less than that of the above organisms. L. bulgaricus and Pseudomonas aeruginosa were not measurably affected. Control

samples demonstrated that the inhibition displayed was not caused by either the slight reduction in pH or the reduced surface tension in rancid milk. Sterile active lipase showed no direct effect on the growth of S. lactis other than through its lipolytic action on the fat in milk. The results of the investigation indicated that inhibition was caused by some component of rancid milk and that the extent of inhibition of S. lactis was in direct proportion to the degree of lipolysis occurring in the milk.

Costilow and Speck (27) studied the inhibition of S. lactis in milk by fatty acids. In an effort to determine the cause of the inhibitory property of rancid milk on S. lactis, all of the fatty acids common to milk fat were tested for their effect upon the growth of this organism. Caprylic, capric and lauric acids inhibited growth of S. lactis, with the degree of inhibition increasing as the concentration of the acid increased. The effect of myristic acid was doubtful and none of the other acids tested was found to be inhibitory. These workers disagree with the belief of Tarassuk and Smith (112) by stating that the inhibitory property of rancid milk and milk containing fatty acids is not the result of low surface tension, but is due to some other unexplained toxic effect of the individual fatty acids.

Effect of lactic streptococci on the growth of miscellaneous organisms

S. lactis has been found to influence the growth of several organisms. Nashif and Nelson (88) observed that a reduced pH caused by extensive growth of S. lactis in cream did not result in any appreciable inhibition of the growth of Ps. fragi but did cause marked reduction in lipase production and activity. The lipase of Ps. fragi showed no measurable activity below pH 4.9.

Albert et al. (1) found that when litmus milk was simultaneously inoculated with S. lactis and B. linens the B. linens population declined rapidly.

Claydon (24) observed a skunk-like odor in commercial cream and resulting butter, which had originally been attributed to Pseudomonas mephitica by Claydon and Hammer (25). Further study demonstrated this defect to be the result of associative action of Pseudomonas species and S. lactis. The development of the defect in milk, cream and butter appeared to depend on balance of organisms, growth temperature and pH.

Marshall (83) observed the effect on lactic acid fermentation, when B. subtilis was added to a milk culture of S. lactis. Presence of B. subtilis stimulated the lactic fermentation and the higher the B. subtilis concentration in the original milk, the greater the stimulus in the early stages of the fermentation. In the original milk the optimum

ratio of B. subtilis to S. lactis, was approximately 22 to 10,000. The stimulus was more evident at low incubation temperatures.

Cox and Whitehead (29) grew each of four organisms commonly found in contaminated milk, in association with lactic streptococci and determined the acidity produced in a given time. The organisms were B. coli (E. coli), B. subtilis, Bacillus faecalis-alkaligenes (Alcaligenes faecalis) and a staphylococcus. B. subtilis stimulated the production of acid. B. coli was variable, causing an increase in acid production in some experiments and a decrease in others. The staphylococcus had a perceptible stimulating effect and B. faecalis-alkaligenes had a slight stimulating effect which was barely beyond experimental error.

The associative growth of B. cereus and S. lactis was investigated by Garvie and Stone (49). When each of three strains of B. cereus was grown in turn in milk at 22° C. with each of two strains of S. lactis and each of two strains of the coli-aerogenes group, the growth of the spore-formers was not affected. Regardless of initial proportions of B. cereus and S. lactis, the streptococci greatly outnumbered the spore-former at the end point of the clot-on-boiling test. B. cereus did not develop in milk held at 15° C.

Some miscellaneous factors affecting organism activity in cream and butter

Fouts (38) investigated the effect of lactic acid on the hydrolysis of fat in cream by pure cultures of several lipolytic organisms. O. lactis and all of the species of bacteria studied were inhibited somewhat by the growth of butter culture organisms in cream. However, the yeast Myc. lipolytica showed increased growth in the presence of the butter culture organisms. Excessive amounts of lactic acid added to cream inhibited growth of O. lactis, Myc. lipolytica and Ach. lipolyticum; however, all of the organisms grew at titratable acidities of 1.0 per cent and the first two species grew sufficiently at an acidity of 2.08 per cent to cause lipolysis.

Nelson (90) observed that the lipase of G. candidum was inactive at pH 4.0 and destroyed at pH 3.0. Lipolysis was retarded when cream experienced a natural rapid lactic acid fermentation and the rate of lipolysis decreased in direct proportion to the increase in acidity.

Garrison (48) noted that many of the fluorescent Pseudomonas organisms were inhibited by acid produced by lactic culture organisms. This investigator was unable to isolate fluorescent bacteria from extremely sour cream.

The use of yeast cultures for the prevention of mold on butter has been investigated by Blok (17) and Bogdanov

and Maksimova (18). These workers have reported that yeast cultures alone and mixed cultures of yeast cells and lactic streptococci protected butter against mold growth for 1.5 months when 10 ml. of cell suspension was used per liter of butter.

METHODS

Pure Cultures of Organisms Used

The following pure cultures of organisms were used to inoculate sterile cream: Streptococcus lactis, Geotrichum candidum, Torula cremoris, Pseudomonas fragi, Aerobacter aerogenes, Lactobacilli casei and Bacillus subtilis.

Source and characterization of organisms

Two cultures of S. lactis were used during the progress of the research project. One was secured from the Iowa State College Dairy Bacteriology Laboratory where it was designated as "Strain 712". The other strain was isolated from raw milk delivered by a producer to the Iowa State College Creamery. Both strains agreed with the characterization for S. lactis indicated by Breed et al. (20).

G. candidum was isolated from raw cream secured from the Iowa State College Creamery and agreed with characteristics listed by Skinner et al. (108).

T. cremoris was secured from the Iowa State College Dairy Bacteriology Laboratory where it carried the designation "Strain B". The organism agreed with the characteristics found in Hammer (54) and keys of Stelling-Dekker and Lodder found in Skinner et al. (108).

B. subtilis was secured from the Iowa State College Dairy Bacteriology Laboratory where it was designated as "Strain 1". The organism agreed with characterizations found in Breed et al. (20) and Smith et al. (109).

L. casei was secured from the Iowa State College Dairy Bacteriology Laboratory where it was designated as "Strain 4". The characteristics of the organism agreed with those found in Breed et al. (20).

Ps. fragi was isolated from raw milk secured from the Iowa State College Creamery. The characteristics were in agreement with those found in Breed et al. (20).

A. aerogenes was isolated from a sample of raw cream secured from Texas Technological College Creamery. The characteristics were in agreement with those found in Breed et al. (20).

Culture purification and activation prior to use

All cultures, except S. lactis which was carried in litmus milk, were carried on standard agar slants and transferred at least once a month when not in use. All cultures were activated in a broth medium suited to the particular culture and transferred a minimum of three times in litmus milk, at favorable incubation temperatures in order to assure inoculation with a vigorous culture. Broth media were used for activation in order that growth could be observed and vigorous activity demonstrated prior to use.

All cultures were plated on appropriate media and examined microscopically for purity prior to inoculation.

Reagents

The oleic, palmitic and stearic acids used in the W.I.A. recovery trials were A.C.S. standard and secured from Baker Chemical Co., Phillipsburg, N. J.

The asbestos used in packing Gooch crucibles for the W.I.A. determination was long fiber, acid-and-alkali-washed according to the A.O.A.C. Method (8), with the alkali and acid digestions being extended for 3 days each.

Dicalcite speedex, the filter aid used in the W.I.A. determinations, was secured from the Great Lakes Carbon Corp., Chicago, Ill.

Filter paper used for the tryptophan determination was Whatman folded No. 12 for the first filtration and Schleicher and Schuell white ribbon No. 589 for the second filtration.

The para-dimethylaminobenzaldehyde used as the color developing reagent in the tryptophan determination was secured from the Eastman Kodak Co., Rochester, N. Y., and was purified according to the method of Gilman and Blatt (50). The purified reagent was stored at 5° C. in a brown glass bottle and the same lot (No. 8421) was used throughout the research project. The color developing reagent was prepared by dissolving 0.4 g. of the purified para-dimethylaminobenzaldehyde

in 5 ml. of acetic acid and then mixing with 92 ml. of phosphoric acid and 3 ml. of concentrated hydrochloric acid.

The DL- tryptophan and L-tryptophan used in preparation of the standard curve and in recovery trials were secured from the Nutritional Biochemicals Corp., Cleveland, Ohio.

Acetone used in the tryptophan analysis was distilled, with the last 5 per cent being discarded to avoid pigment, and diluted with 10 per cent distilled water. The 90 per cent acetone was neutralized to the end point of phenolphthalein with 0.1 N NaOH immediately before using.

Alumina cream used in the tryptophan determination was prepared according to the A.O.A.C. Method (8).

All miscellaneous chemical reagents used were A.C.S. standard and were secured from the Baker Chemical Co., Phillipsburg, N. J.

Source of Cream and Butter

Inoculated cream samples

The cream used in this work was secured from the creamery of either the Iowa State College or the Texas Technological College. The cream was grade A quality and was standardized to 35 per cent fat, using grade A milk. The purpose of standardizing was to eliminate fat and serum

variables and thus establish uniformity of composition for the benefit of organism response and to simplify the computation of subsequent analytical values.

Uninoculated commercial samples

In one portion of the investigation the fermentation of three qualities of unsterilized, uninoculated cream was followed. One of these samples secured from a local bottling plant was fresh (current day) surplus grade A cream being channeled to sweet cream butter and ice cream mix. The other two samples were mixtures of a 2-day accumulation of both evening and morning cream secured from producers routinely delivering to a local churning plant. It was necessary to take a 2-day accumulation of cream for the latter two samples because no appropriate producers could be found whose 1-day production would provide a sufficient quantity of cream. The two producer samples were selected in such manner as to provide medium and poor quality cream. For the purpose of uniformity and in order to simplify calculations, all three samples of cream were standardized to 35 per cent fat, using sterile skim milk. None tested above 40 per cent fat originally.

Sour cream samples for a series of tryptophan and other analyses were secured from a small local churning plant making 89 score or grade C butter.

Butter samples analyzed for free tryptophan were secured from normal distribution outlets.

Experimental Procedures

Preparation of cream

In each separate experiment the cream was mixed sufficiently to assure homogeneity and immediately divided in such manner as to place 750 g., a quantity sufficient for future sampling requirements, into each of eight pyrex one-liter florence flasks. These flasks were tightly plugged with non-absorbent cotton and capped with two layers of parchment secured by rubber bands. The cream was then steamed for a minimum of 1 hour on each of 3 consecutive days in either an Arnold steamer or an autoclave operated without pressure. Throughout the 3-day preparation period, the cream was refrigerated at 5° C., except during the steaming interval. Two of the flasks of cream, designated as controls, were not inoculated. One control was incubated at 10° C. and the other at 30° C. throughout a 10-day period, which was the duration of each of the studies performed. There was one exception in which uninoculated controls were prepared according to normal procedure, held at 10, 20 and 30° C. and examined at regular sampling intervals to assure that sterility prevailed and no enzyme activity occurred.

Inoculation

Three of the remaining six flasks of sterilized cream were inoculated with 0.1 ml. of an 18-hour litmus milk culture of the organism selected for study in that particular lot of cream. The three remaining flasks were each inoculated with 0.1 ml. of the same culture plus 0.1 ml. of a litmus milk culture of S. lactis, in order that the growth and fermentation pattern of the organism in the presence of S. lactis could be observed. Following inoculation, the cream was thoroughly agitated to assure uniform distribution of the inoculum.

Incubation

In order to simulate as approximately as possible the temperatures at which cream for buttermaking may be held, incubation at 10, 20 and 30° C. was employed. Flasks of cream containing each inoculum were incubated at each of these temperatures. It was considered that the 10° C. temperature would correspond to the holding conditions prevailing among producers who used refrigeration facilities for their cream during holding prior to marketing. The 20° C. temperature would approximate the holding temperature of cream by those who had no refrigeration but who made conscientious use of such natural cooling facilities as are available, such as water tanks. The 30° C. temperature was intended to correspond to the conditions of exposure by producers who

provided no protection against high temperature. Uniform incubation conditions as described above prevailed throughout the experiment on all organisms studied.

Frequency and method of sampling

In all series of trials the two control flasks were sampled following sterilization and immediately prior to incubation. These two samples were analyzed and the average of the results recorded as the initial or 0-day analysis on the entire lot of cream. The two controls were sampled and analyzed again at the conclusion of a 10-day incubation period at 10 and 30° C.

Samples for microbiological and other analyses were secured from all inoculated flasks after incubation for 1, 3, 5, 7 and 10 days, with the flasks being thoroughly agitated prior to sampling. In addition, samples for microbiological analyses were removed immediately before inoculation and within 10 minutes after inoculation, following thorough agitation to assure uniform dispersion of the cells. These initial samples on 0 day were to determine if the prepared cream was sterile and to determine the initial organism population inoculated into the cream. Sterile 1.1 ml. pipettes were used to secure the samples for microbiological examination. For the other analyses performed, it was necessary on each sampling occasion to secure from 95 to 100 g. of cream. Sterile 100 ml. pipettes and 50 ml.

burettes were found to be the most satisfactory sampling devices.

Media and methods of enumerating organism populations

All media and reagents used in media were secured from the Difco Laboratories, Detroit, Mich.

Enumeration of S. lactis and total counts. Tryptone-glucose-extract agar with 1 per cent skim milk added, prepared according to the American Public Health Association (2), was used for enumeration of S. lactis and for all other standard plate counts. Plates were incubated at 32° C. In all instances where inoculated organism populations were enumerated on special media, duplicate standard agar plates were prepared.

Yeast and mold counts. Potato dextrose agar prepared according to Standard Methods (2) and acidulated to pH 3.5 with sterile 2 per cent tartaric acid immediately before pouring, was used for all yeast and mold determinations. The plates were incubated at 25° C. for 4 or 5 days.

Enumeration of individual species of organisms in cream containing S. lactis and G. candidum and in cream containing S. lactis and T. cremoris. On standard agar plates incubated at 32° C. for 48 hours there was sufficient difference in colony appearance to distinguish S. lactis from T. cremoris or G. candidum.

Coliform counts. Desoxycholate agar prepared according

to Standard Methods (2) was used for all coliform counts. The agar was boiled to accomplish solution, then placed in final containers and steamed for 1 hour in the autoclave without pressure and stored in a refrigerator at 5° C. Incubation of plates was at 37° C. for 24 hours.

Enumeration of S. lactis and A. aerogenes when grown as mixed cultures. It was not always possible to differentiate S. lactis and A. aerogenes colonies on standard agar; therefore, S. lactis populations were determined by subtracting the coliform counts on desoxycholate agar from the total populations on standard agar.

Enumeration of lactobacilli. L. casei counts were made on a medium developed by Rogosa et al. (106) which adequately supported lactobacilli and inhibited most other organisms. It later was observed that this medium supported mold growth, but this characteristic was of no consequence when inoculated cream was examined. Preliminary trials using three strains of L. casei and three strains of S. lactis demonstrated that this medium supported growth of L. casei and inhibited growth of S. lactis. Plates were incubated at 32° C. for 72 hours.

Enumeration of S. lactis and L. casei when grown as mixed cultures. S. lactis colonies were easily distinguishable from L. casei colonies after growing on standard agar for 48 hours at 32° C.

B. subtilis counts. A carbohydrate-free medium contain-

ing 0.3 per cent beef extract, 0.5 per cent peptone and 1.5 per cent agar was used to enumerate B. subtilis. The plates were incubated at 32° C. for 48 hours.

Enumeration of S. lactis and B. subtilis when grown as mixed cultures. B. subtilis counts were made on the carbohydrate-free medium. An attempt to follow B. subtilis populations in the presence of S. lactis on standard agar containing added skim milk was abandoned. S. lactis completely dominated the plates and apparently reduced the pH of the medium by carbohydrate fermentation to a point below the growth tolerance of B. subtilis. S. lactis populations were secured from standard agar plates incubated at 32° C. for 48 hours. The colonies were easily distinguished from B. subtilis.

Enumeration of Ps. fragi. Standard agar containing 1 part of crystal violet dye to 125,000 parts of agar was used for the enumeration of Ps. fragi. The plates were incubated at 32° C. for 48 hours.

Enumeration of S. lactis and Ps. fragi when grown as mixed cultures. The crystal violet dye inhibited S. lactis without interfering with the development of Ps. fragi. The dye was also effective in inhibiting Gram-positive organisms when Gram-negative organism counts were performed on commercial raw cream. S. lactis counts were determined by subtracting the Ps. fragi populations on the dye-containing plates from the total populations on the standard agar plates

which were incubated at 32° C. for 48 hours.

In determining the optimum level of crystal violet dye to use for inhibiting S. lactis in the presence of Ps. fragi, concentrations of 1 to 1000,000, 1 to 111,000, 1 to 125,000, 1 to 142,000, 1 to 165,000 and 1 to 200,000 were tried. Ps. fragi grew with no apparent inhibition at all concentrations. Some S. lactis growth occurred at 1 to 142,000 but none at 1 to 125,000.

Lipolytic counts. Lipolytic counts were performed by the modified nile blue sulfate technique of Long and Hammer (81), with standard agar plates containing fat emulsion being flooded for 30 minutes with nile blue sulfate containing 1 part dye to 1,500 parts distilled water. The plates were rinsed with water, incubated a few hours at 45° C. and the colonies surrounded by blue fat globules enumerated with the aid of a magnifying lens.

Proteolytic counts. Proteolytic counts were performed by enumerating those colonies which produced protein hydrolysis on standard agar plates containing 0.6 ml. of sterile skim milk added at the time the plates were poured.

Analyses of samples

Titratable acidity, pH and organoleptic values were determined within a few minutes after the samples were secured. Samples for tryptophan and W.I.A. determination were promptly weighed into 4-ounce bottles and refrigerated

until analyses could be performed. The tryptophan samples were stored at 0° C. and analyzed as soon as possible, usually the following day, but in no instance were they held longer than 1 week. The samples for W.I.A. were stored at -20° C. and analyzed as soon as possible, with analyses usually completed within 10 to 15 days. In no case were these samples held longer than 1 month.

Titratable acidity. Titratable acidity was performed on 9 g. of cream using 0.1 N NaOH and ten drops of 1 per cent alcoholic phenolphthalein. Nine ml. of distilled water was used to rinse the pipette.

Organoleptic examination. Organoleptic examination was made on all samples at the time they were withdrawn for analysis. Appropriate numerical and letter scores and criticisms were recorded.

W.I.A. Water-insoluble acid determinations were performed according to the A.O.A.C. Method (8). All glassware was thoroughly cleaned and then rinsed with petroleum ether to avoid the possibility of picking up any W.I.A. during analysis. All results were calculated and reported as mg. of W.I.A. per 100 g. of fat.

As a check on the efficiency of the method, recovery experiments were performed using oleic, stearic and palmitic acids which were dissolved in ether and added to the cream, and routine determinations performed. The results are shown in Table 1.

Table 1

Per cent water-insoluble acid recovered when known quantities of oleic, stearic and palmitic acids were added to cream

W.I.A. in original cream (g.)	Acid added (g.)	Total W.I.A. present (g.)	W.I.A. recovered (g.)	W.I.A. recovered (%)
		Oleic acid added		
0.0130	0.1801	0.1931	0.1941	100.6
0.0130	0.3118	0.3248	0.3132	96.4
0.0130	0.4957	0.5087	0.4986	98.0
		Stearic acid added		
0.0446	0.0369	0.0815	0.0781	106.9
0.0446	0.1264	0.1710	0.1638	95.8
0.0446	0.4442	0.4888	0.4473[a]	91.5
		Palmitic acid added		
0.0446	0.0665	0.1111	0.1137	102.3
0.0446	0.1591	0.2037	0.1952	95.9
0.0446	0.5634	0.6080	0.5245[a]	86.3

[a]Considerable difficulty was experienced in filtering these two samples because the W.I.A. content was so high it caused clogging of the filters. The low percentage recovered would suggest the loss of some W.I.A. The sample containing approximately 0.5 g. of oleic acid filtered more slowly than most samples but did not clog the filter mat as much as the corresponding quantity of stearic and palmitic acids.

Medium fritted glass filters were tried in place of asbestos mats in Gooch crucibles for filtering the W.I.A. and gave comparable analytical results, with a little more rapid filtration, but fritted glass filters were not available in sufficient numbers, hence were not used.

Free tryptophan. Free tryptophan determinations were performed according to the method of Duggan (32). There must be no delay in analysis procedure between the addition of acetone and the initial filtration. High free tryptophan values result if the acetone is allowed to remain in contact with the original sample for an extended interval prior to centrifugation and filtration. This was encountered accidentally when an interruption necessitated a few hours delay at this stage during analysis and was demonstrated by repeated trials.

In cases where high free tryptophan values were anticipated, the color was developed on a smaller aliquot portion of the filtrate, using 1 ml. rather than the usual 2 ml. The greater the quantity of tryptophan in the sample being analyzed, the greater the intensity of color and the greater the time required for maximum color development. The more intense colors offered greater opportunity for error. In working with tryptophan solutions of known strength while making the standard curve, it was found that as much as 20 minutes was required for maximum color development in solutions containing 30 to 40 micrograms per ml.,

and 30 minutes for solutions containing 50 or more micrograms per ml. Since the free tryptophan in the 2 ml. portion of the filtrate normally used for color development represents the equivalent of approximately three times the tryptophan content of the serum of the cream (formula conversion), it was found to be advisable to take 1 ml. aliquots for color development when tryptophan values in excess of 10 to 15 p.p.m. in the serum were anticipated. However, these are higher values than normally encountered in samples of milk, cream or butter. It was found that once maximum color intensity was attained, the color was stable for 40 to 60 minutes. In all cases care must be exercised to assure reading the sample at maximum color intensity.

Particular care must be taken to avoid ruptured filter paper or any other condition which permits protein in the filtrate. Concentrated hydrochloric acid is added simultaneously with the indicator reagent in developing the color and any protein present may be partially hydrolyzed, resulting in a false, abnormally high value.

In performing the tryptophan analysis on butter, the Duggan (32) method continually yielded results lower than the value of the cream from which the butter was made. The results were reasonably consistent. In scrutinizing the formula for the calculation of tryptophan, it was observed that the salt of the butter is included in the serum containing the tryptophan. Also, while it is customary to consider

butter as containing 80 per cent fat, some samples contain substantially more than 80 per cent fat. When the tryptophan content of butter was calculated on the basis of the actual serum content 100 - (fat + salt) , the resulting values more closely approximated those of the original cream. Even then the agreement of results must be considered to be more or less fortuitous because some of the serum fraction in butter arises from water used for washing, part of which is subsequently worked into the butter.

Colors were measured on a Klett-Summerson colorimeter, using a K.S. 54 filter which has transmission limits of 520 to 580 millimicrons. A standard curve was prepared for determining values. The following formula was used for calculation of results:

$$\text{p.p.m.} = \frac{A \times \frac{B}{C}}{g \times (1.00 - F)}$$

A = Net micrograms of free tryptophan in the aliquot determined from the standard curve.

B = Total milliliters of sample solution.

C = Milliliters of aliquot used (usually 2 ml. but reduced to 1 ml. when high values were anticipated).

g = Grams of original sample (25 g. of cream and 50 g. of butter).

F = Per cent fat plus salt in the sample, expressed as decimals (in analyzing butter in this work, the salt was also added to the fat).

To prepare a standard curve for converting colorimetric readings to free tryptophan values, distilled water solutions of DL-tryptophan were prepared by dissolving 10 mg. of DL-tryptophan in 100 ml. of distilled water to make a solution containing 0.1 mg. per ml. Five separate solutions were prepared, two using distilled water at room temperature and shaking vigorously for 3 or 4 hours as tryptophan is difficult to dissolve, and three by heating to 70° C. and holding for 5 minutes with continuous agitation. Determined values of free tryptophan in the heated solutions were identical with those of the unheated solutions. In each case the solutions were diluted to give a second solution containing 0.01 mg./ml. The solutions were kept under refrigeration at 5° C., and in no case was a standard solution used which was more than 3 days old.

The routine procedure for development of color and tryptophan analysis was performed on portions of each of these solutions representing a range from 1 to 80 mcg. of free tryptophan. Twenty-two points along the range were designated for determination, with 12 of the points being at values ranging from 1 to 20 mcg. and the remaining 10 points being between the range of 20 to 80 mcg. of free tryptophan. Figure 1 is the graph used for calculating free tryptophan values.

The five determinations for each designated point,

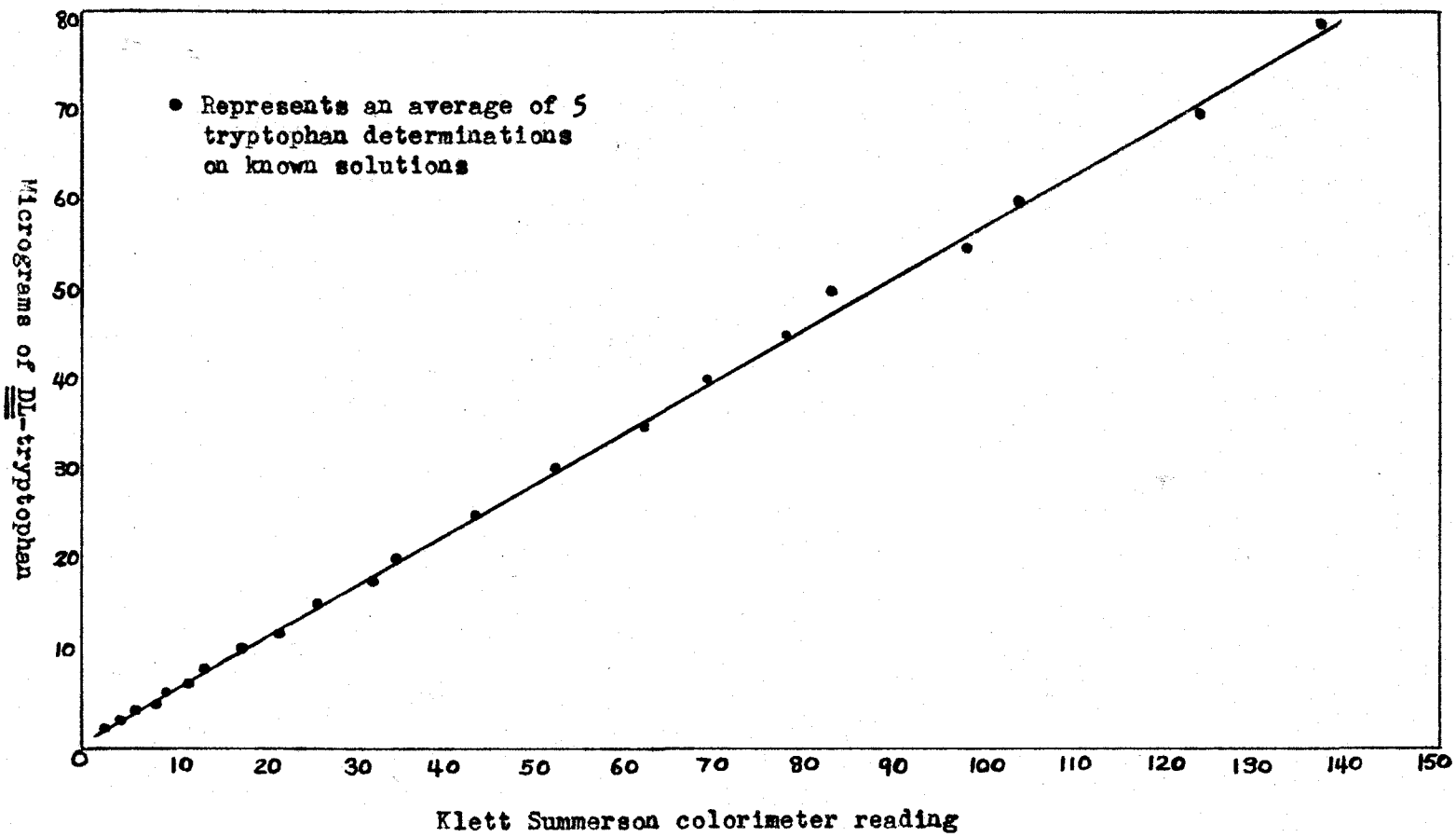


Fig. 1. Standard curve for computing free tryptophan

one determination for each solution, were averaged and the standard curve plotted on these points. The determined values of the different solutions and of duplicate samples from the same solution consistently checked within 10 per cent.

A 5 ml. microburette was used for dispensing the tryptophan solutions except in instances where less than 0.5 ml. was required, in which cases a graduated 1 ml. pipette was used. Duggan (32) recommended reading samples of tryptophan extracts 25 minutes after addition of color developing reagents and 10 minutes after addition of glacial acetic acid and hydrogen peroxide. However, Duggan pointed out that samples containing high concentrations of free tryptophan may require longer for maximum color development. As previously noted, it was necessary, with the high values, to repeat readings after 20 and sometimes after 30 minutes to assure reading at maximum color intensity. In each trial, blanks containing all reagents, but no tryptophan solution, were prepared and the colorimeter reading adjusted to zero with these blanks.

A solution of L-tryptophan was prepared and analyses on this solution were found to correspond identically with equivalent quantities of solutions of DL-tryptophan. This was done to obtain assurance that both forms of tryptophan were equally responsive in color development.

Recovery trials were performed in order to assure the accuracy of the method. Known portions of a solution of free tryptophan were added to skim milk and to cream, the free tryptophan content of which had previously been determined. The samples were analyzed in the routine manner. Tables 2 and 3 show the efficiency of recovery. In regard to the recovery trials involving skim milk, it should be pointed out that skim milk samples are more easily and probably more accurately analyzed than cream samples because there is no interfering fat to retard the first filtration, although the data in the two tables do not entirely support this assumption. In the recovery samples containing more than 10 mcg. of free tryptophan, color was developed on smaller aliquot portions of the sample in order to increase accuracy by reducing the color intensity.

Table 2

Per cent DL-tryptophan recovered when known quantities were added to grade A skim milk containing 0.2 p.p.m. of free tryptophan

Sample number	Tryptophan added (p.p.m.)	Total tryptophan present (p.p.m.)	Tryptophan recovered (p.p.m.)	Tryptophan recovered (%)
0	0	0.2	0.2	----
1	0.5	0.7	0.6	85.7
2	1.0	1.2	1.1	91.7
3	2.0	2.2	2.0	90.9
4	3.0	3.2	3.2	100.0
5	4.0	4.2	4.1	97.6
6	5.0	5.2	5.0	96.1
7	6.0	6.2	6.0	96.8
8	7.0	7.2	7.1	98.6
9	8.0	8.2	8.1	98.8
10	9.0	9.2	9.4	102.2
11	10.0	10.2	10.3	101.0
12	12.0	12.2	12.2	100.0
13	15.0	15.2	15.0	98.7
14	18.0	18.2	17.4	95.6
15	20.0	20.2	19.8	98.0
16	25.0	25.2	27.0	107.1
17	30.0	30.2	32.8	108.6
18	40.0	40.2	41.6	103.5
19	50.0	50.2	44.8	89.2
20	60.0	60.2	62.8	104.1

Table 3

Per cent DL-tryptophan recovered when known quantities were added to cream containing 2.4 p.p.m. of free tryptophan in the serum

Sample number	Tryptophan added to the serum (p.p.m.)	Total tryptophan present in the serum (p.p.m.)	Tryptophan recovered from the serum (p.p.m.)	Tryptophan recovered from the serum (%)
1	0	2.4	2.4	----
2	1.0	3.4	3.2	94.1
3	2.0	4.4	4.8	109.1
4	3.0	5.4	5.1	94.8
5	4.0	6.4	6.6	103.1
6	5.0	7.4	7.0	94.6
7	8.0	10.4	10.2	98.1
8	10.0	12.4	11.2	93.2

RESULTS

Explanation of Figures

Samples of cream were prepared, sterilized, inoculated, sampled and analyzed according to procedures previously described.

Graphs are presented to illustrate the variations in each of the values determined during the incubation period. The following legend prevails throughout the entire series of figures:

S. lactis count . . . □	Score . . . . . ○
Other organism count . △	W.I.A. . . . . ⊗
Titratable acidity . . ⊙	Tryptophan . . . . ●
pH . . . . . ▲	

In all figures, except the last three which apply to a group of commercial samples, counts, acidity and pH are plotted on one figure, with W.I.A., tryptophan and score plotted on another figure. Three incubation temperatures, 10, 20 and 30° C., were used with each group of samples. Therefore three separate sets of graphs, one representing each temperature, are shown on each figure except in two instances where values were so large that only two graphs could be placed on one figure.

In constructing the graphs, time in days ranging from

0 to 10 is shown on the horizontal axis and values of the various quality factors determined are shown on the vertical axis. A common denominator was determined for the values of all of the various factors considered in order to enable the depiction of several quality factors on one graph and thus avoid the confusion of too many graphs.

In plotting microorganism population, the logarithms of the counts are used throughout.

pH is plotted at the actual numerical pH value.

In plotting titratable acidity, one scale unit equals 0.1 per cent. For example, 0.45 per cent is plotted as 4.5.

Free tryptophan is plotted as one scale unit equal to 1 part per million or 1 microgram per gram.

In plotting the W.I.A. values, one scale unit equals 100 mg. of W.I.A. per 100 g. of fat. For example, a W.I.A. value of 1,050 is plotted as 10.5.

On the vertical scale most of the values fall between 0 and 15, with occasional high values extending beyond this maximum and one extremely high value extended to 83. The graphs are constructed to best depict the bulk of the data. In the case of some unusually high values it has been necessary to indicate off-chart extensions beyond the boundary of the graph.

Organoleptic grade is indicated on the right side of each graph on the perpendicular scale. The U.S.D.A. letter grading system is used with grades descending from AA to

N.G. A brief explanation of this grading system follows. Grade AA corresponds to 93 score sweet cream, A is equivalent to 92 score sweet cream, B represents 90 score sour cream and C corresponds to 89 score sour cream. These are the marketable grades. In addition, C.G. represents cooking grade[1] and N.G. indicates no grade. All cream used for culture inoculation in this work was grade AA at the time inoculated.

Analyses of Controls

Initial controls

Before proceeding with inoculated samples it was deemed advisable to follow the progression of the various fermentation factors in a preliminary control sample which was prepared, sterilized, incubated and sampled in exactly the same manner and analyzed for the same quality factors as the subsequent inoculated samples. The results for each of the three incubation temperatures are shown in figure 2. There was no microorganism growth or significant change in pH, titratable acidity, W.I.A., tryptophan or organoleptic grade at any of the three incubation temperatures during the 10-day

[1]Since completion of this work the U.S.D.A. has announced a new grading system effective April 1, 1954, which eliminates cooking grade, but which leaves all other grades fundamentally the same.

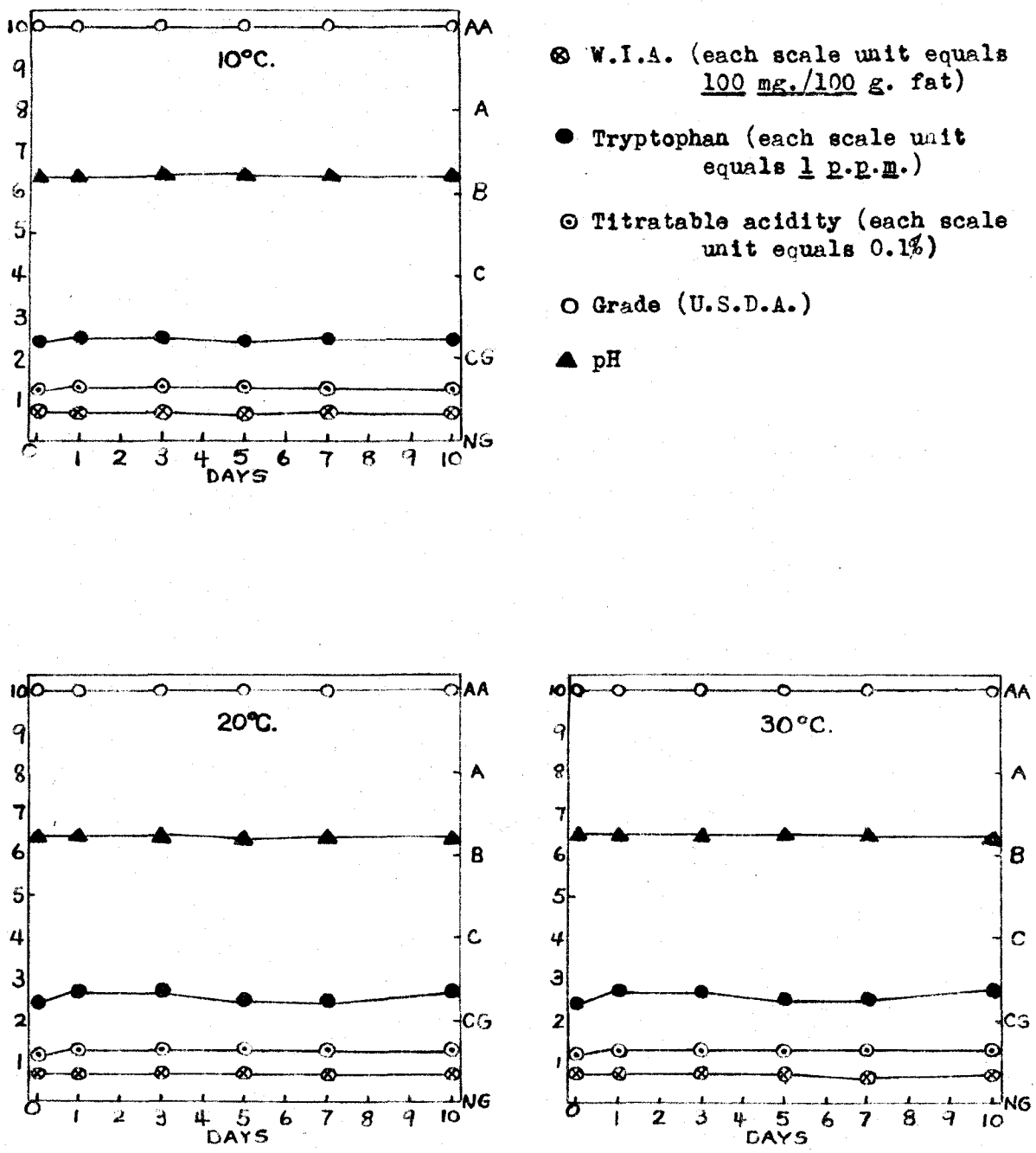


Fig. 2. Control. W.I.A., tryptophan, titratable acidity, grade and pH values on sterilized, uninoculated excellent quality cream.

holding period. The results of this preliminary control established that intermittent heating, as performed, accomplished complete organism destruction and lipase and protease inactivation. It was therefore assumed that any change in titratable acidity, pH, W.I.A., tryptophan or score values which occurred in similarly prepared samples probably would occur as a result of organism inoculation.

Companion controls on inoculated samples

Throughout the subsequent research involving inoculated samples, uninoculated controls of the same sterilized cream were incubated at 10 and 30° C. for 10 days. These two portions were sampled and analyzed for all quality factors being determined, following sterilization and immediately before being placed at their respective incubation temperatures. The results constituted duplicate initial analyses representative of the entire group of cream samples. The average of these duplicate results was recorded on the 0-day value for each factor for the entire group of samples. The controls were left undisturbed at their assigned incubation temperatures for the 10-day incubation period, after which they were again sampled and analyzed for all factors studied. There was only one instance in which a significant change occurred in any factor in the controls during the incubation period. In this case all the results were discarded and the series repeated, in order to eliminate from consideration

the possibility of results being influenced by any factor not induced by the inoculated organisms.

There were instances in which undesired contaminating organisms were detected in one of the inoculated samples during the incubation period. In all such cases the results were discarded and the entire program of analyses on the particular series of samples was repeated.

Analyses of Inoculated Samples

It was possible to perform the analytical work on a maximum of six individual samples being incubated at any one time. Three samples constituted a group; therefore two groups of samples from the same original quantity of cream were paired and incubated simultaneously. The first pair was the control group and the group inoculated with G. candidum. The next pair was a group of samples inoculated with S. lactis and a companion group inoculated with S. lactis plus G. candidum. Throughout the remainder of the work on inoculated samples the arrangement was continued whereby one group of samples was inoculated with the particular organism whose activity was being determined and a companion group of samples from the same original portion of cream was inoculated with the organism and also with S. lactis. This arrangement assured uniformity between the parallel or companion groups of cream samples and also

minimized the number of controls necessary.

Sterile cream inoculated with G. candidum

Figure 3 shows the changes induced in population, titratable acidity and pH as a result of inoculating sterile cream with G. candidum and incubating at 10, 20 and 30° C. The population increased fairly uniformly, with the most rapid rate and the highest maximum occurring at 30° C. There was virtually no change in acidity or pH at 10° C., but at 20° C. there was a moderate increase in titratable acidity beginning on the fifth day and a corresponding slight drop in pH. At 30° C. the titratable acidity development was moderate through the seventh day and then an abrupt increase occurred accompanied by a moderate decline in pH.

The changes in W.I.A., tryptophan and score attributable to G. candidum are illustrated in figure 4. The graphs show a definite correlation between W.I.A. increase and deterioration in organoleptic quality. Also, W.I.A. increased enormously as the temperature increased, within the range studied. G. candidum was by far the most lipolytic organism encountered, as evidenced by high W.I.A. values. The minor tryptophan fluctuations are of no consequence with the pronounced increase at 30° C. occurring after the cream had deteriorated to N.G.

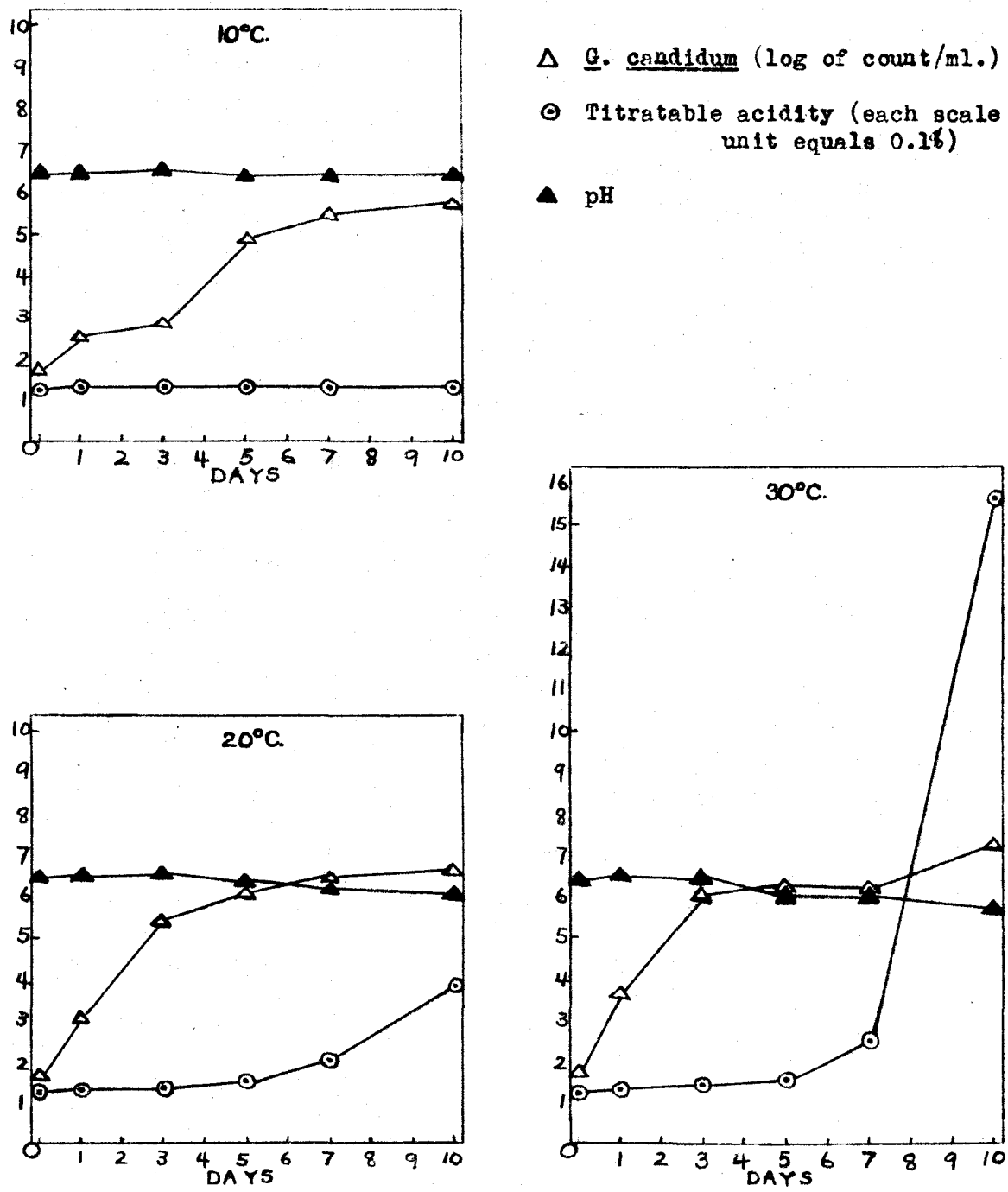


Fig. 3. Titratable acidity, pH and counts of G. candidum in cream containing this organism.

W.I.A. (each scale unit equals
100 mg./100 g. fat)

Tryptophan (each scale unit
equals 1 p.p.m.)

Grade (U.S.D.A.)

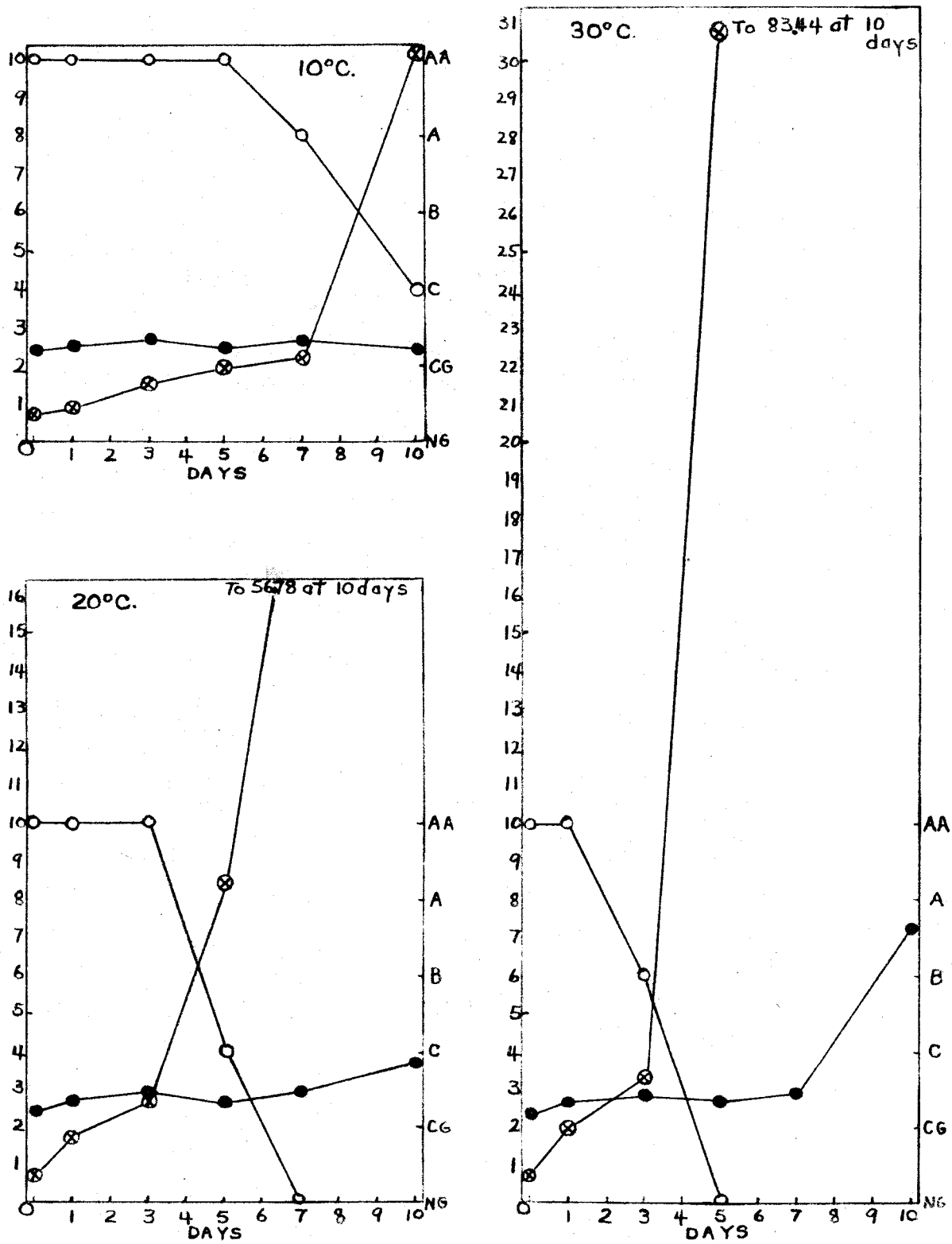


Fig. 4. W.I.A., tryptophan and grade values on cream containing G. candidum.

Sterile cream inoculated with S. lactis

Figure 5 shows the changes which occurred in population, titratable acidity and pH when a freshly isolated strain of S. lactis was inoculated into sterile cream. Temperature has a decided effect upon S. lactis population during an extended incubation period. At 10° C. the count increased uniformly through the fifth day, after which it leveled off, remaining high and virtually stationary. In delayed response to organism population, titratable acidity increased and pH decreased through the seventh day and then leveled off. In the 20° C. sample the population and titratable acidity increased and the pH decreased abruptly on the first day. After the third day the population began a gradual decline and titratable acidity and pH remained fairly constant. The greatest change also took place in all three factors during the first day in the 30° C. sample, after which the count decreased uniformly but markedly throughout the remainder of the incubation period. The pH changed only slightly after the first day and the titratable acidity gradually increased through the seventh day.

The manner in which W.I.A., tryptophan and score responded to S. lactis is shown in figure 6. The trend of the W.I.A. values shown on the graphs is of no consequence in quality, however, uniformity of pattern seems to be significant. At all three temperatures a maximum occurred

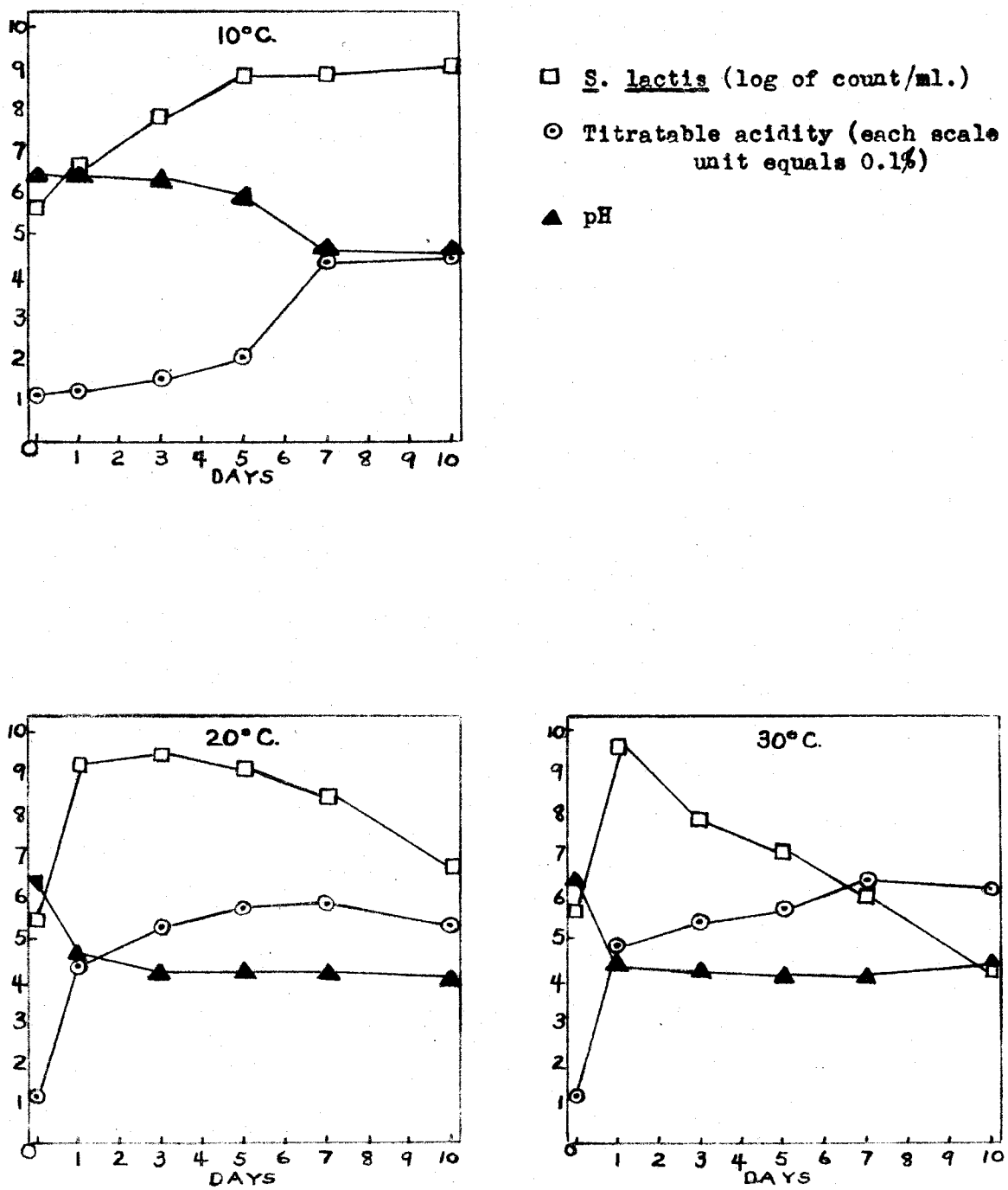
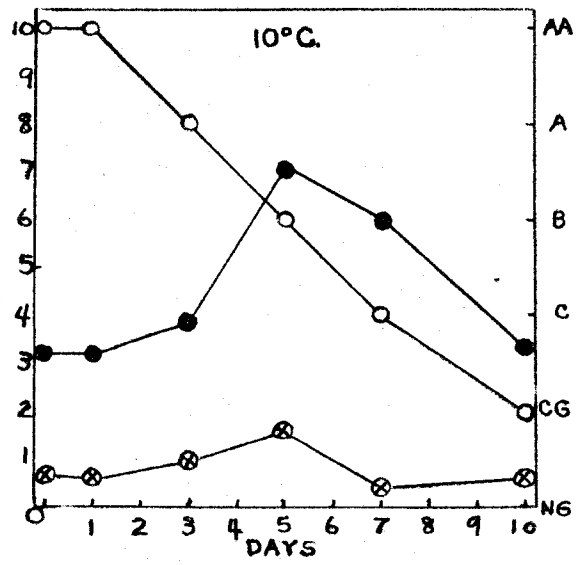


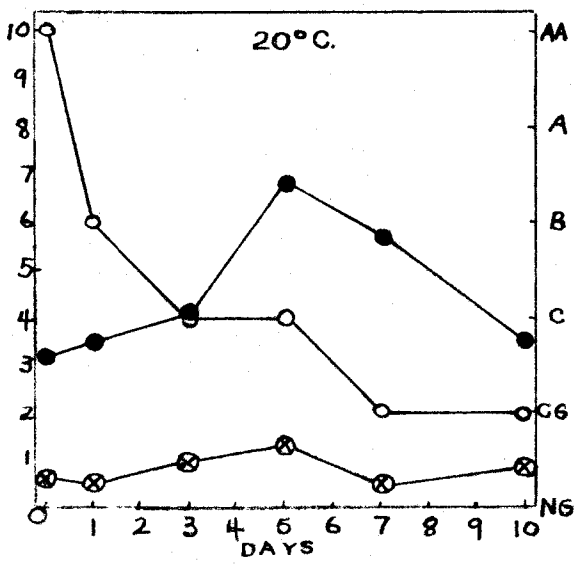
Fig. 5. Titratable acidity, pH and counts of S. lactis in cream containing this organism.



⊗ W.I.A. (each scale unit equals 100 mg./100 g. fat)

● Tryptophan (each scale unit equals 1 p.p.m.)

○ Grade (U.S.D.A.)



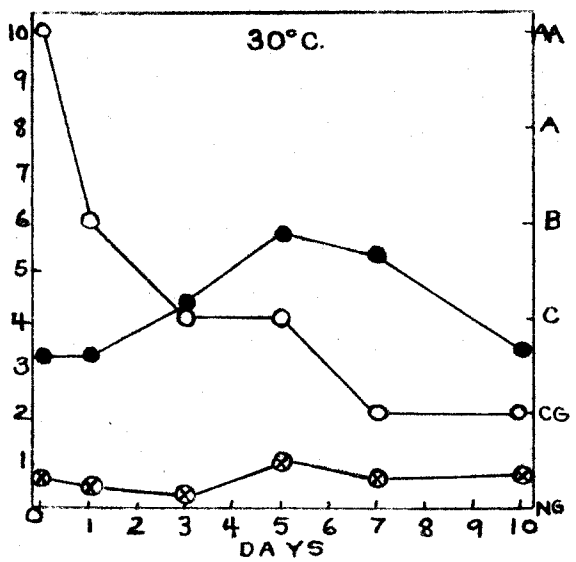
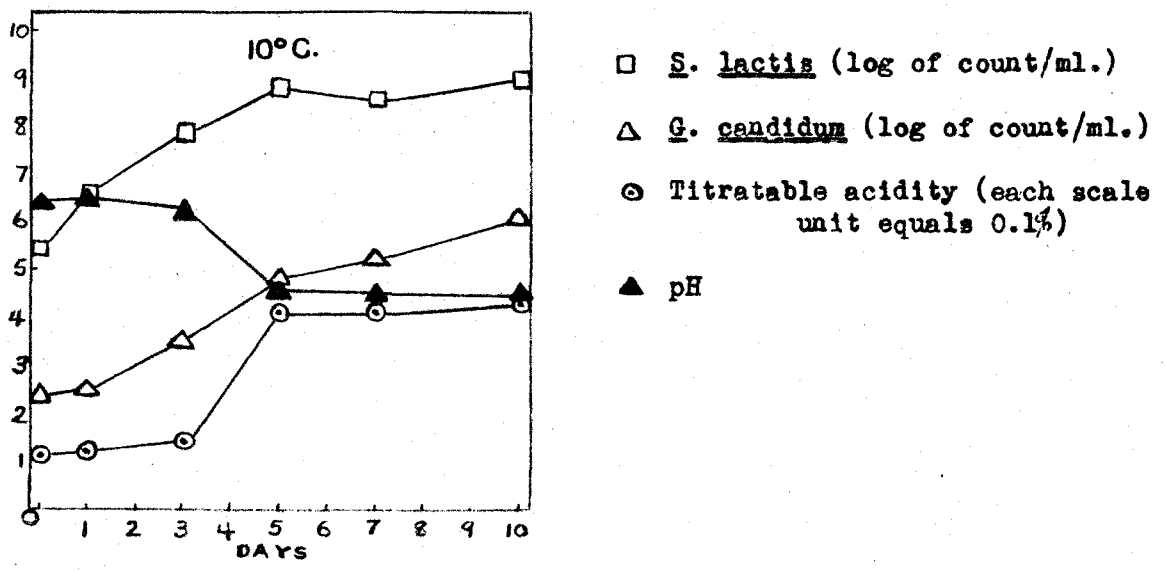


Fig. 6. W.I.A., tryptophan and grade values on cream containing S. lactis.

at 5 days, followed by a definite decline and a subsequent slight increase. On the first day a slight decrease is barely perceptible in the 10 and 20° C. curves, but a definite decrease is apparent in the 30° C. curve. The fluctuations are beyond the normal range of analytical error and must have occurred in response to some condition existing in the inoculated cream. Also of particular interest is the uniform pattern of the tryptophan curve at all three temperatures. In each sample the increase was gradual to the third day, greater to the fifth day, followed by a uniform decline to the approximate initial value on the tenth day. As in the case of the W.I.A. variations, these fluctuations also must be attributed to some influence resulting from S. lactis inoculation of the cream. The decline in quality at all temperatures seems to be related to increases in S. lactis population and changes in titratable acidity and pH. Organoleptic deterioration was more rapid at 30 and 20° C. but terminated with C.G. at all three temperatures on the tenth day.

Sterile cream inoculated with S. lactis and G. candidum

The changes occurring in organism counts, titratable acidity and pH when sterile cream was inoculated with S. lactis and G. candidum and incubated at 10, 20 and 30° C. are shown in figure 7. A comparison of figures 3 and 7 reveals that the presence of S. lactis exhibited practically



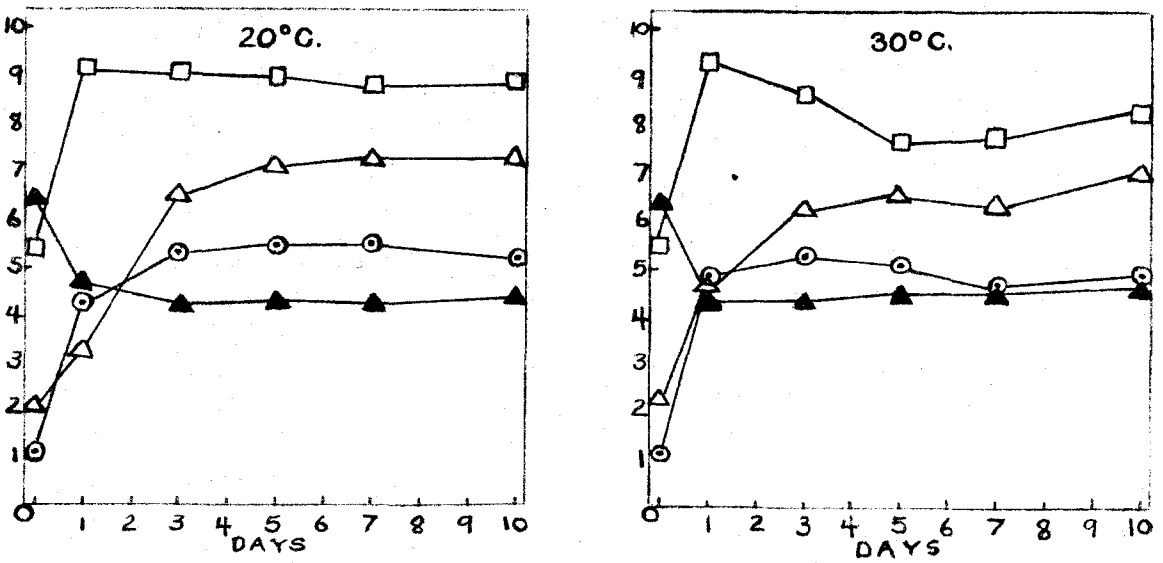


Fig. 7. Titratable acidity, pH and counts of S. lactis and G. candidum in cream containing both of these organisms.

no influence on rate of increase or maximum population attained by G. candidum, the mold count curves being almost identical at each temperature on the two figures. However, a comparison of figures 5 and 7 indicates that while the presence of G. candidum did not affect S. lactis population at 10° C., the presence of the mold definitely diminished the rate of S. lactis decline after the first day at 20° C. and the third day at 30° C. At 20° C. the S. lactis population remained approximately stationary after reaching maximum the first day. In the 30° C. sample there was a moderate decline in S. lactis count from the first to the fifth day, at which point the decrease was arrested and there was a slight secondary increase.

A further comparison of figures 5 and 7 shows that the titratable acidity curves produced by the two organisms at 10 and 20° C. correspond to the acidity curve of S. lactis alone. However, at 30° C. the mold obviously exhibited an inhibitory effect on the acid-producing ability of S. lactis because the maximum titratable acidity attained is lower than developed by S. lactis alone. A comparison of titratable acidities in figures 3 and 5 suggests that S. lactis slightly inhibited the acid-producing ability of G. candidum at 20° C. and materially inhibited its acid-producing ability at 30° C. This curtailment of titratable acidity, particularly at 30° C., probably was due to the greatly

decreased W.I.A. formation by G. candidum in the presence of S. lactis as indicated by figure 8. A comparison of the pH curves in figures 3, 5 and 7 shows that the pH change occurring in the presence of the two organisms is almost entirely in response to, and dominated by S. lactis.

Figure 8 shows the changes in W.I.A., tryptophan and score occurring as a result of incubating sterile cream inoculated with S. lactis and G. candidum. A comparison of the W.I.A. curves in figures 4 and 8 indicates that S. lactis has a pronounced inhibitory effect on the ability of G. candidum to produce W.I.A. At 10° C. the W.I.A. increased only slightly in the presence of both organisms and attained a maximum value of 234 at 10 days, whereas the maximum value produced by G. candidum alone at the same time and temperature (figure 4) was 1,027. At 20 and 30° C. the W.I.A. curves (figure 8) show an unusual response to the combined activity of the organisms. The rate of increase up to the third day was only slight, then quite pronounced to the fifth day, followed by a substantial drop at 20° C. and a moderate drop at 30° C. on the seventh day. After the seventh day there was an increase of considerable magnitude. Attention should be focused to figure 6 which shows the W.I.A. curves produced by S. lactis alone. At all three temperatures there was a decrease in W.I.A. between the fifth and seventh day, which was followed by a slight

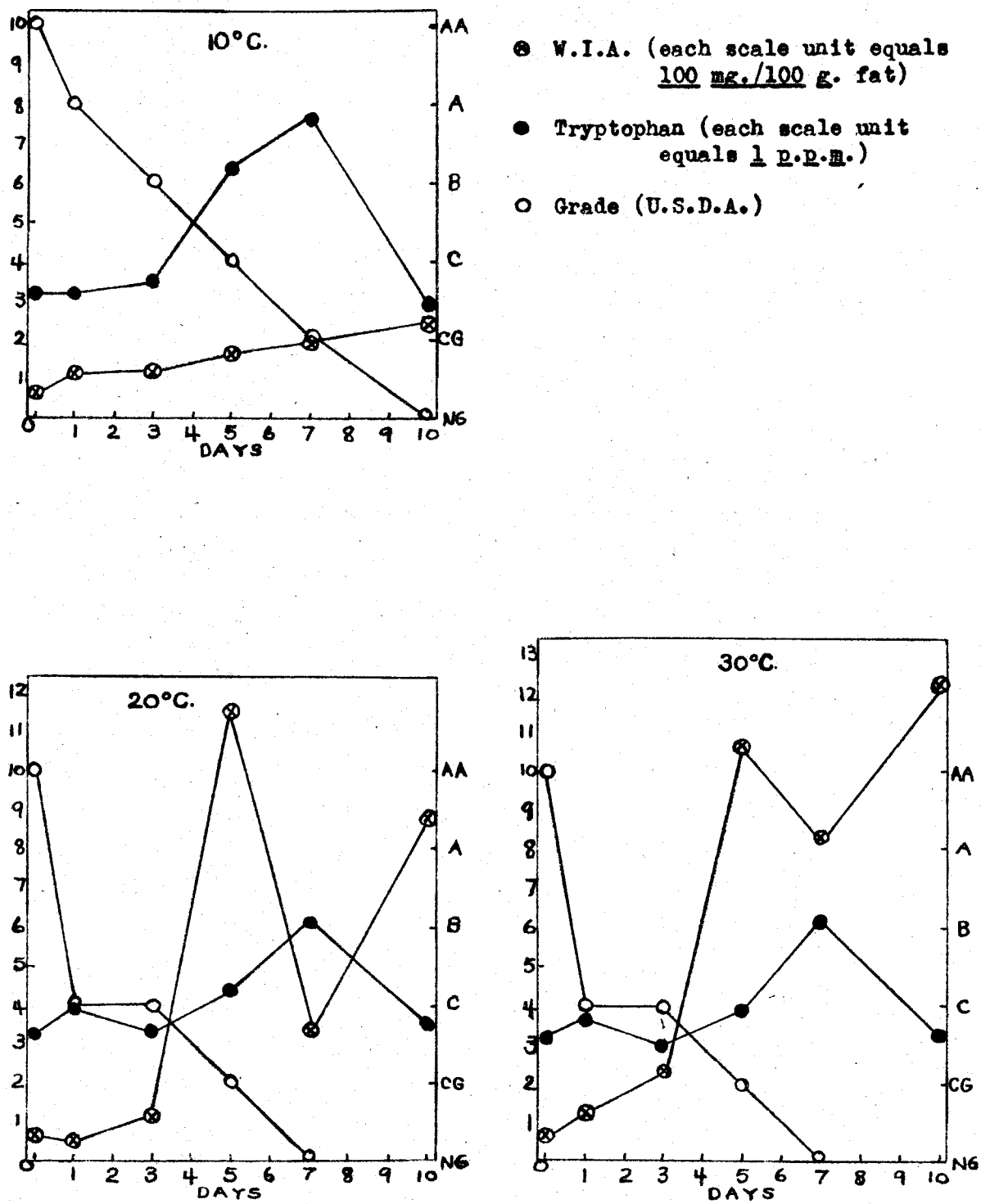


Fig. 8. W.I.A., tryptophan and grade values on cream containing both S. lactis and G. candidum.

secondary increase. Apparently S. lactis was responsible for the decreases shown in figure 8 at 20 and 30° C. on the seventh day.

In figure 8 the pattern of the curves representing tryptophan values indicates that S. lactis was the dominant organism and G. candidum did not participate in proteolytic activity. The tryptophan curves in figure 8 follow the same general trend but differ slightly from the tryptophan curves in figure 6. In figure 8, the 20 and 30° C. curves show an insignificant but uniform fluctuation. At all three temperatures the curves begin ascending on the third day and reach a maximum on the seventh day. Then they decline rather sharply to a 10-day value approximately equivalent to the initial value. The same pattern prevails in the three graphs in figure 6, except the maximum value is attained on the fifth day and the rate of subsequent decline is slower, but the total decline is the same. Obviously the fluctuating free tryptophan values must be attributed to S. lactis.

The organoleptic quality decline indicated in figure 8 seems to be closely related to the initial population increase of the two organisms and to the titratable acidity and pH change shown in figure 6. However, after the third day there was also fairly close relationship between organoleptic deterioration and W.I.A. and tryptophan increase.

Sterile cream inoculated with S. lactis (I.S.C. 712)

A strain of S. lactis secured from the Iowa State College Dairy Bacteriology Laboratory designated as strain 712 was inoculated into sterile cream and the routine analyses were performed. Although an attempt was made to invigorate this organism prior to inoculation, it was found to be a slow fermenting organism, which is evidenced by the titratable acidity and pH curves on figure 9. The acid production potential for this strain is much weaker than is normal for S. lactis. A comparison of the S. lactis population curves in figures 5 and 9 shows that the 712 strain attained lower maximums, showed no decline at 20° C. and much less decline at 30° C. than the more vigorous freshly isolated strain of S. lactis. One reason for including the data on the 712 strain is to show the similarity between the W.I.A. and free tryptophan curves produced by the two strains of S. lactis. In the case of the less vigorous S. lactis strain (figure 10) the increase in tryptophan from time of inoculation until the fifth day was only slight. Then there was an abrupt increase to the seventh day followed by an abrupt decline to the tenth day, with the general pattern being uniform at all temperatures. The more vigorous strain of S. lactis followed the same pattern except that accelerated increase began on the third day and the decline began on the fifth day.

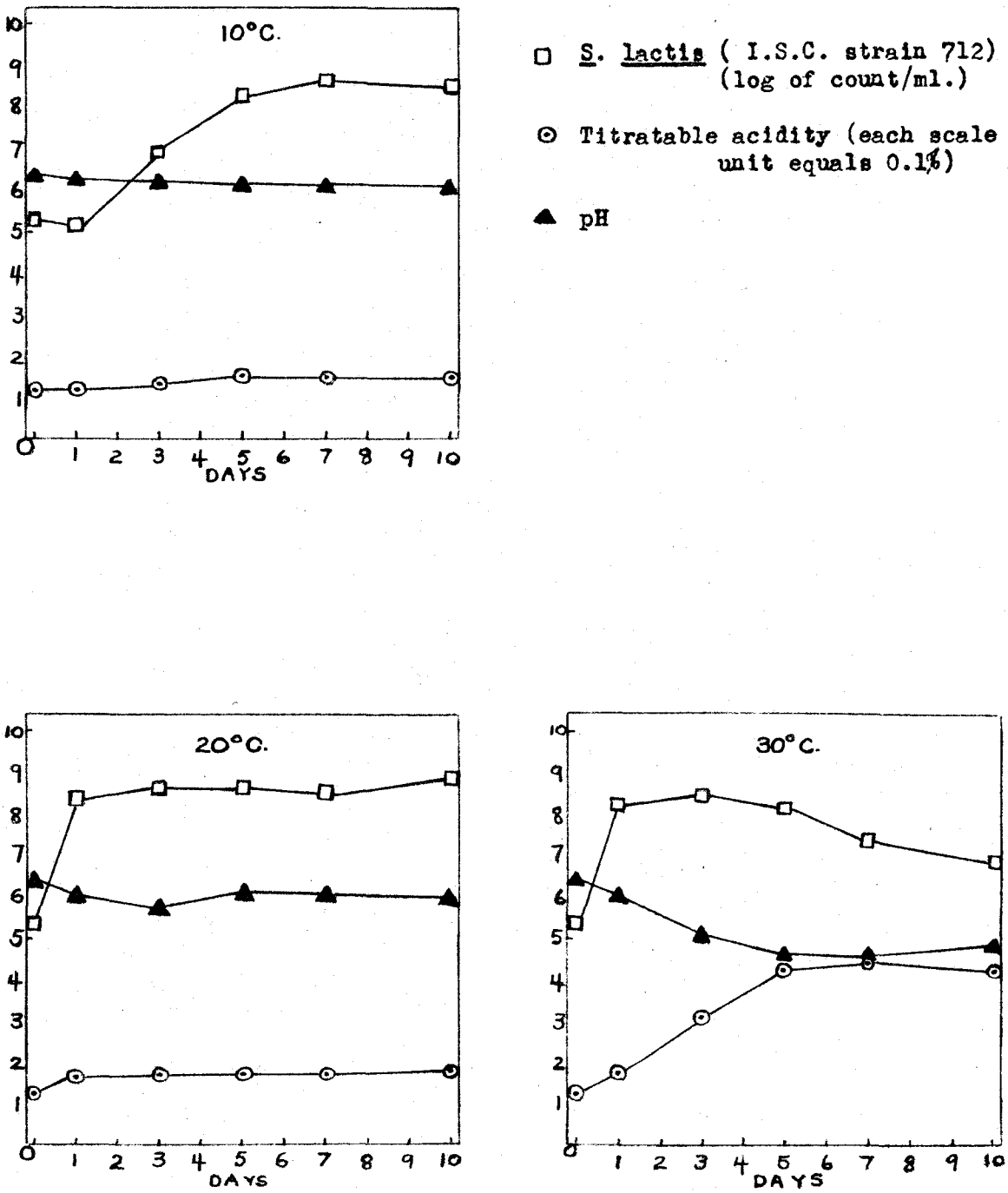
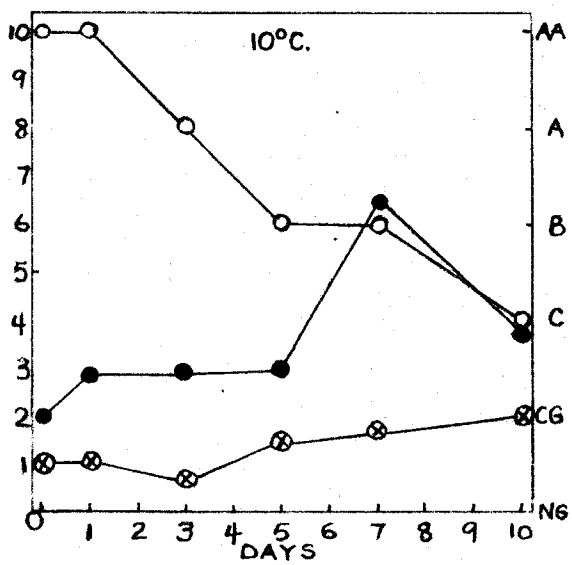


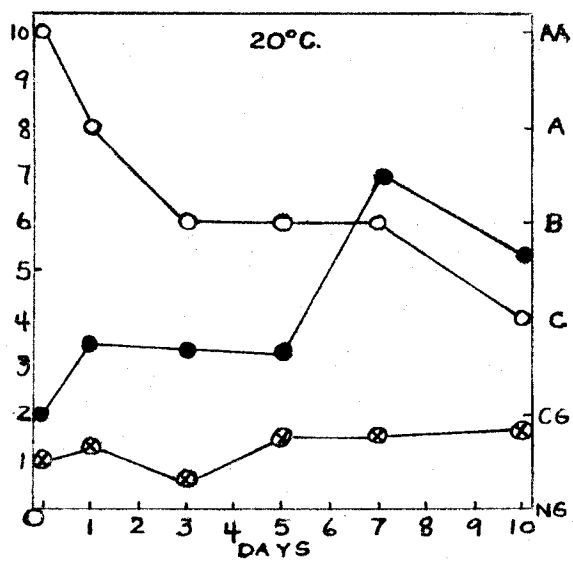
Fig. 9. Titratable acidity, pH and counts of S. lactis (I.S.C. strain 712) in cream containing this organism.



⊗ W.I.A. (each scale unit equals 100 mg./100 g. fat)

● Tryptophan (each scale unit equals 1 p.p.m.)

○ Grade (U.S.D.A.)



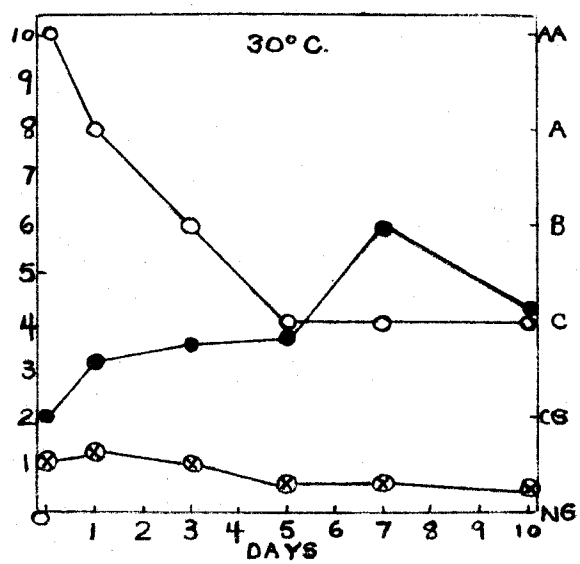


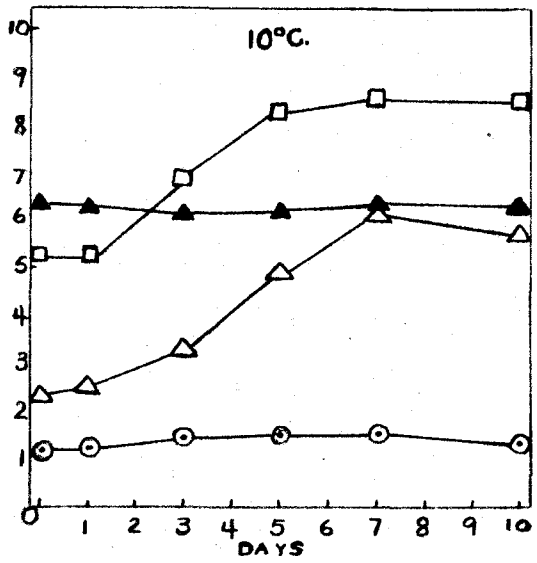
Fig. 10. W.I.A., tryptophan and grade values on cream containing S. lactis (I.S.C. strain 712).

The curves representing the W.I.A. values in figures 6 and 10 do not follow the same pattern, but it seems important to note that at all temperatures on both curves a decrease in W.I.A. value occurred at some time.

Sterile cream inoculated with S. lactis (I.S.C. 712) and G. candidum

The weak S. lactis strain was inoculated into cream along with G. candidum and the results are presented primarily for comparison with similar cream samples inoculated with the same strain of G. candidum and the more active S. lactis. A comparison of figures 9 and 11 shows that G. candidum tended to retard the rate of population increase of strain 712 at 20 and 30° C. and also restrained the subsequent rate of decrease at 30° C. The characteristic of G. candidum in minimizing the rate of decrease of S. lactis is evident when figures 5 and 7 are compared.

The uniform tryptophan curves previously observed in figures 6 and 10, which depict the result of inoculations with S. lactis only, are again evident in figures 8 and 12 which represent inoculations with both S. lactis and G. candidum. The accumulation of evidence illustrated by the tryptophan curves at all three temperatures on these four figures further emphasizes that the fluctuation of free tryptophan values represents normal S. lactis activity in

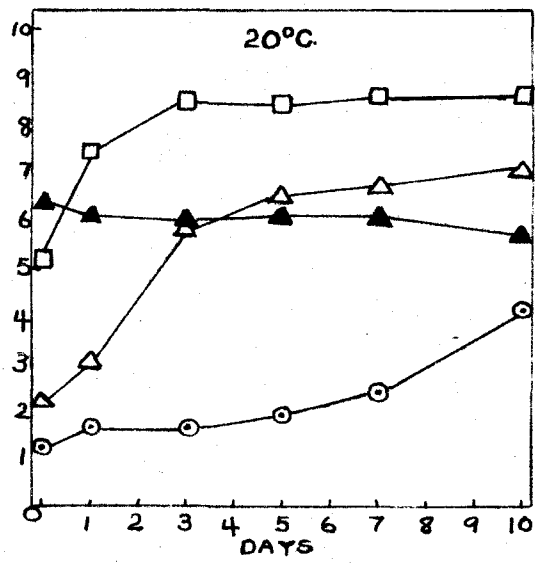


□ S. lactis (I.S.C. strain 712) (log of count/ml.)

△ G. candidum (log of count/ml.)

⊙ Titratable acidity (each scale unit equals 0.1%)

▲ pH



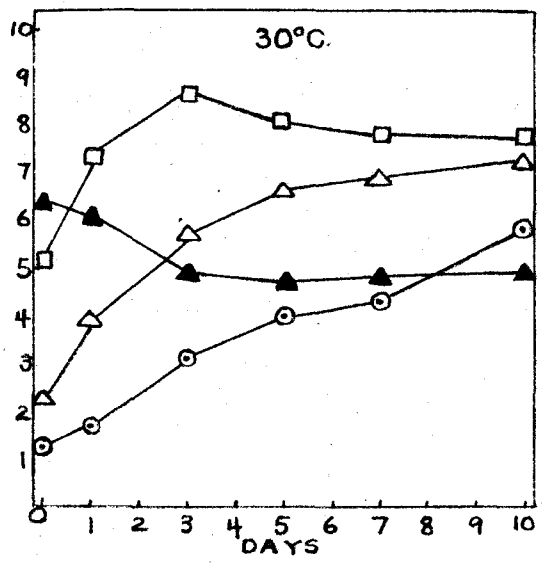


Fig. 11. Titratable acidity, pH and counts of S. lactis (I.S.C. strain 712) and G. candidum in cream containing both of these organisms.

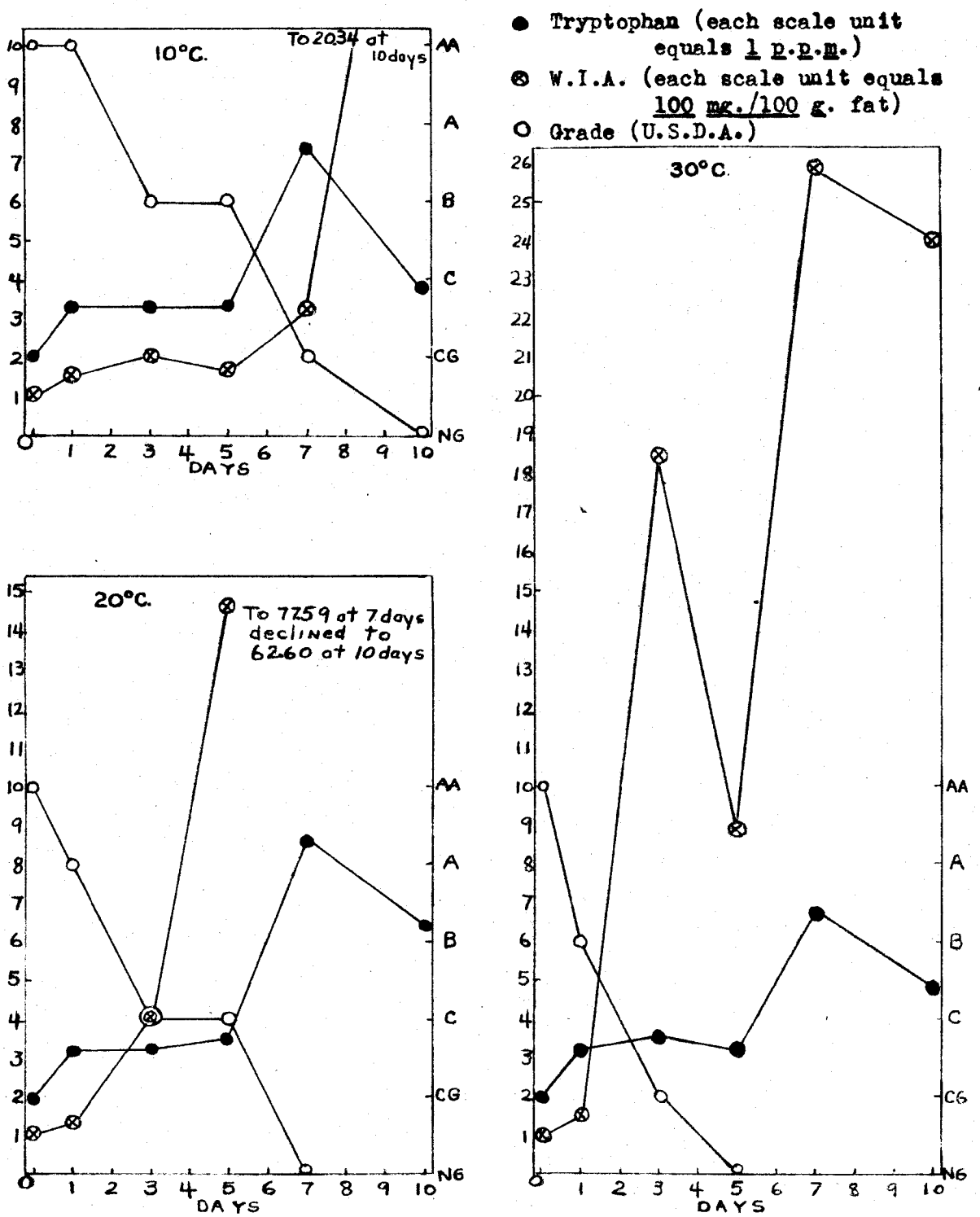


Fig. 12. W.I.A., tryptophan and grade values on cream containing both S. lactis (I.S.C. strain 712) and G. candidum.

cream.

A study of the W.I.A. values in figures 8 and 12 readily reveals the inferior ability of the weak strain of S. lactis to inhibit the lipase of G. candidum. In the presence of S. lactis 712, G. candidum produced greater W.I.A. values than when inoculated alone. This circumstance should be interpreted as a coincidence and not as additional lipolysis attributed to S. lactis. However, the weak S. lactis strain maintained the pH at about 6.0 for an extended period which seems favorable for the lipase of G. candidum. The more vigorous S. lactis culture reduced the pH low enough to partially inhibit the lipase. Another feature of the W.I.A. values shown in figures 8 and 12 should be observed. At both 20 and 30° C. a decrease in W.I.A. began on the third, fifth or seventh day, depending on the temperature and the vigor of the S. lactis culture. Because of previous decreases in W.I.A. encountered in the presence of S. lactis and the absence of such decreases when G. candidum is alone, it seems reasonable to attribute these decreases to an activity of S. lactis.

Sterile cream containing added oleic acid and inoculated with S. lactis

The recurrent tendency of S. lactis to cause a decrease in W.I.A. was considered worthy of investigation, especially since the decrease was observed with two different strains as

indicated in figures 6 and 10. Decreases also were encountered in results not reported in this work. A weighed quantity of oleic acid was dissolved in a small amount of ether and transferred to cream which was subsequently sterilized by the routine method. The amount of oleic acid was calculated to be 576.8 mg. per 100 g. of fat. Upon analysis the initial W.I.A. values prior to inoculation were determined to be 859, 864 and 846 for the 10, 20 and 30° C. samples, respectively. The samples were then inoculated with S. lactis and the ensuing changes which occurred in the W.I.A. values are indicated in figure 14. A consistent decrease extending through the fifth day occurred at all three temperatures and was followed by a slight rise to the seventh day. The W.I.A. value in the 20° C. sample on the seventh day is inconsistent with the normal pattern and is suspected of being an analytical error or undetected contamination by a lipolytic organism. The amounts of W.I.A., in mg. per 100 g. of fat, which disappeared during the interval from initial inoculation to the fifth day were 211 at 10° C., 143 at 20° C. and 148 at 30° C. These quantities, together with previously indicated smaller decreases, are sufficient to justify a conclusion that S. lactis utilized W.I.A.

Tryptophan and score values also were followed and are indicated in figure 14. The increase and subsequent decrease in free tryptophan which appeared at all temperatures has been

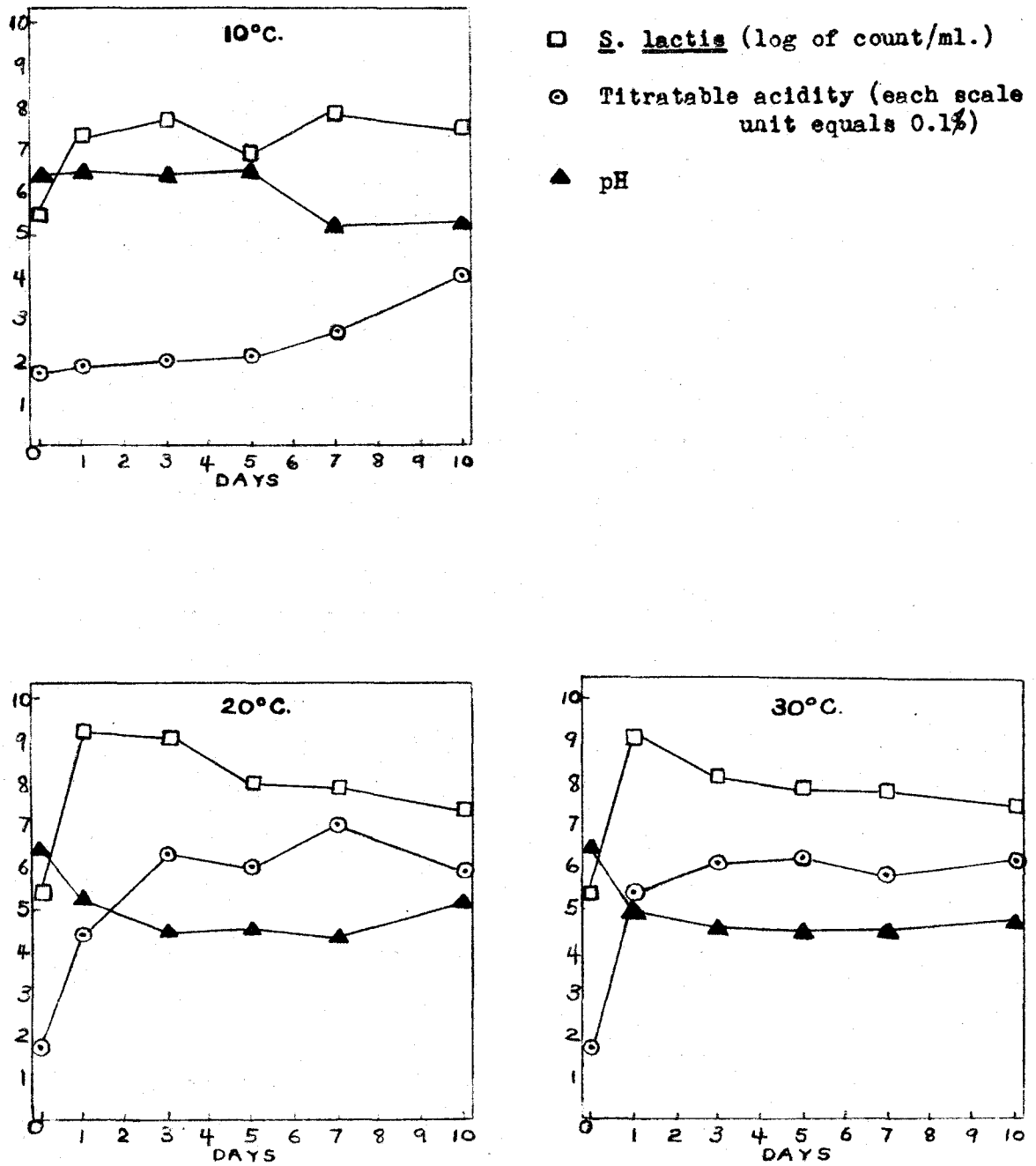
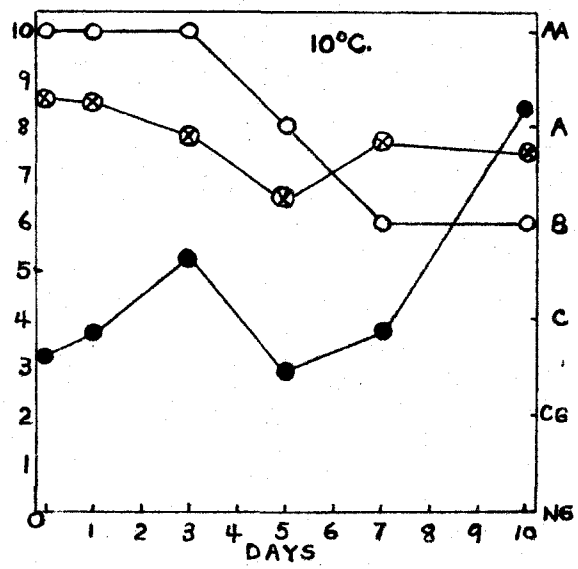


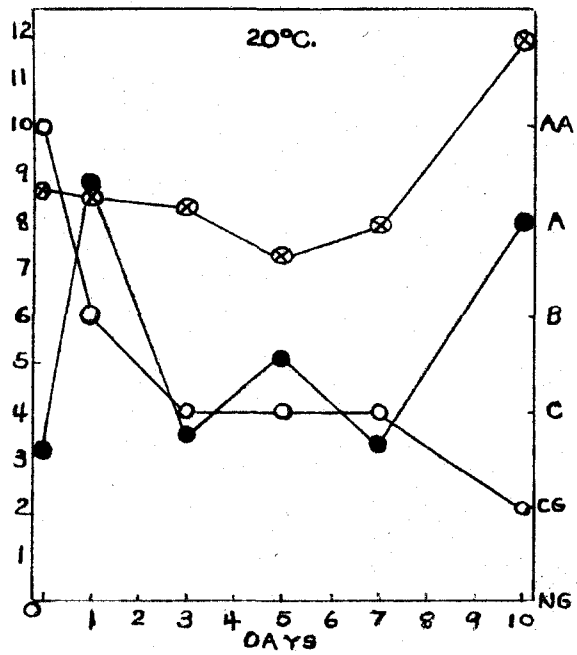
Fig. 13. Titratable acidity, pH and counts of S. lactis in cream containing this organism and added oleic acid.



⊗ W.I.A. (each scale unit equals 100 mg./100 g. fat)

● Tryptophan (each scale unit equals 1 p.p.m.)

○ Grade (U.S.D.A.)



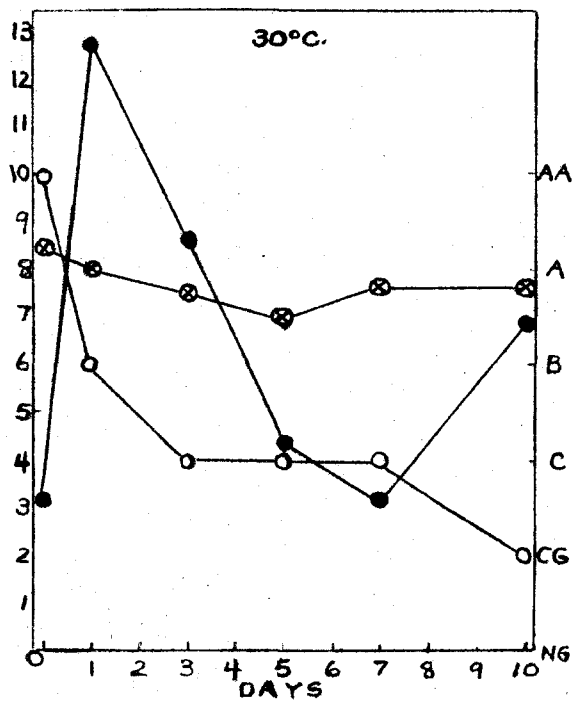


Fig. 14. W.I.A., tryptophan and grade values on cream containing S. lactis and added oleic acid.

noted previously and seems to be a normal response to S. lactis. However, in the presence of oleic acid the fluctuation occurs earlier in the time cycle than previously and in this case is followed by a secondary increase which heretofore has not appeared.

Figure 13 shows normal response of organism population, titratable acidity and pH in the cream containing added oleic acid. The presence of the additional oleic acid showed no inhibitory effect upon S. lactis activity.

Sterile cream inoculated with T. cremoris

Changes occurring in organism population, titratable acidity and pH when T. cremoris is inoculated into sterile cream are shown in figure 15. At 10° C. the organism increase was moderately slow, attained maximum on the fifth day and then remained stationary. At 20 and 30° C. the increase was more rapid, reached maximum on the third day and gradually declined; however, at 30° C. there was a secondary increase after the seventh day. The increases and subsequent decreases in titratable acidity at 20 and 30° C. are due to gas accumulation which began to diminish after the third day.

Score, W.I.A. and tryptophan values are shown in figure 16. At all temperatures the W.I.A. values show a general tendency to increase, although there is a uniform slight decline on the third day. The tryptophan values

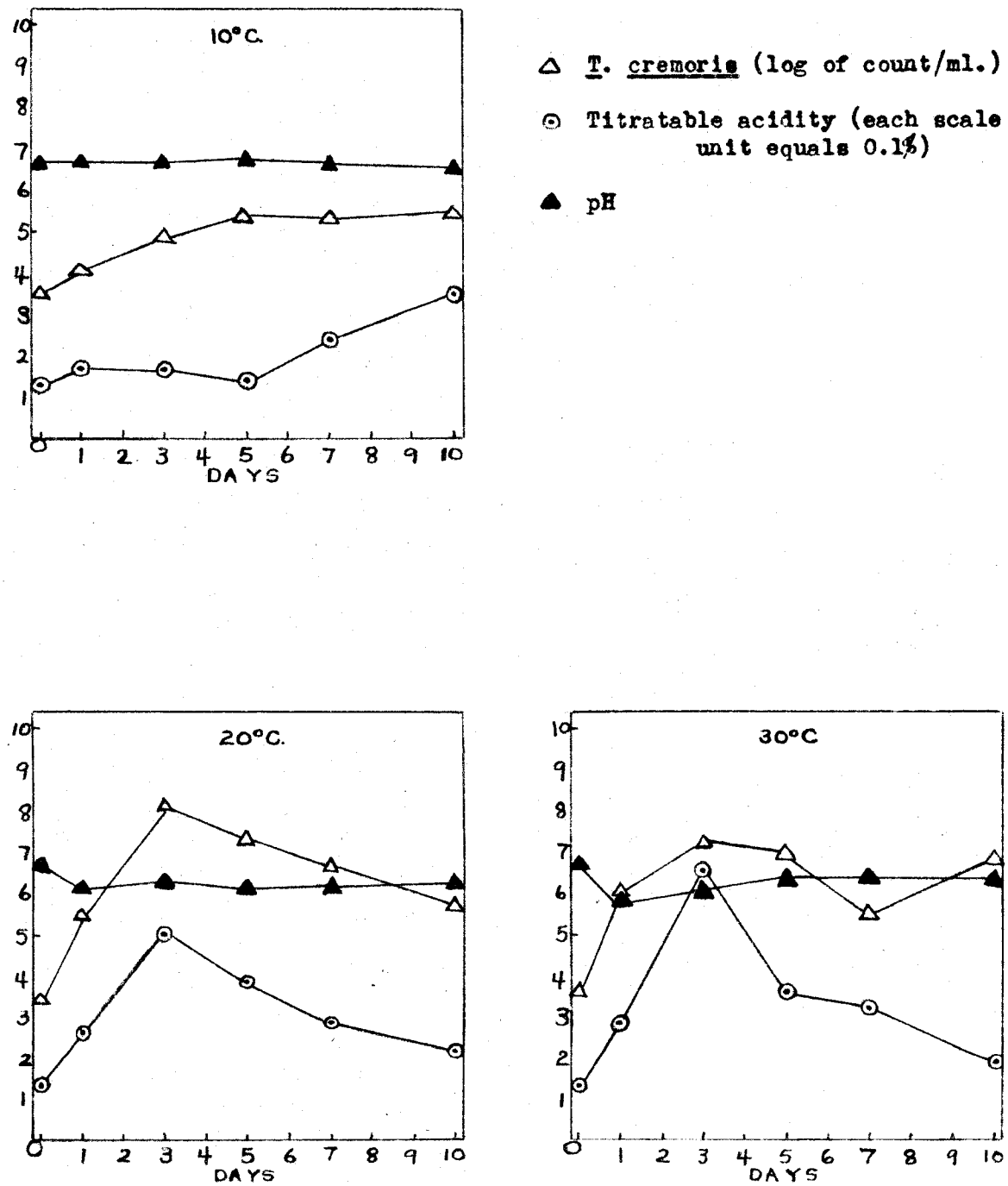
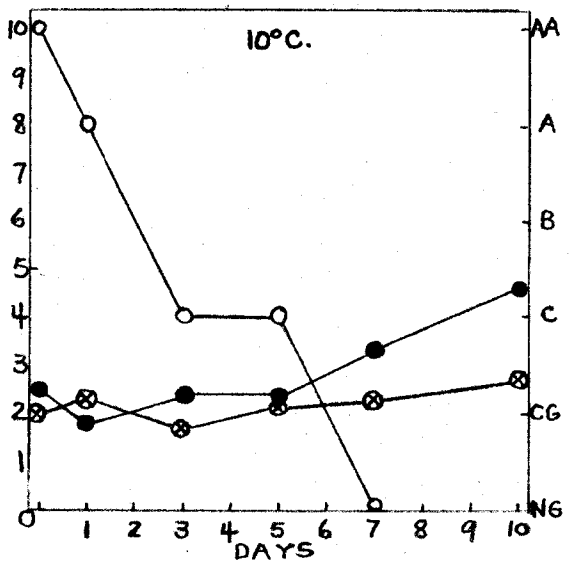


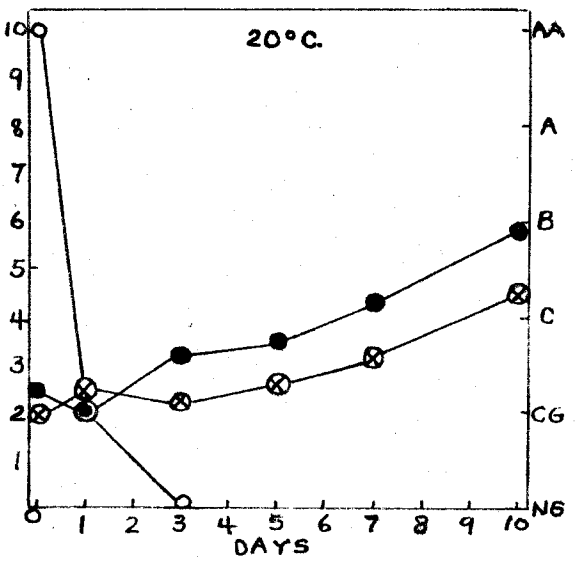
Fig. 15. Titratable acidity, pH and counts of T. cremoris in cream containing this organism.



⊗ W.I.A. (each scale unit equals 100 mg./100 g. fat)

● Tryptophan (each scale unit equals 1 p.p.m.)

○ Grade (U.S.D.A.)



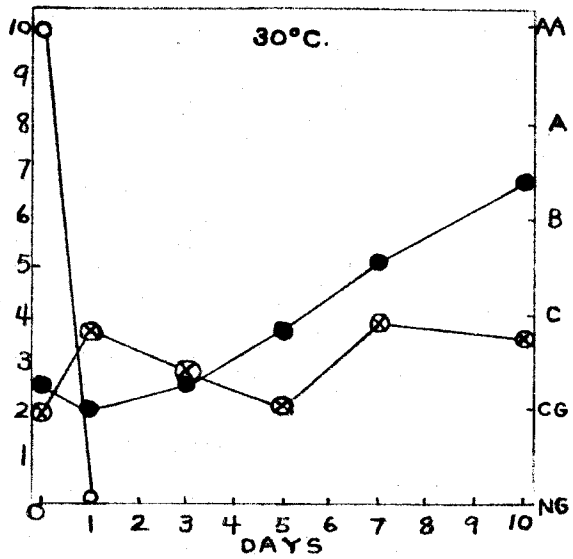


Fig. 16. W.I.A., tryptophan and grade values on cream containing T. cremoris.

decreased uniformly at all temperatures on the first day and then gradually ascended throughout the remainder of the incubation period. The organoleptic deterioration of the cream was so rapid that no W.I.A. or tryptophan fluctuations of consequence occurred during its commercial life.

Sterile cream inoculated with T. cremoris and S. lactis

The changes in organism population, titratable acidity and pH occurring when S. lactis and T. cremoris are inoculated into sterile cream are recorded in figure 17. A comparison of figures 15 and 17 indicates that the presence of S. lactis did not seem to exhibit a significant effect on the population of T. cremoris at 10° C., but showed a marked inhibition at 20 and 30° C. Also there was a lag in the growth rate of T. cremoris, with the maximum population occurring later when S. lactis was present. The titratable acidity fluctuations reflect the influence of both organisms, with the late decreases apparently due to diminishing gas retention. The pH did not drop as low as normal for a culture containing S. lactis, suggesting that T. cremoris interferred with the acid-producing ability of S. lactis, although the growth curve of S. lactis does not reflect any inhibitory influence on cell population.

Figure 18 shows the influence of S. lactis and T. cremoris on W.I.A., tryptophan and score. A comparison of

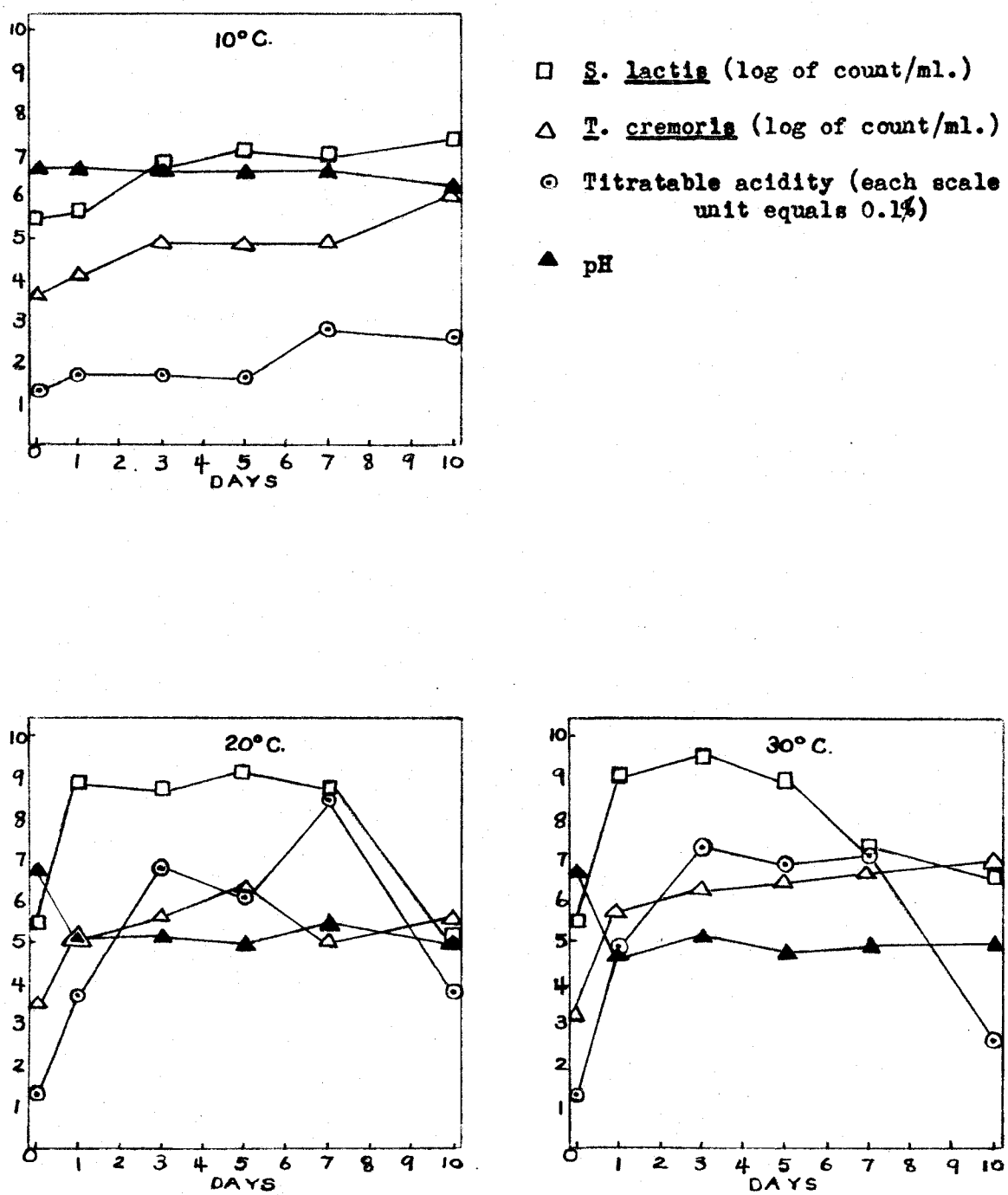


Fig. 17. Titratable acidity, pH and counts of S. lactis and T. cremoris in cream containing both of these organisms.

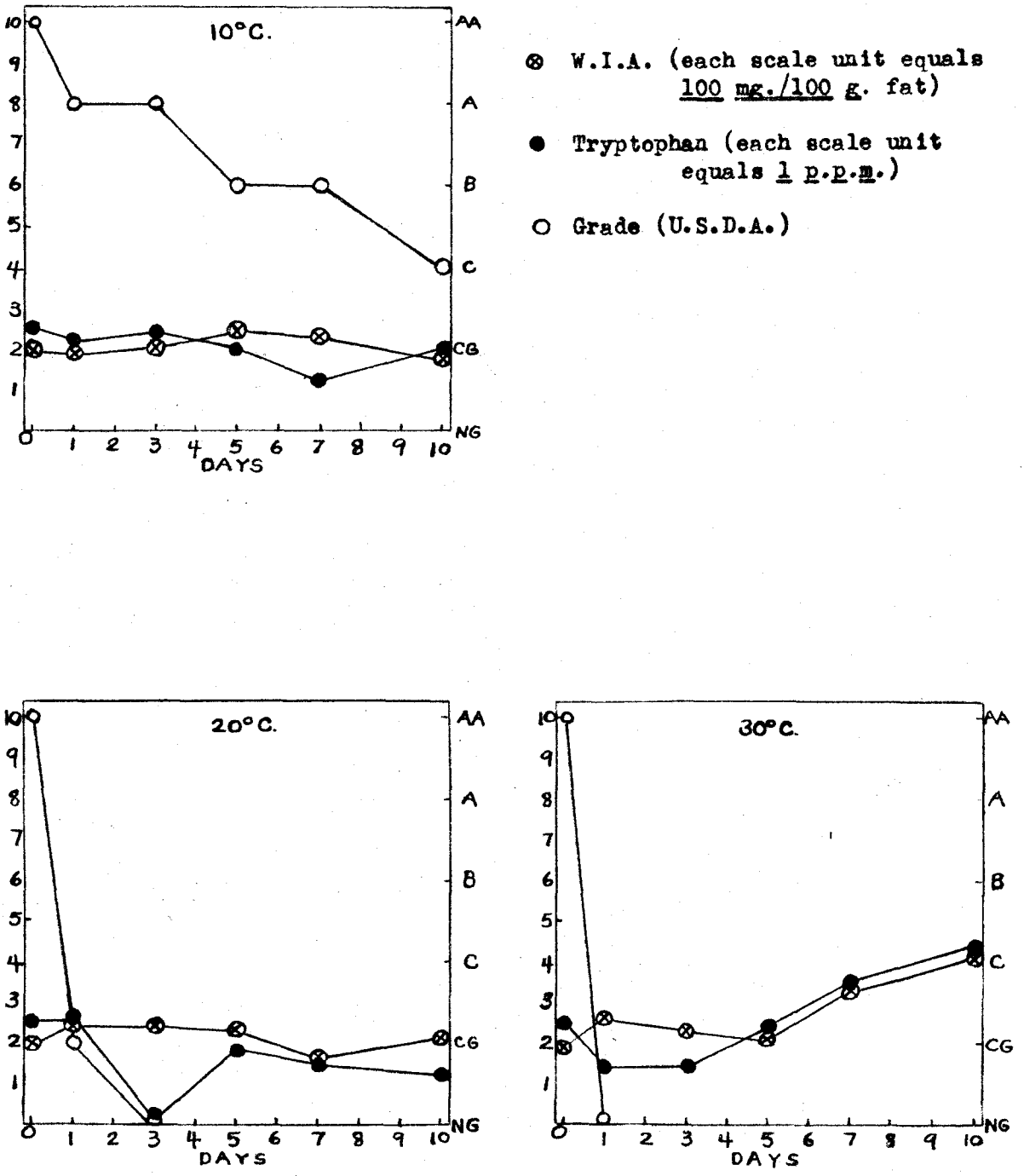


Fig. 18. W.I.A., tryptophan and grade values on cream containing S. lactis and T. cremoris.

the W.I.A. curves in figures 6, 16 and 18 show that the tendency of T. cremoris to cause a slight early increase in W.I.A. is more dominant than the inclination of S. lactis to cause a decrease during the corresponding period. The early slight decrease in tryptophan caused by T. cremoris alone also occurred when the yeast was grown in association with S. lactis. W.I.A. and free tryptophan values did not increase to quantities of any consequence at any of the three temperatures. At 20 and 30° C., addition of S. lactis did not alter the rate of organoleptic deterioration induced by T. cremoris. However, at 10° C. S. lactis exhibited a significant protective effect on the quality, even though the population attained by T. cremoris in the presence of S. lactis was approximately the same as when unaccompanied. The quality factors plotted do not show any reason for the superior flavor of the 10° C. cream containing both organisms as compared to the 10° C. cream containing only T. cremoris, but in the former the yeasty flavor was much less intense and the body was less foamy.

Sterile cream inoculated with Ps. fragi

Changes occurring in count, titratable acidity and pH as a result of incubating sterile cream inoculated with Ps. fragi are shown in figure 19. Maximum population was attained on the third day at 10 and 20° C. and on the first

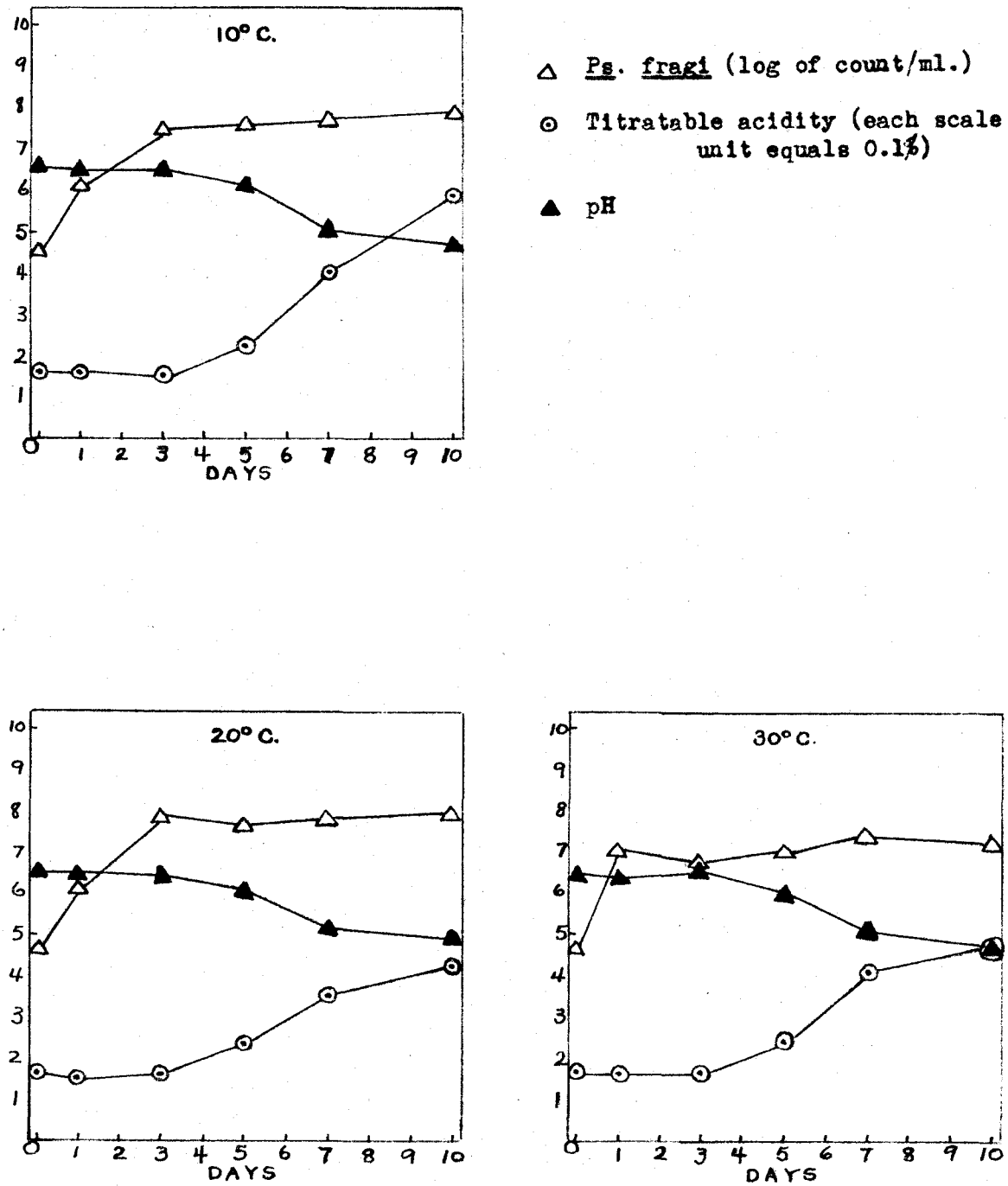


Fig. 19. Titratable acidity, pH and counts of Ps. fragi in cream containing this organism.

day at 30° C. Also it is significant that there was no appreciable population decline throughout the incubation period. The greatest maximum population was attained at 20° C., and the maximum at 10° C. was greater than at 30° C. Both the titratable acidity and pH curves show the same pattern at all temperatures.

W.I.A., tryptophan and score values are plotted on figure 20. The increases in W.I.A. were the most significant feature of Ps. fragi action. After the third day the increases were extremely abrupt at all three temperatures and at 10° C. this organism was even more lipolytic than G. candidum. The fluctuations in the tryptophan values in the early days of the incubation period lack uniformity. The 3-day, 20° C. sample was accidentally destroyed after it was too late to secure another. After the third day at 30° C. and after the fifth day at 10 and 20° C. there was a pronounced increase in free tryptophan at all temperatures, with a subsequent decline occurring after the seventh day. The greatest degree of proteolysis occurred at 10 and 20° C.; however, this may be due to protease inhibition by the extremely high free fatty acid accumulation at 30° C., rather than low temperature preference of the protease. Although Ps. fragi is noted for its lipolytic activity, it also was one of the most proteolytic organisms studied. Organoleptic deterioration seemed to be more closely related to increases in W.I.A. and free tryptophan than to any other

W.I.A. (each scale unit equals
100 mg./100 g. fat)

Tryptophan (each scale unit
equals 1 p.p.m.)

Grade (U.S.D.A.)

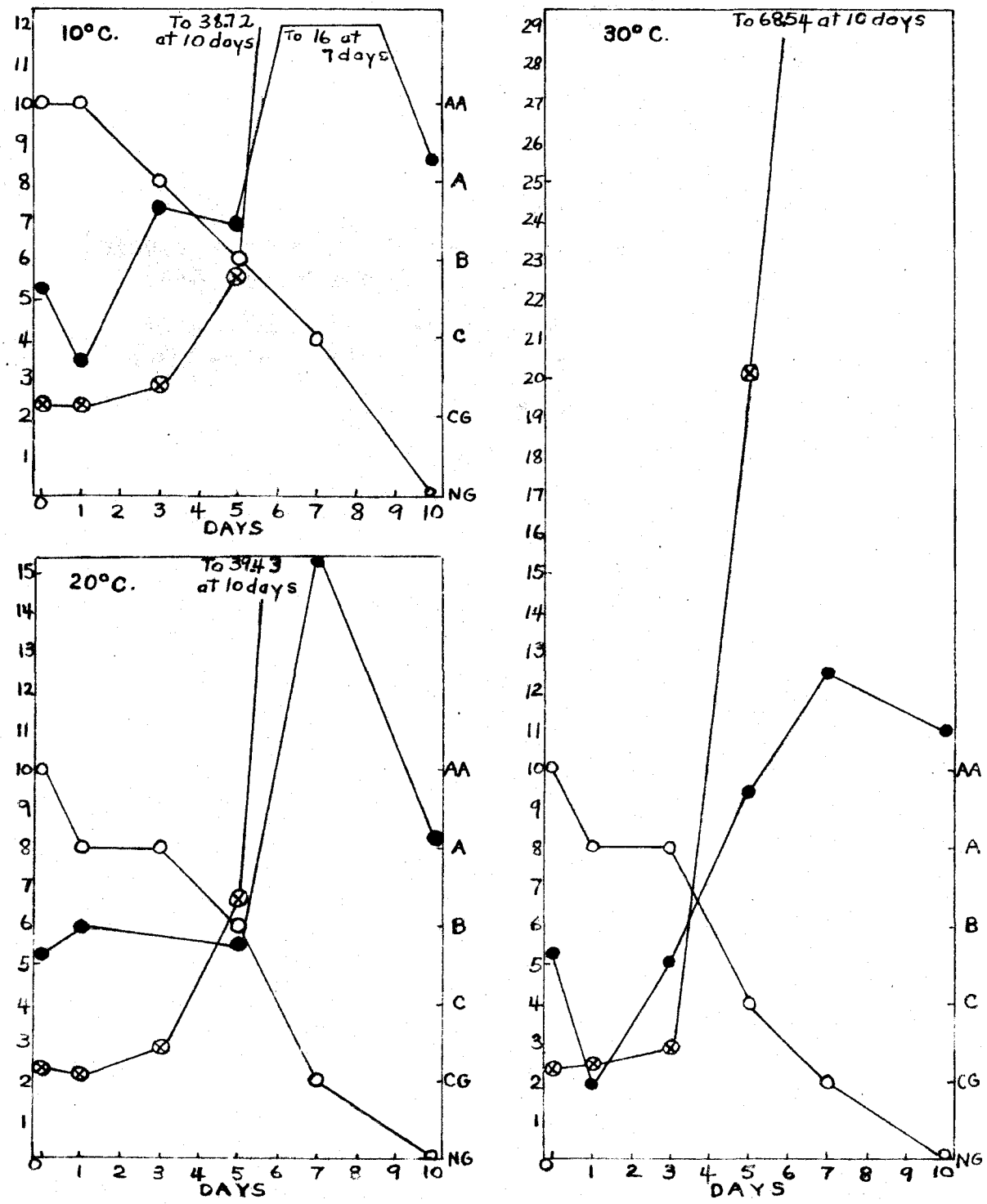


Fig. 20. W.I.A., tryptophan and grade values on cream containing Ps. fragi.

factor considered.

Sterile cream inoculated with S. lactis and Ps. fragi

The changes which occur in organism population, titratable acidity and pH as a result of incubating sterile cream inoculated with S. lactis and Ps. fragi are shown in figure 21. A comparison of figures 19 and 21 shows that the presence of S. lactis exhibited a pronounced inhibitory effect upon the Ps. fragi population at each of the three incubation temperatures. When associated with S. lactis, the maximum Ps. fragi counts attained were much lower and there was a numerical decline during the later stages of holding which did not occur when Ps. fragi was grown alone. At 30° C. S. lactis caused the virtual disappearance of Ps. fragi with the counts being 10 and 14 per ml. at the seventh and tenth days, respectively. The S. lactis growth response seemed to be normal at each of the three temperatures, indicating that the association with Ps. fragi produced no effect on S. lactis population. The increase in titratable acidity paralleled the increase in S. lactis population, with the maximums occurring simultaneously. Beginning with the third day at 10 and 20° C. and with the first day at 30° C., there was a decrease in titratable acidity which was slight at 20 and 30° C. and pronounced at 10° C. The pH curves followed the normal

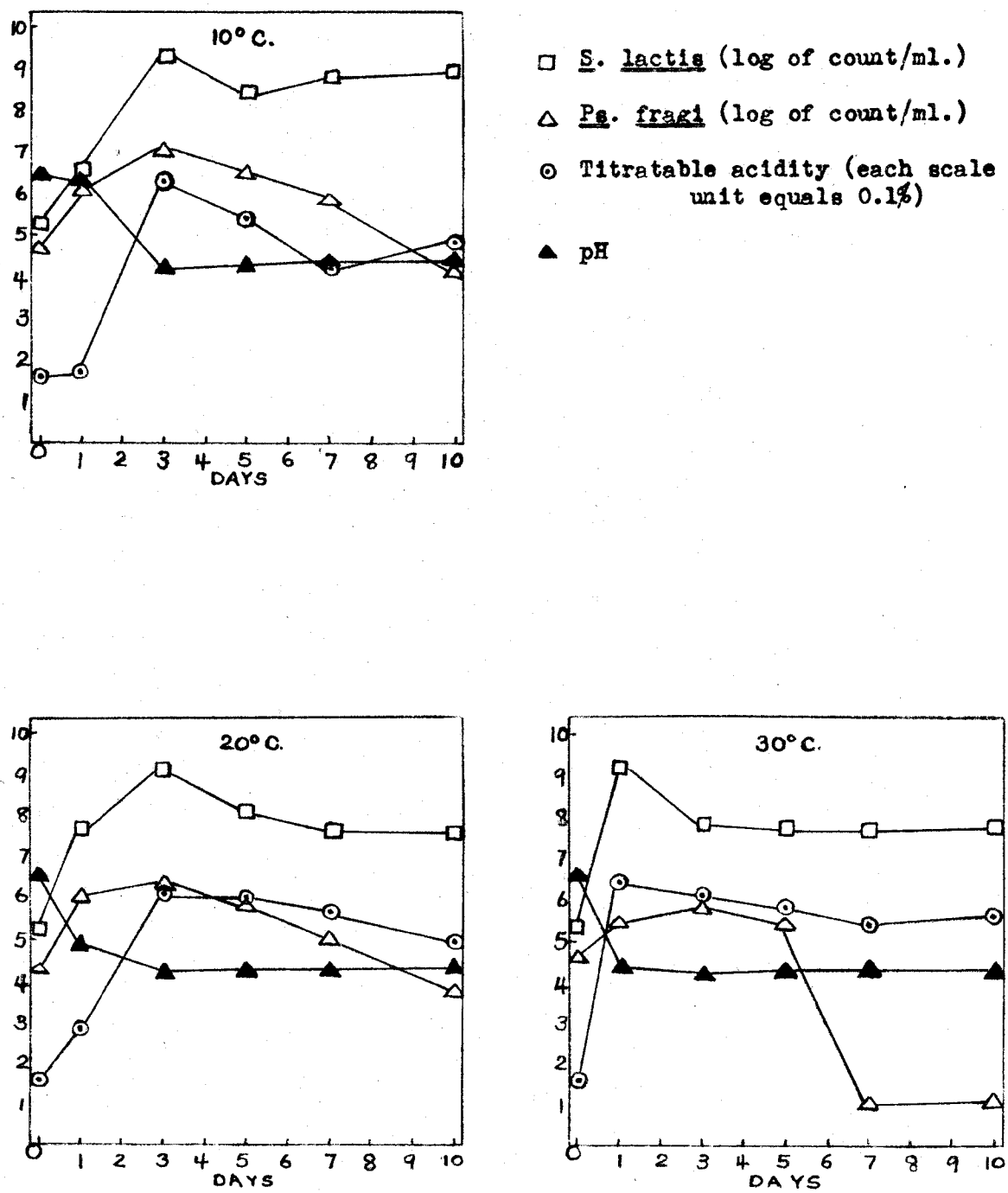


Fig. 21. Titratable acidity, pH and counts of S. lactis and Ps. fragi in cream containing both of these organisms.

pattern at all temperatures, with barely perceptible gradual increases occuring coincident with the declines in titratable acidity. There is no apparent explanation for these decreases in titratable acidity, for both organisms show an opposite trend when functioning independently.

Figure 22 shows the changes occurring in score, W.I.A. and tryptophan values in sterile cream inoculated with S. lactis and Ps. fragi. The inhibitory effect of S. lactis upon Ps. fragi becomes much more evident when the W.I.A. curve is examined at each of the three temperatures. At 20 and 30° C. the influence of S. lactis was sufficient to inhibit activity of any lipase elaborated from Ps. fragi, which is in extreme contrast with W.I.A. values encountered when Ps. fragi was inoculated alone. At 10° C. the lipase was able to cause considerable fat hydrolysis, although barely more than half as much as caused by Ps. fragi when growing alone. Possible reasons for the large amount of lipolysis occurring at 10° C. may be found by examining figure 21. The Ps. fragi population attained was substantially higher at 10 than at 20 or 30° C. The pH decline was slower in the 10° C. sample, but the greater Ps. fragi population at 10° C. seems to represent the most logical explanation for the higher W.I.A. value, because the W.I.A. increase did not begin until the third day, at which time the pH was down to 4.22. The characteristic of S. lactis

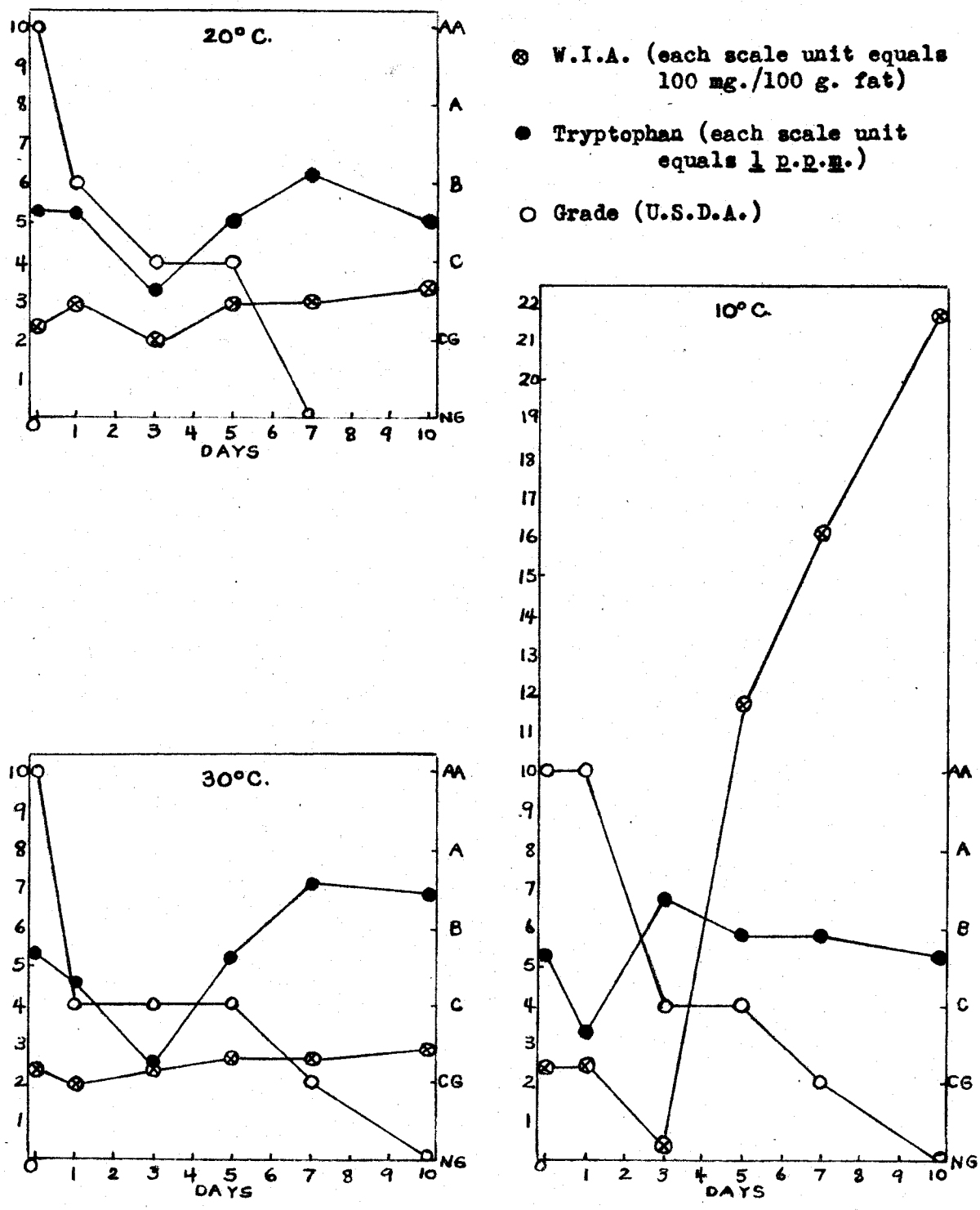


Fig. 22. W.I.A., tryptophan and grade values on cream containing both S. lactis and Pg. fragi.

to utilize W.I.A. early in the incubation period was again apparent, with the decrease appearing on the first day at 30° C. and on the third day at 10 and 20° C.

The tryptophan curves at all three temperatures in figure 22 show an early decline, followed by a rise and a secondary decrease. The early decrease may be attributed to Ps. fragi, since the same tendency appears at 10 and 30° C. on figure 20, but after passing the first declining interval the curves more nearly correspond to those produced by S. lactis, as shown in figure 6. The proteolytic characteristic of Ps. fragi definitely was subordinated by S. lactis at all temperatures because the maximum values are much lower than those appearing in figure 20. Deterioration in organoleptic grade does not seem to be closely related to any one factor except that the early decline corresponds to the organism population increase.

Sterile cream inoculated with A. aerogenes

The changes which occurred in organism population, titratable acidity and pH values in sterile cream inoculated with A. aerogenes are shown in figure 23. At 10° C. the organism population showed a consistent uniform decrease throughout the incubation period. No significant change occurred in titratable acidity or pH. At 20 and 30° C. the counts and titratable acidity increased, with more rapid deterioration occurring at 30° C. Gas formation was

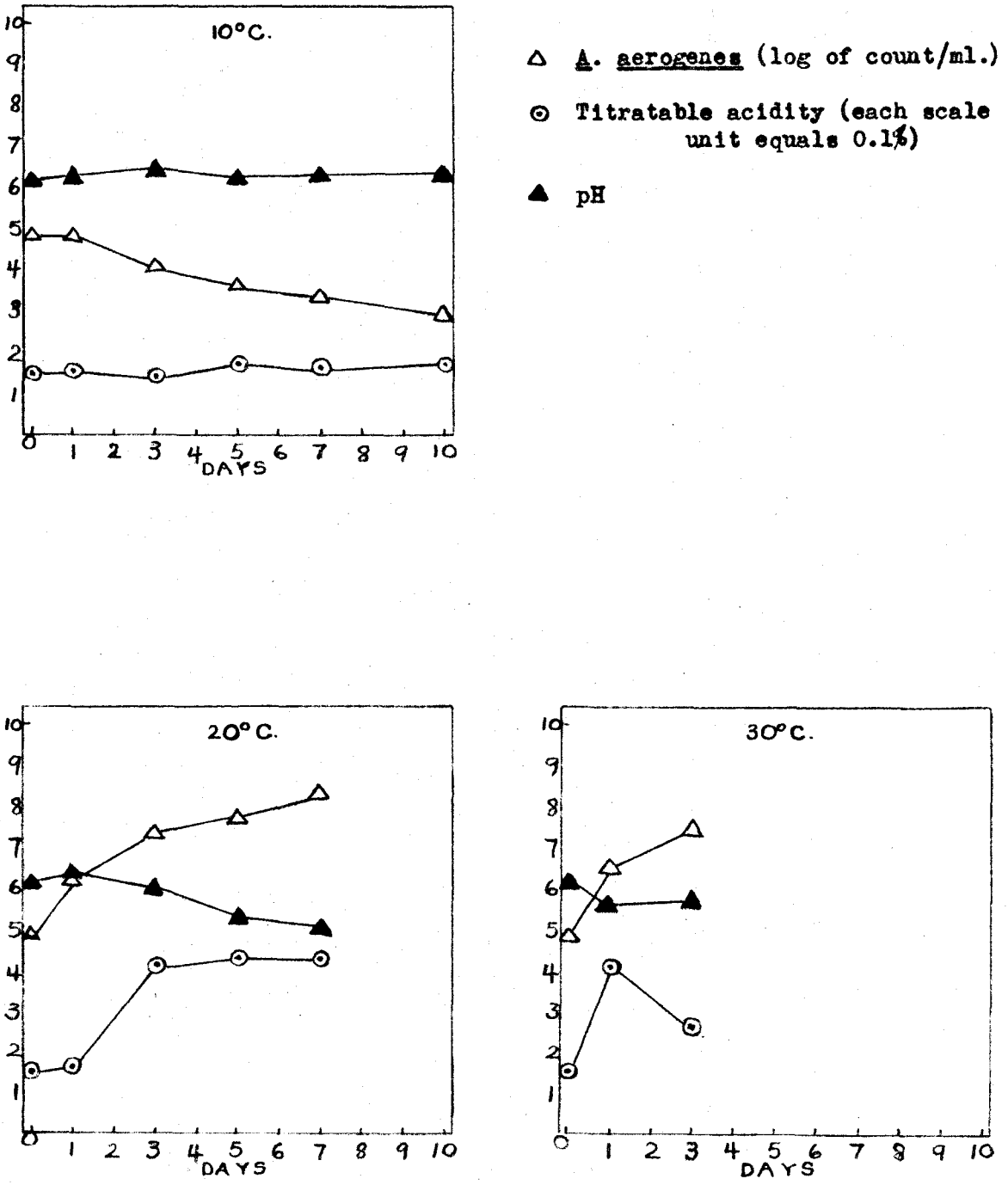


Fig. 23. Titratable acidity, pH and counts of A. aerogenes in cream containing this organism.

so great in the 30° C. sample that the plug was blown out and the cream foamed over after incubation for about 40 hours. A portion of the contents was salvaged, refrigerated and analyzed on the third day. The decline in titratable acidity apparently was caused by loss of gas. The 30° C. sample was discarded on the third day and the 20° C. sample on the seventh day because of pronounced deterioration. The pH decreases at the two higher temperatures were less than normal for the degree of acidity indicated, but were reasonable, since most of the acidity was due to CO_2 development.

Figure 24 shows the changes which occurred in W.I.A., tryptophan and score values when sterile cream was inoculated with A. aerogenes and incubated at 10, 20 and 30° C. Very slight gradual increases occurred in W.I.A. at all temperatures. However, at 30° C. the commercial life of the sample was too short to determine a long-term trend. The free tryptophan values seem unusual but maintain a uniform trend of an early increase followed by a decrease, although the increase at 20° C. is only slight. The decline in organoleptic quality seemed to be more closely related to organism population than to any other factor. From the standpoint of flavor and physical effect on cream, A. aerogenes at 20 and 30° C. was one of the most objectionable organisms encountered because of the foamy body and unclean flavor produced. At 10° C. the population was insufficient

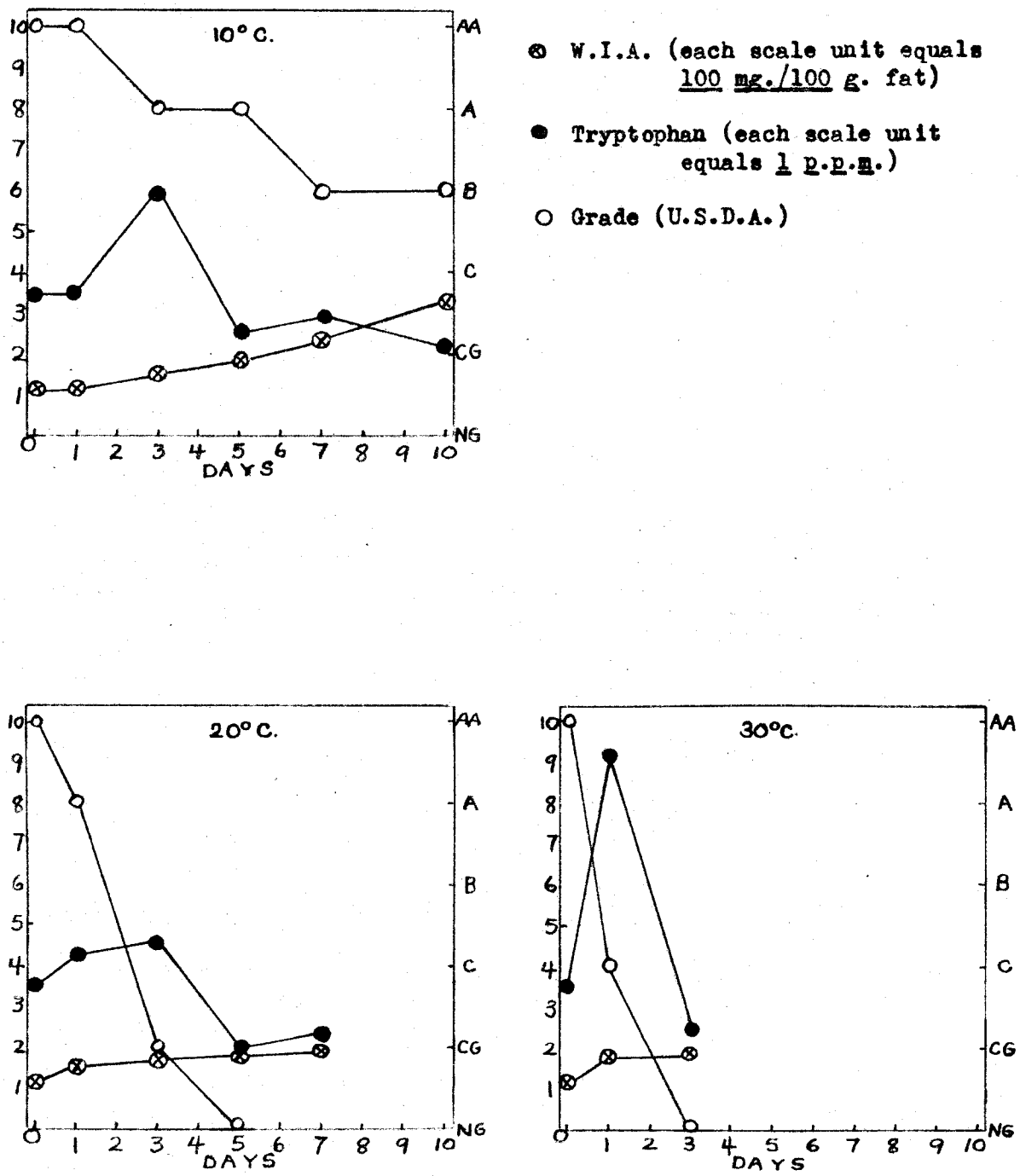
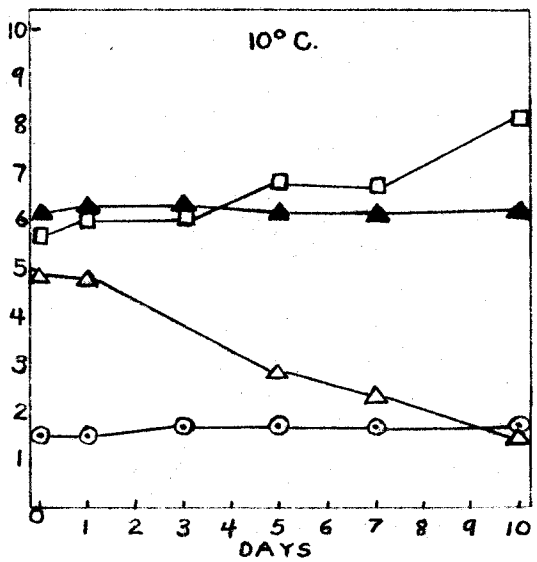


Fig. 24. W.I.A., Tryptophan and grade values on cream containing A. aerogenes.

to produce the unclean flavor and gassy body, but the gradual development of a stale flavor was evident.

Sterile cream inoculated with A. aerogenes and S. lactis

Changes in population counts, pH and titratable acidity induced by incubating sterile cream inoculated with A. aerogenes and S. lactis are shown in figure 25. At 10° C. the count on the coliform organism decreased from an initial 74,000 to 38 per ml. on the tenth day. The pattern of decrease was the same when A. aerogenes exhibited an inhibitory effect on S. lactis because the population increase was much slower than the normal curve shown in figure 5. There was no apparent change in titratable acidity or pH at 10° C., a further indication that S. lactis was inhibited. At 20° C. the S. lactis increase was normal, but a comparison of figures 23 and 25 shows that S. lactis inhibited the coliform population to some extent. The sample held at 30° C. foamed over after about 40 hours incubation. A portion was salvaged, refrigerated and analyzed on the third day. The drop in A. aerogenes population after the first day may have been due to antagonism from S. lactis or to refrigeration. The titratable acidity increases were due to acid and gas development, with the pH decrease again being less than normal for the corresponding titratable acidity because of the CO_2 present.

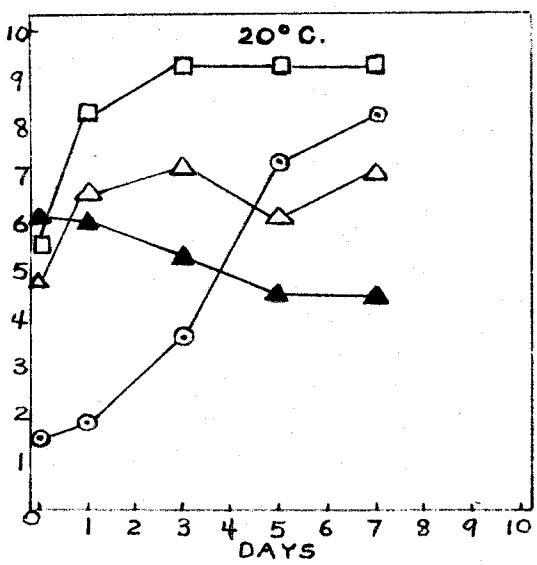


□ S. lactis (log of count/ml.)

△ A. aerogenes (log of count/ml.)

⊙ Titratable acidity (each scale unit equals 0.1%)

▲ pH



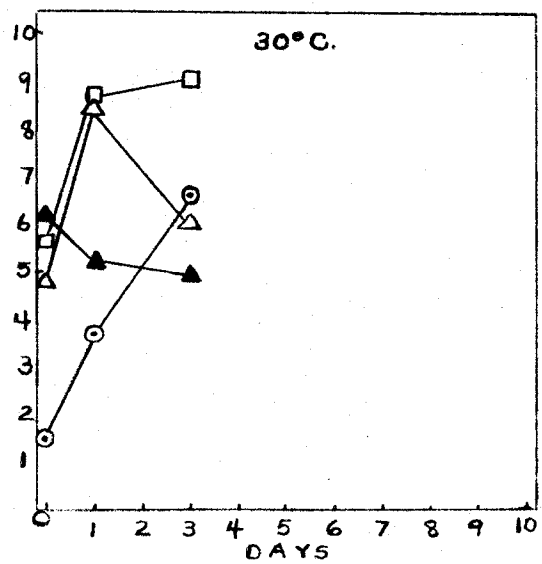


Fig. 25. Titratable acidity, pH and counts of S. lactis and A. aerogenes in cream containing both of these organisms.

Figure 26 indicates the changes which occurred in W.I.A., tryptophan and score when S. lactis and A. aerogenes were inoculated into sterile cream. No change of any consequence occurred in the W.I.A. values at either of the three temperatures, although at 30° C. there was an early decrease, probably attributable to S. lactis. Combination of the two organisms produced marked increases in tryptophan at both 20 and 30° C., with the value at 20° C. reaching an extreme of 32 p.p.m. on the third day. These tryptophan values are much greater than either organism was able to produce singly, and in the 20° C. sample the value was twice as much as the cumulative effect of the two organisms functioning alone. Organoleptically detectable deterioration was severe at the two higher temperatures, with a sour, unclean flavor and gassy body becoming evident early in the incubation period. At 10° C. the keeping quality was fairly good, with A. aerogenes seeming to survive long enough to interfere with normal acid development by S. lactis.

Sterile cream inoculated with L. casei

The changes which occurred in organism population, pH and titratable acidity as a result of incubating sterile cream inoculated with L. casei are shown in figure 27. At 10° C. there was no change in titratable acidity, very little change in pH except for a slight decline on the tenth day and the organism count increased only slightly through

W.I.A. (each scale unit equals 100 mg./100 g. fat)

Tryptophan (each scale unit equals 1 p.p.m.)

Grade (U.S.D.A.)

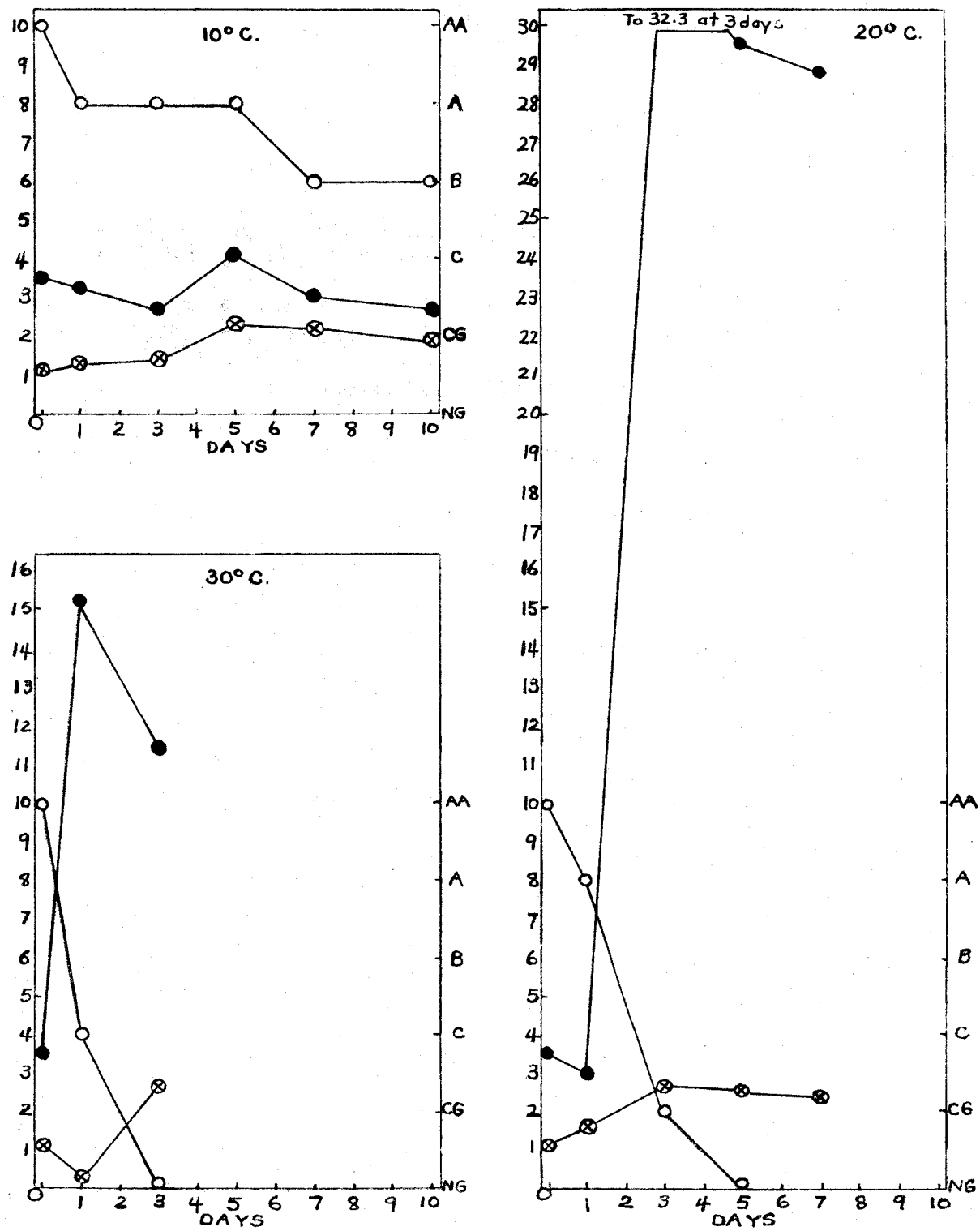
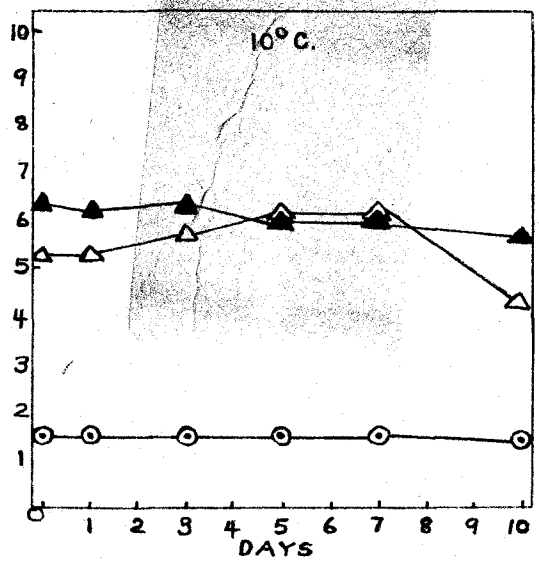


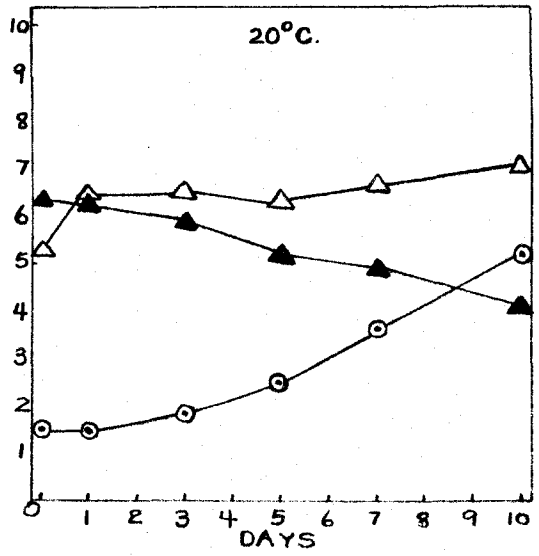
Fig. 26. W.I.A., tryptophan and grade values on cream containing both S. lactis and A. aerogenes.



△ L. casei (log of count/ml.)

⊙ Titratable acidity (each scale unit equals 0.1%)

▲ pH



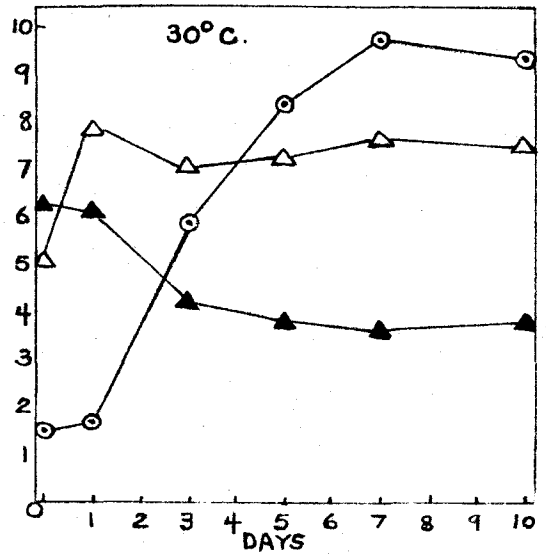


Fig. 27. Titratable acidity, pH and counts of L. casei in cream containing this organism.

the first seven days and then decreased. At 20 and 30° C. the curves in figure 27 are in appropriate relation to each other, with 30° C. obviously being the best of the three temperatures for L. casei activity, as indicated by the high organism population, considerable acid production and low pH.

Figure 28 illustrates changes which occurred in W.I.A., tryptophan and score when L. casei was inoculated into sterile cream. The 10° C. graph illustrating these same factors for cream inoculated with S. lactis plus L. casei is included. The W.I.A. fluctuations at each of the three temperatures are beyond the range of experimental error but not sufficient to be of any consequence other than to suggest a slight degree of fat hydrolysis and simultaneous fatty acid utilization. The tryptophan curves follow the same pattern at all three temperatures, which seems unusual when the extreme differences in organism populations at the various temperatures are considered. The tryptophan fluctuations are extremely irregular with an increase on the first day, a decrease to the fifth, an abrupt increase to the seventh and a sharp decrease to the tenth day. A possible explanation lies in the fact that as the organism population increased and the pH decreased, the protease elaborated by the organisms was inhibited by the low pH to an extent approximately proportionate to the amount of enzyme produced at the higher temperature. The logic of such reasoning is

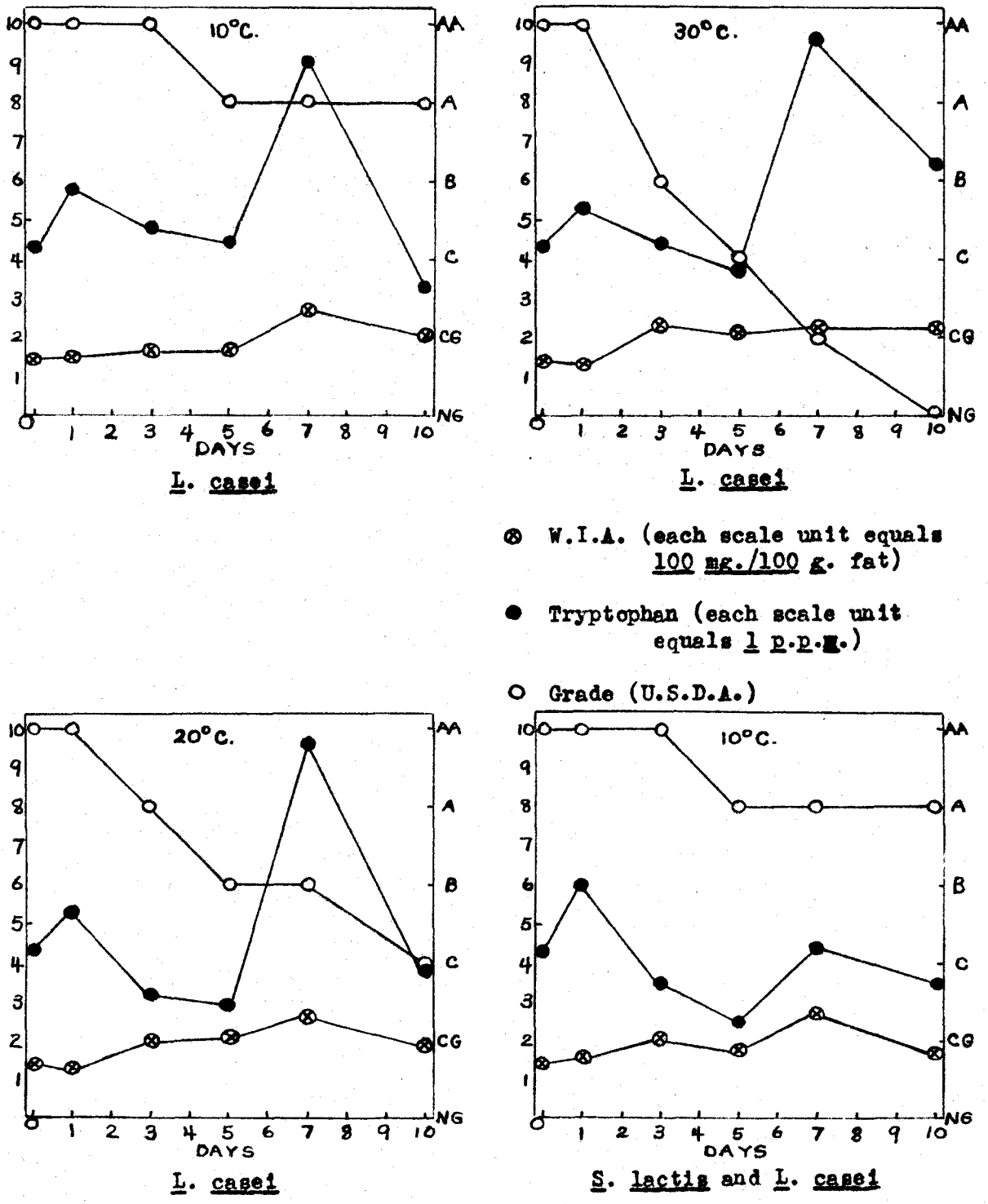


Fig. 28. W.I.A., tryptophan and grade values on cream containing L. casei, or S. lactis and L. casei as indicated.

admittedly subject to question, but a similar situation, indicated in figure 6, was encountered previously with S. lactis. The organoleptic deterioration seems to be closely related to incubation temperature and was related to organism population and acidity development.

Sterile cream inoculated with L. casei and S. lactis

Figure 29 indicates the variations which occur in population, pH and titratable acidity when incubating sterile cream inoculated with S. lactis and L. casei.

A comparison of figures 27 and 29 shows the L. casei population curve to be similar when grown alone and when grown with S. lactis at each of the three temperatures. Included in the similarity is a decrease between the seventh and tenth day at 10° C. Apparently the presence of S. lactis had no influence on the L. casei population. A comparison of figures 5 and 29 indicates that the presence of L. casei had a slight inhibitory influence on the rate of increase and maximum population attained by S. lactis. Especially significant is the rapid S. lactis decrease in the 30° C. sample, with the 10-day count showing a population of only 20 per ml. At 10° C. neither organism was able to attain sufficient population to materially influence titratable acidity and pH. At 20° C. the titratable acidity increase and pH reduction were greater than accomplished by either organism alone, whereas at 30° C. the values corre-

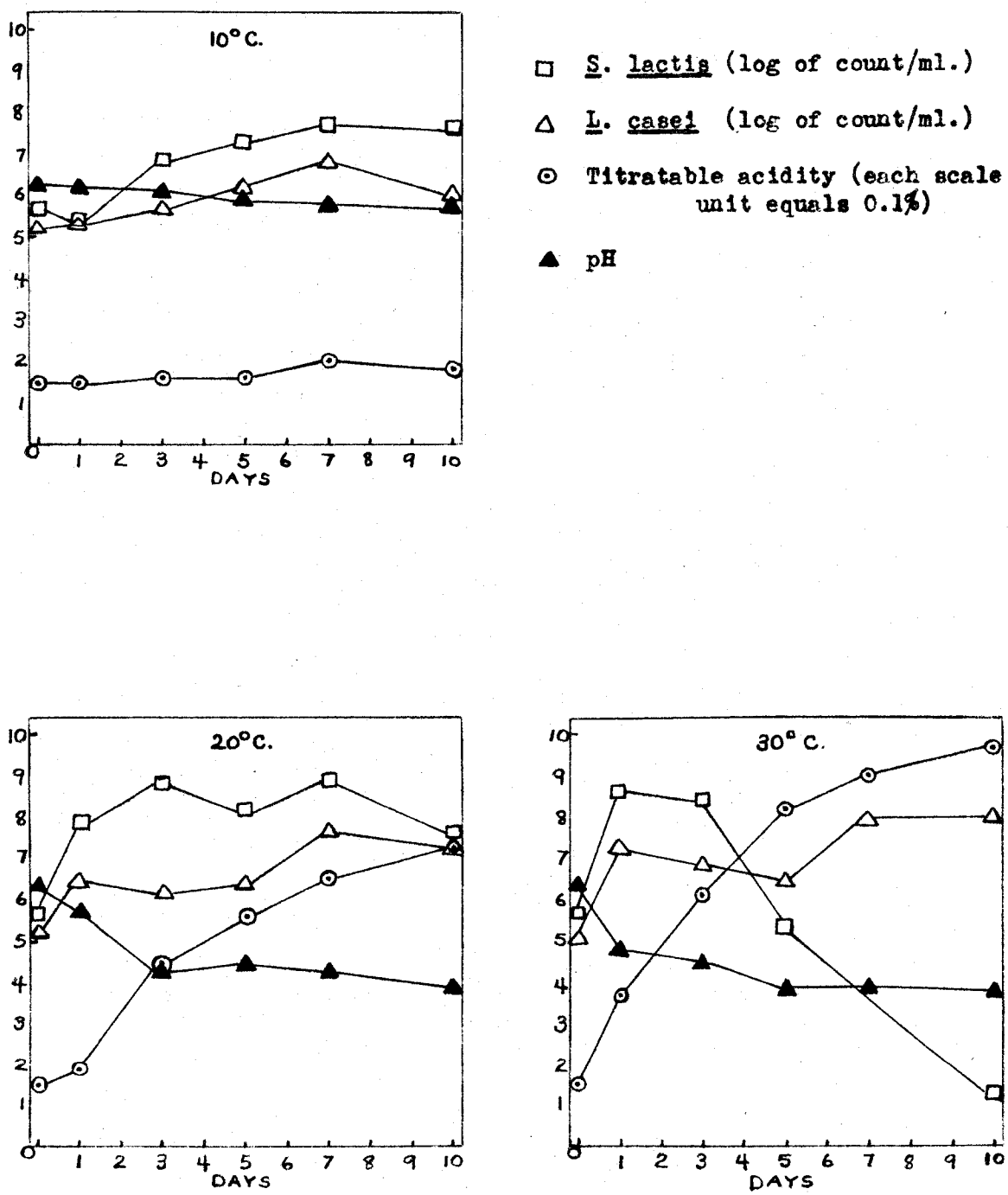


Fig. 29. Titratable acidity, pH and counts of S. lactis and L. casei in cream containing both of these organisms.

spond closely with those produced by L. casei alone.

Figure 30 illustrates the variations which occurred in W.I.A., tryptophan and score values, with incubation at 20 and 30° C., when sterile cream was inoculated with S. lactis and L. casei. The results at 10° C. are included in figure 28. The W.I.A. fluctuations are greater than the range of experimental error and show a uniform decrease from the seventh to the tenth day. Also the slight decrease consistently appearing when S. lactis is present occurred on the first day at 20 and 30° C. but was delayed until the fifth day at 10° C. The W.I.A. variations were insufficient to influence quality. At 10° C. the two organisms caused a fluctuation in free tryptophan (figure 28) which was similar to the tryptophan values created by L. casei when unaccompanied, except that the 7-day value was much lower. At 20 and 30° C. the two organisms together produced extremely high tryptophan values with a saw tooth curve prevailing after the third day. The greater organism population at 30° C. probably accounts for the tryptophan value being greater at 30 than at 20° C. on the first day. The fact that subsequent values were all lower at 30 than at 20° C., even though the same pattern prevailed, may be explained by the fact that the lower pH at 30° C. may have been inhibitory to the protease enzyme. At 10° C. the organoleptic deterioration was slight, and sweet cream quality was

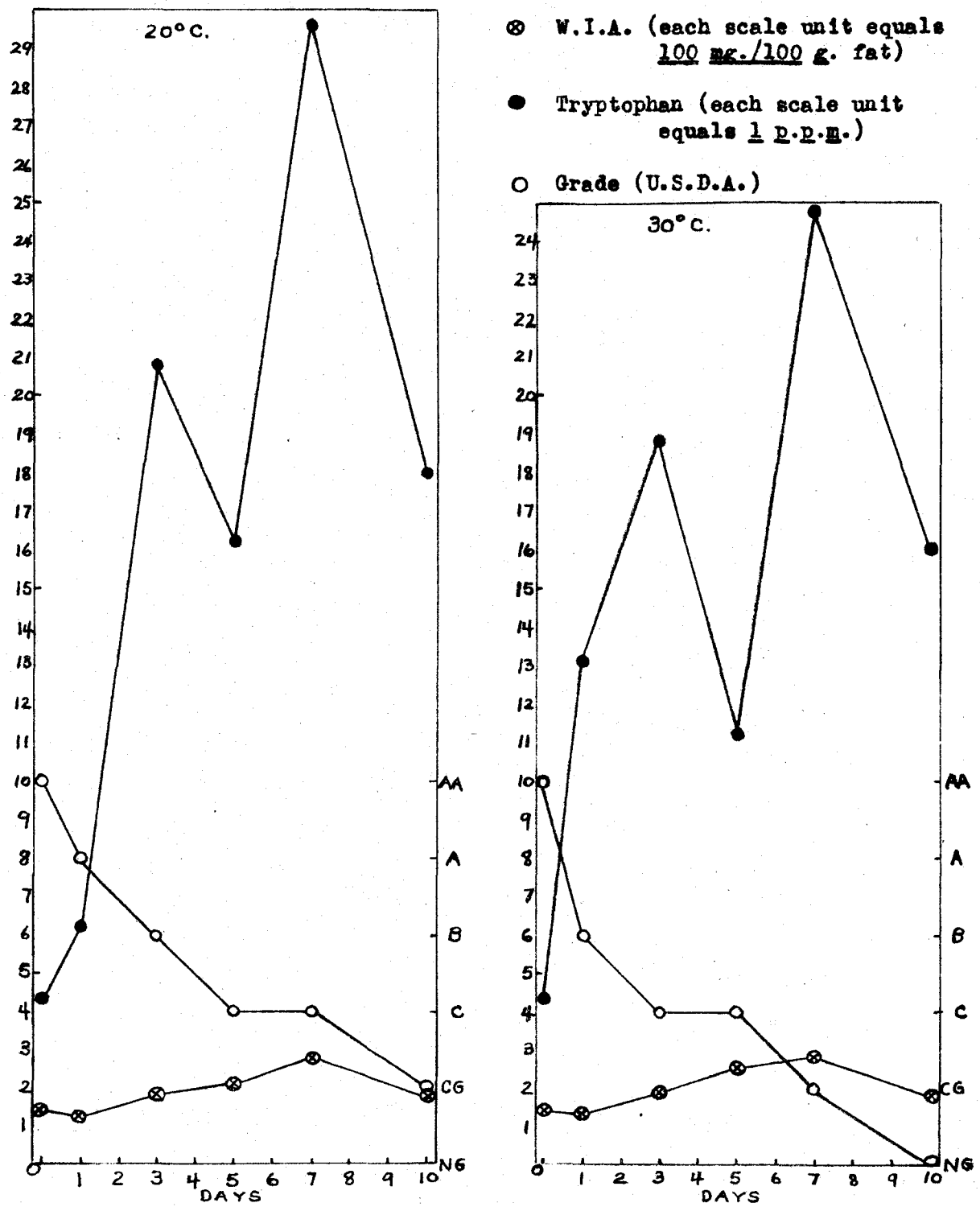


Fig. 30. W.I.A., tryptophan and grade values on cream containing both S. lactis and L. casei.

retained throughout the incubation period. Quality decline at 20 and 30° C. seemed to be in response to changes in titratable acidity and pH, with high acidity being the principal criticism.

Sterile cream inoculated with B. subtilis

Changes in organism population, pH and titratable acidity occurring as a result of inoculation with B. subtilis are shown in figure 31. The population curves show a more rapid rate of increase as the temperature increases. The highest count was attained on the third day at 30° C., after which there was a noticeable decline until the sample was discarded on the seventh day. At 10 and 20° C. the population increases continued throughout the incubation period. There was no significant change in pH. Slight increases of 0.01 to 0.03 per cent titratable acidity were encountered; however; after these increases occurred, inoculations of the cream into litmus milk produced alkalinity.

Variations occurring in W.I.A., tryptophan and score values when B. subtilis is inoculated into sterile cream are shown in figures 32, 33 and 34. There was a noticeable increase in W.I.A. on the fifth and seventh days at 30 and 20° C., respectively, (figures 33 and 34) but the increase was not associated with a corresponding change in organoleptic quality. The rapid increase in free tryptophan

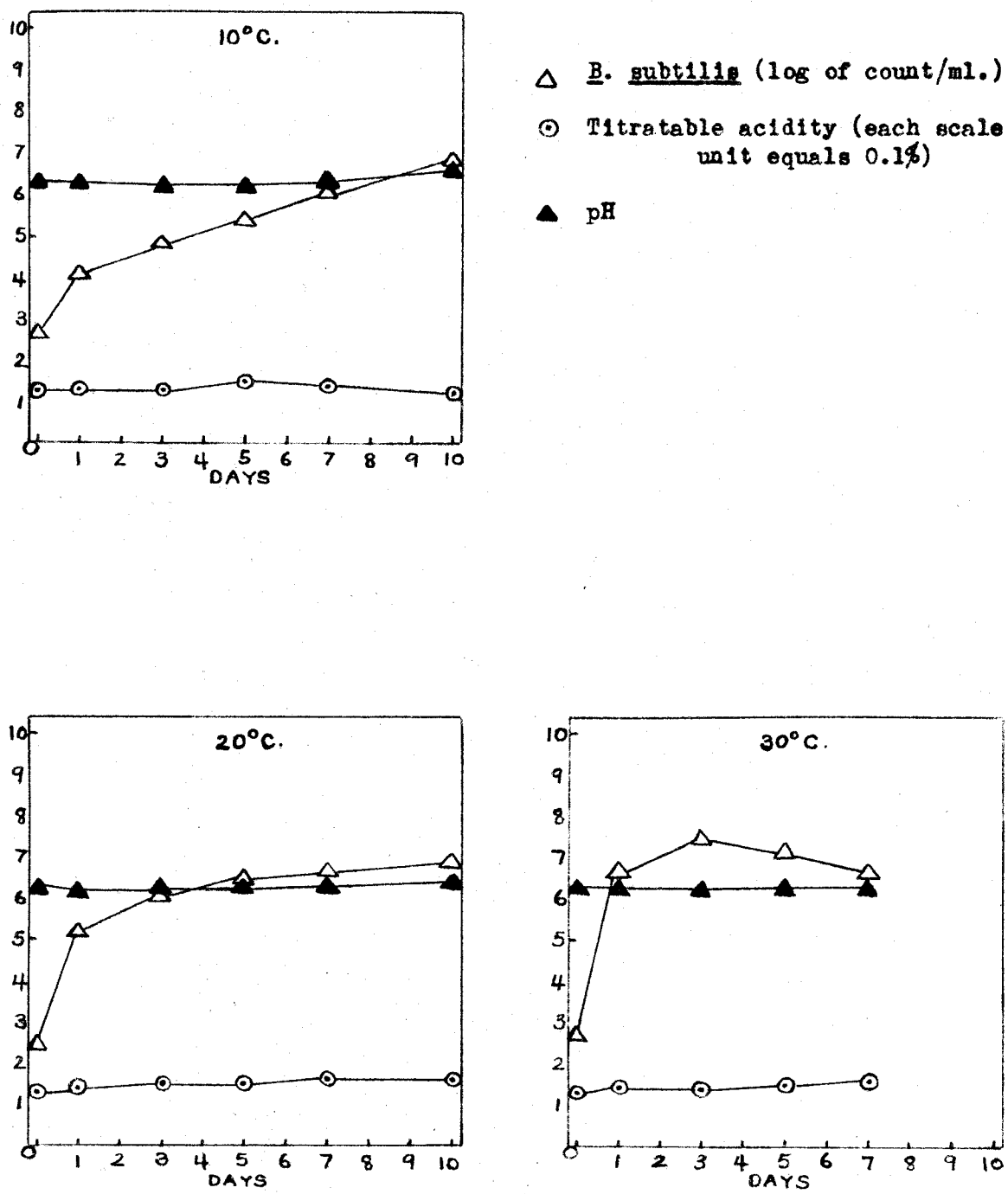


Fig. 31. Titratable acidity, pH and counts of B. subtilis in cream containing this organism.

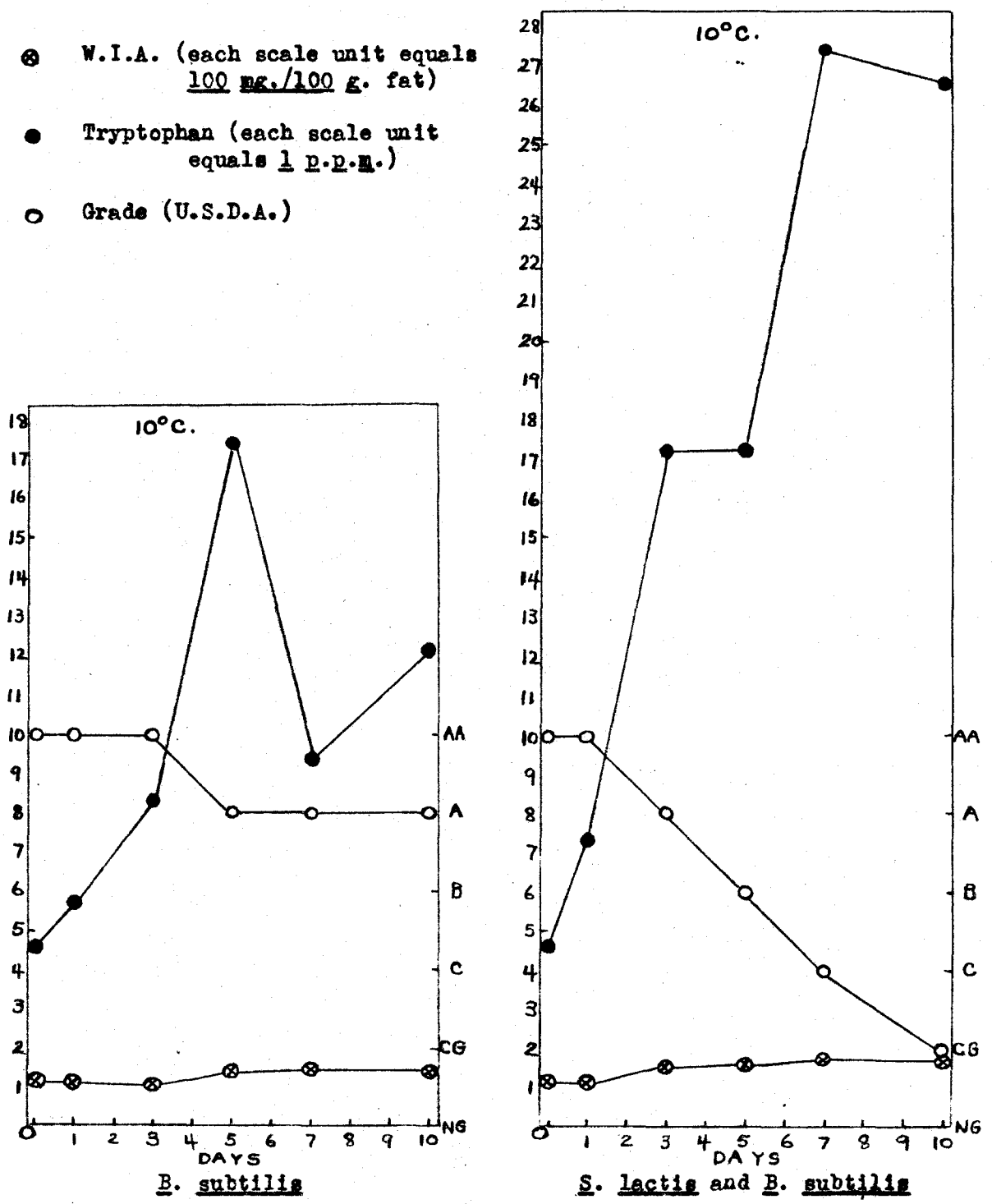
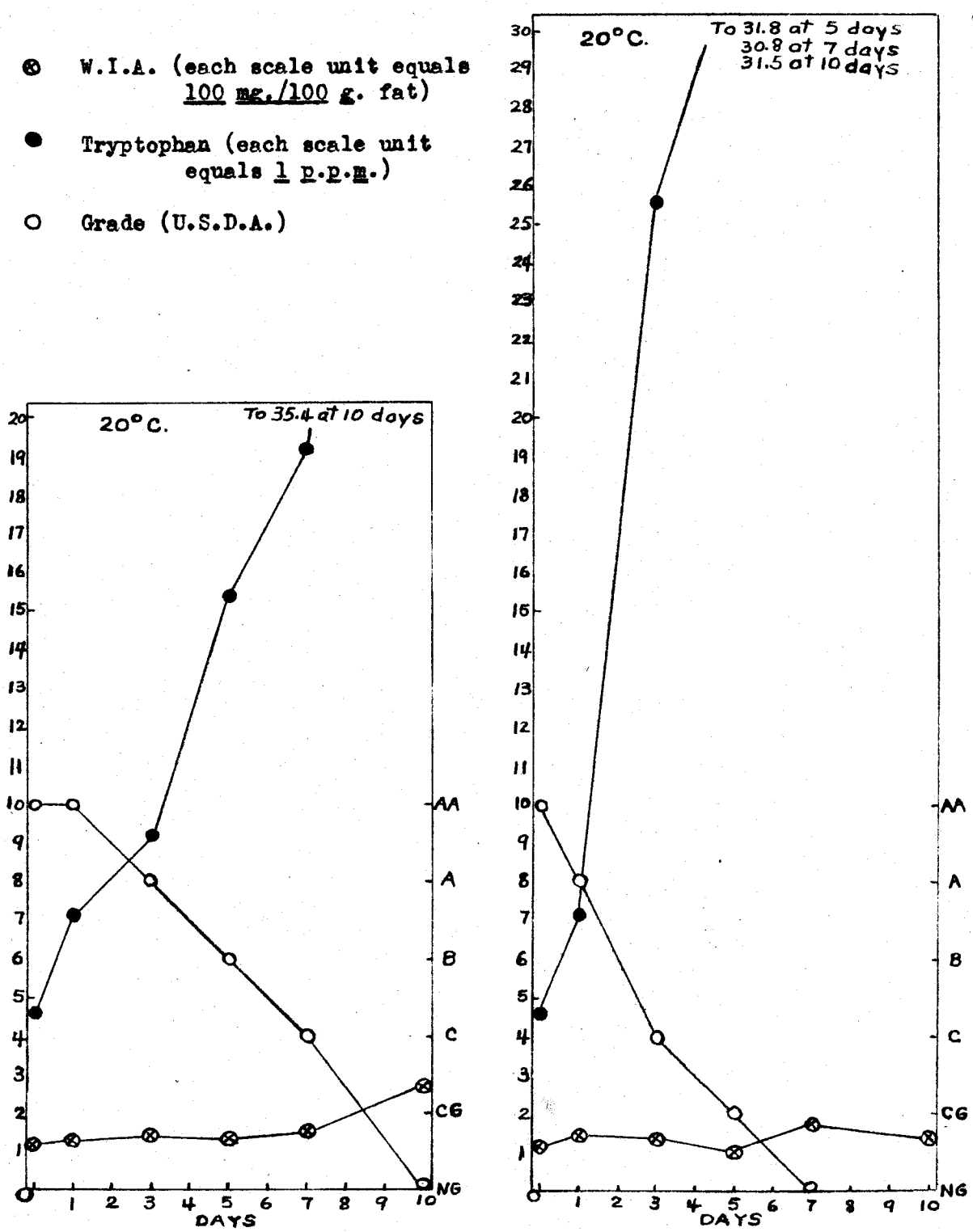
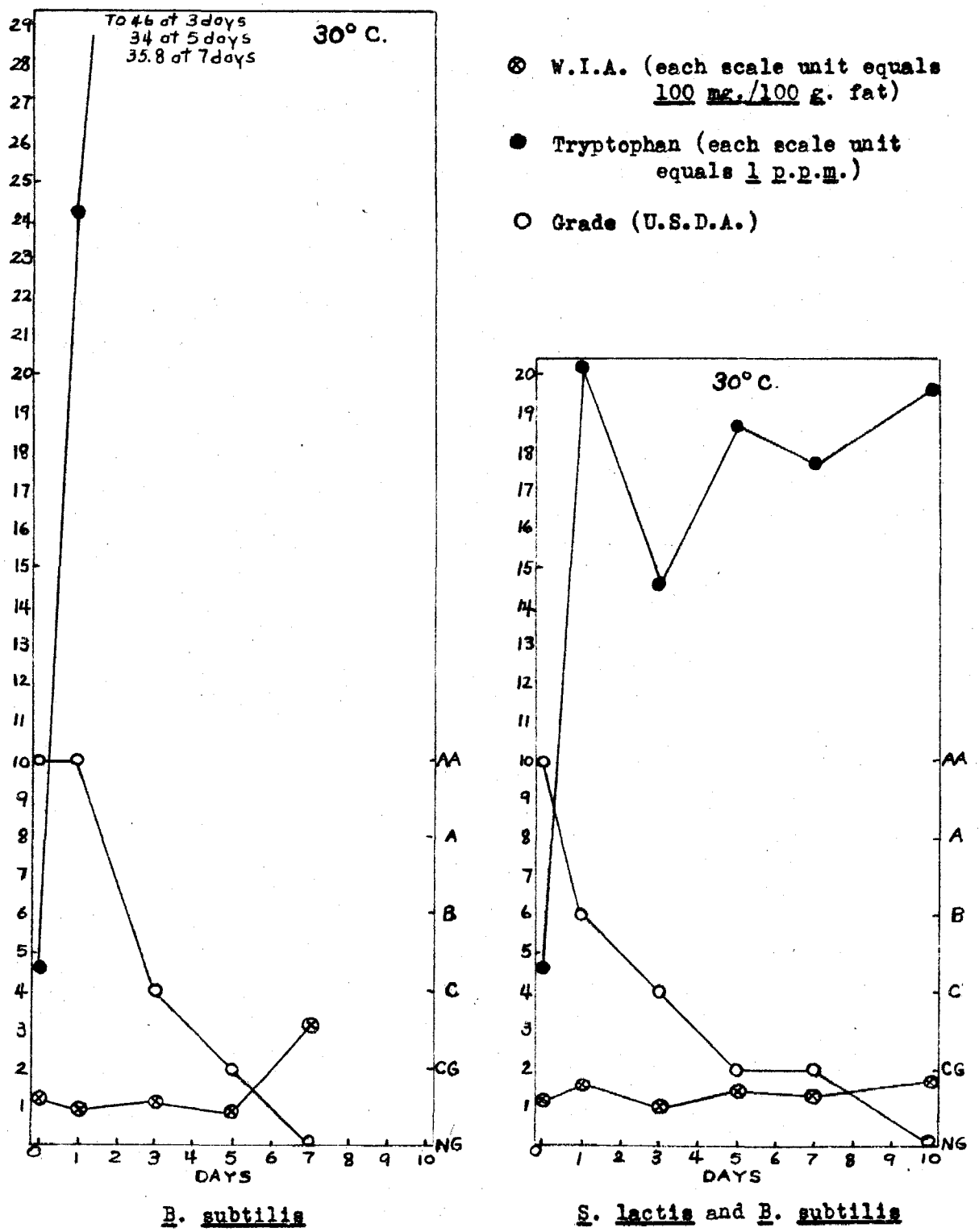


Fig. 32. W.I.A., tryptophan and grade values on cream containing B. subtilis or S. lactis and B. subtilis as indicated.



B. subtilis S. lactis and B. subtilis

Fig. 33. W.I.A., tryptophan and grade values on cream containing B. subtilis or S. lactis and B. subtilis, as indicated.



B. subtilis

S. lactis and B. subtilis

Fig. 34. W.I.A., tryptophan and grade values on cream containing B. subtilis or S. lactis and B. subtilis, as indicated.

and the high maximum values attained indicate the extremely high degree of proteolysis caused by B. subtilis. In figure 32 there is a decided dip in the curve occurring on the seventh day at 10° C. At 20° C. the increase in free tryptophan was continuous throughout the incubation period with the maximum being 35.4 p.p.m. on the tenth day. At 30° C. the maximum of 46 p.p.m. occurred on the third day, after which there was a decline to 34 and 35.8 p.p.m. on the fifth and seventh days, respectively. The degree of proteolysis increased as the temperature increased, with the increase being much more rapid at 30° C. Deterioration in organoleptic quality seemed to increase with population and degree of proteolysis, with sweet cream quality prevailing throughout the incubation period at 10° C.

Sterile cream inoculated with B. subtilis and S. lactis

Figure 35 indicates the variations occurring in organism population, titratable acidity and pH in sterile cream inoculated with S. lactis and B. subtilis. The population curve for S. lactis at all temperatures is normal. The presence of S. lactis appeared to become inhibitory to B. subtilis at about the time the maximum S. lactis population occurred. Also the decrease in B. subtilis population was coincident with the decline in pH. An extreme drop in B. subtilis population occurred on the seventh day at 10° C. and on the fifth day at 20 and 30° C. The decrease was

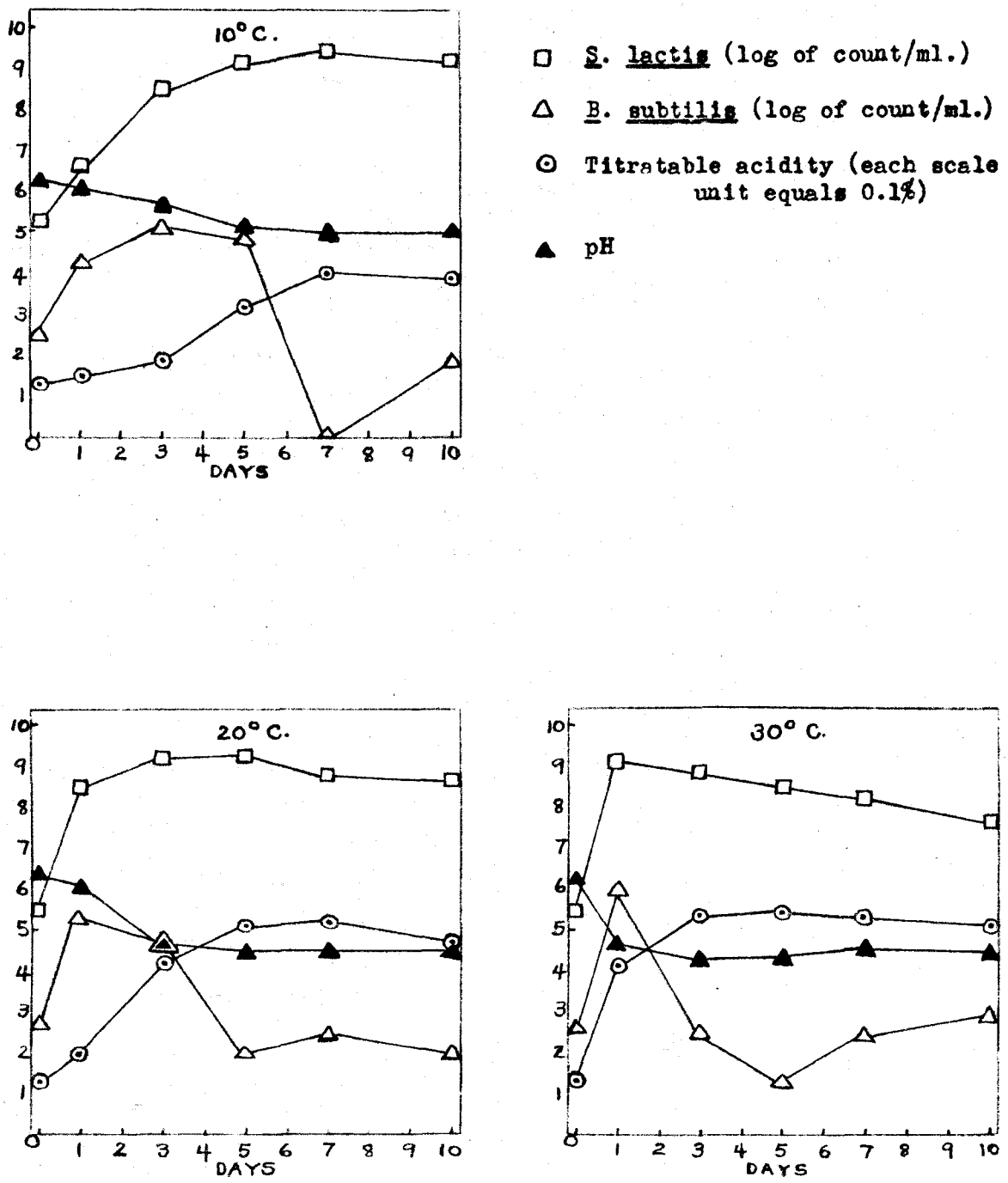


Fig. 35. Titratable acidity, pH and counts of S. lactis and B. subtilis in cream containing both of these organisms.

followed by a secondary increase which may be due to selection of B. subtilis cells which survived the low pH. The increases in titratable acidity represented normal response to S. lactis development.

The changes occurring in W.I.A., tryptophan and score values when sterile cream was inoculated with S. lactis and B. subtilis are shown in figures 32, 33 and 34. Only slight fluctuations occurred in W.I.A. values and these are of no consequence. At 10° C. (figure 32) the total free tryptophan produced by the two organisms was greater than the sum of their individual free tryptophan productions. At 20° C. (figure 33) the rate of increase was more rapid but the maximum values attained were slightly less with both organisms present than when B. subtilis was alone. At 30° C. (figure 34) it is apparent that the presence of S. lactis materially inhibited the proteolytic ability of B. subtilis. An examination of figure 35 shows that the pH at 30° C. was slightly lower than at 20° C. and the population diminished substantially at both temperatures after the first day. Figures 32, 33 and 34 suggest that early organoleptic deterioration was in response to organism development, while later deterioration was coincident with free tryptophan development. It is noteworthy that in the cream containing both organisms, organoleptic quality deterioration was more rapid at 20 than at 30° C., probably due to the higher pH

and greater proteolysis at 20° C.

Analysis of Uninoculated Samples of Commercial Cream

Three samples of commercial raw cream representing excellent, medium and poor quality were subdivided into three portions which were incubated at 10, 20 and 30° C., respectively. All samples were incubated for a period of 10 days, or until the quality deteriorated to N.G. The following microorganism determinations were made: Total count, coliforms, lipolytics, proteolytics, Gram-negatives (using crystal violet in the agar as previously described under methods), lactobacilli, yeasts and molds. In some cases it was found to be impossible to distinguish individual counts in these designated categories. For example, several of the selective media supported mold growth to the extent that accurate population estimates were impossible after the mold count increased to significant figures.

In addition, analyses were performed to determine the titratable acidity, pH, tryptophan, W.I.A. and flavor. Determinations for these factors and the microorganism counts were made on 0, 1, 3, 5, 7 and 10 days unless the extent of deterioration necessitated discarding the samples prior to the tenth day.

Excellent quality cream (grade AA or 93 score)

The microorganism populations determined during the incubation period of this sample are shown in table 4. The values on the other factors are shown in figure 36.

Incubation at 10° C. The initial total count (table 4) was in accord with that expected of excellent quality raw cream. Maximum population was attained on the fifth day with little subsequent change. The original coliform count was higher than expected for cream of this quality and the coliform population continued to increase throughout the 10-day interval. The progressive increase of the lipolytic population probably was due primarily to Pseudomonas strains which increase in cream at this incubation temperature, a fact which was demonstrated previously in this research using Ps. fragi. The proteolytic and Gram-negative counts increased uniformly throughout the incubation period, with the Gram-negative count showing a particularly rapid increase on the seventh and tenth days. The initial lactobacillus count was low and showed little increase during the early days of incubation, but a substantial increase was evident on the seventh and tenth days. No significant changes occurred in the yeast and mold population which remained negligible.

The titratable acidity at 10° C. (figure 36) showed no particular change until the seventh day and then increased

Table 4

Microbial populations of excellent quality cream sample

	Holding temperature		
	10° C.	20° C.	30° C.
		0 day	
Total count	64,000		
Coliforms	4,300		
Lipolytics	7,000		
Proteolytics	18,000		
Gram-negatives	10,300		
Lactobacilli	10		
Yeasts	2		
Molds	1		
		1 day	
Total count	101,000	54,000,000	300,000,000
Coliforms	19,000	8,800,000	2,490,000
Lipolytics	17,000	9,800,000	1,000,000
Proteolytics	23,000	2,400,000	7,000,000
Gram-negatives	45,000	9,000,000	10,400,000
Lactobacilli	11	9	33,000
Yeasts	1	2	11
Molds	3	1	20
		3 days	
Total count	4,900,000	1,300,000,000	30,000,000
Coliforms	340,000	19,000,000	4,600,000
Lipolytics	1,450,000	6,800,000	7,600,000
Proteolytics	340,000	2,600,000	12,000,000
Gram-negatives	360,000	11,000,000	11,200,000
Lactobacilli	2	19,000	72,000,000
Yeasts	5	20	1,470,000
Molds	2	4	29,000

Table 4 (continued)

	Holding temperature		
	10° C.	20° C.	30° C.
	5 days		
Total count	83,000,000	93,000,000	59,000,000[a]
Coliforms	250,000	340,000	1,100,000
Lipolytics	12,000,000	51,000,000	76,000,000
Proteolytics	1,800,000	Not detectable[b]	Not detectable[a]
Gram-negatives	690,000	1,700,000	8,200,000
Lactobacilli	170	119,000	Not detectable[a]
Yeasts	5		
Molds	1	9,000[c]	16,600,000[c]
	7 days		
Total count	83,000,000	810,000,000	81,000,000[d]
Coliforms	1,800,000	122,000	None
Lipolytics	17,000,000	140,000,000	36,000,000[e]
Proteolytics	7,000,000	Not detectable[b]	Not detectable[b]
Gram-negatives	14,000,000	142,000	None
Lactobacilli	27,500	Not detectable[d]	Not detectable[d]
Yeasts	3		
Molds	0	490,000[c]	19,000,000[c]
	10 days		
Total count	74,000,000	850,000,000[a]	Sample
Coliforms	2,200,000	113,000	discarded
Lipolytics	26,000,000	9,000,000[a]	(N.G.)
Proteolytics	6,000,000	Not detectable[a]	
Gram-negatives	38,000,000	24,000	
Lactobacilli	3,200,000	Not detectable[a]	
Yeasts	6		
Molds	0	21,000,000[c]	

[a]Heavy mold population
[b]Overgrown by non-proteolytic organisms
[c]Could not differentiate
[d]Heavy yeast and mold population
[e]Predominately mold

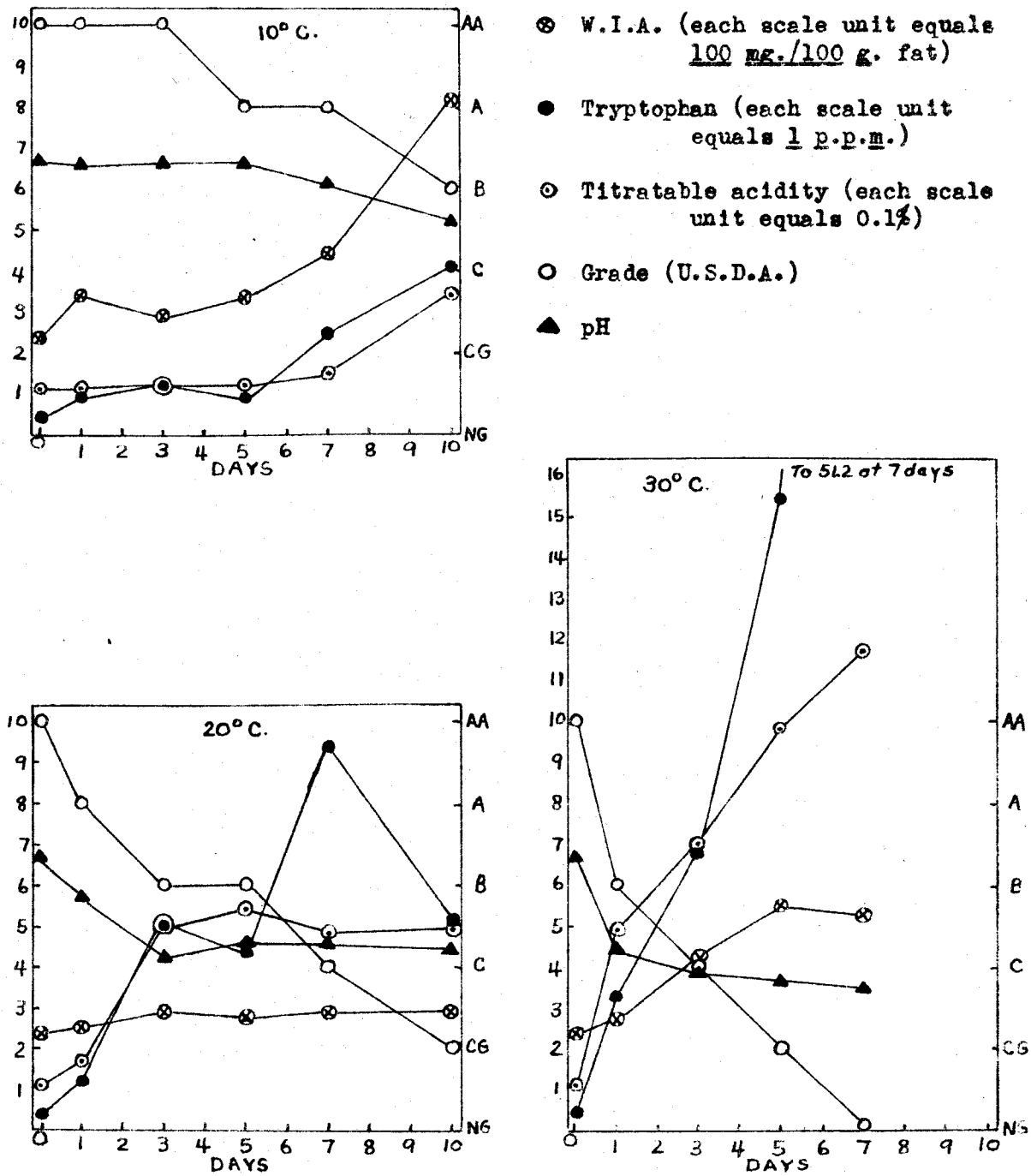


Fig. 36. W.I.A., tryptophan, titratable acidity, grade and pH values on uninoculated excellent quality commercial raw cream.

appreciably by the tenth day. The pH began to decline on the seventh day and continued to the tenth day. Also the free tryptophan value showed little change through the first 5 days and then began a definite increase. From a low initial value the W.I.A. content showed a gradual upward trend through the first 5 days, then increased considerably to 819 mg./100 g. fat on the tenth day. This advance in W.I.A. value corresponded to the increase in lipolytic count and presumably organisms of the Pseudomonas group. In this particular case grade AA cream stored at 10° C. retained its original quality for 3 days and remained grade A for 7 days. Organoleptic deterioration was coincident with increases in titratable acidity, tryptophan and W.I.A.

Incubation at 20° C. The total count increased rapidly, as shown in table 4, reaching a maximum on the third day, then decreased on the fifth day and increased again on the seventh and tenth days. This pattern is presumed to be due to the rapid increase and subsequent decline of S. lactis, followed by an increase in acid-tolerant organisms. The rapid rise through the third day and ensuing decline of coliform organisms is also noteworthy. The lipolytic population increased continuously and the 10-day count may be incomplete due to mold interference. The proteolytic population continued to increase as long as these counts could be made, but beginning with the fifth day mold interference

prevailed. The Gram-negative count increased rapidly during the first 3 days, reached a maximum on the third day and then gradually diminished. These counts were inconsistent with the lipolytic population. Perhaps the crystal violet dye was inhibitory to some Gram-negative lipolytic organisms. The lactobacilli showed progressive increase until the yeasts and molds prohibited identification on the seventh day. The yeast and mold population showed only moderate increase on the third day and then increased rapidly throughout the remainder of the incubation period, with yeast being dominant.

As shown in figure 36, the titratable acidity increased rapidly through the first 3 days and then leveled off. The pH tended to follow, reaching a minimum on the third day, and then increased slightly. Tryptophan values show a considerable degree of fluctuation. This is another instance of tryptophan increases and disappearance during the progression of the incubation period. A maximum W.I.A. value of 291 mg./100 g. fat was attained on the third day. Attention should be focused to the fact that lipolytic counts were much higher at 20 than at 10° C. and W.I.A. values increased markedly at 10° C. Probably the rapid decrease in pH at 20° C. prevented the lipase of the lipolytic organisms from hydrolyzing the fat. The decline in score was coincident with the increase in microorganism population and titratable acidity development. By the seventh day a very slight yeasty

flavor was apparent. In this cream sample, quality can in no way be coordinated with W.I.A. values and only remotely with tryptophan values.

Incubation at 30° C. Table 4 indicates the maximum total count occurred on the first day. However, the actual maximum probably occurred some time between the first and third day. The decline in S. lactis population had become apparent on the third day. Total counts on the fifth and seventh days increased, presumably due to acid-tolerant organisms, because of the continued acid development. The coliform population reached a maximum on the third day, declined on the fifth day and coliforms had completely disappeared on the seventh day. The lipolytic population continued to increase through the fifth day and on the seventh day most of the lipolytic colonies were mold. The proteolytic population reached maximum on the third day and proteolytic bacteria were not subsequently detectable due to mold domination. The Gram-negative population remained reasonably constant from the first through the fifth day, but there was no growth on the seventh day. In some instances mold growth was supported on crystal violet agar, but the crystal violet plates of this particular sample did not show mold growth. Lactobacilli showed an early increase and could not be enumerated after the third day because of yeast and mold interference. The yeast and mold counts

increased rapidly after the first day and reached a maximum on the seventh day.

The titratable acidity (figure 36) increased and the pH decreased throughout the incubation period. The tryptophan content began increasing immediately and reached 51.2 p.p.m. on the seventh day, indicating a tremendous amount of proteolysis, especially after the third day. The W.I.A. value increased continuously through the fifth day and then was slightly lower on the seventh day. The organoleptic quality deteriorated rapidly and the sample was barely commercially acceptable on the fifth day, at which time an intensely sour and slightly yeasty flavor was evident. This experience suggests that even excellent quality cream cannot be held longer than 3 or 4 days at 30° C.

Table 4 shows that the total counts, as expected, increased much more slowly and attained a lower maximum at 10 than at 20 or 30° C. Maximum organism population was attained later at 20 than at 30° C., but after attaining maximum, the 20° C. counts were greater than the 30° C. counts. The coliform counts increased more rapidly, reached a greater maximum and declined less at 20 than at 30° C. This suggests the existence of some antagonism against the coliforms at 30° C., because their optimum growth temperature is known to be around 30 to 40° C. The lipolytic, proteolytic, lactobacillus, yeast and mold populations

increased more rapidly and reached greater maximums at 30° C.

Figure 36 shows the titratable acidity increased faster and to a greater maximum at 30° C., probably due to high-acid-producing organisms such as the lactobacilli and to a limited extent, the formation of free fatty acids by lipase action. The pH drop was much more rapid and to a lower minimum at 30° C. Tryptophan values were much greater at 30° C. W.I.A. increases were comparatively insignificant at 20° C., but were above the 400 mg./100 g. fat maximum standard on the third day at 30° C. and on the seventh day at 10° C. The higher the temperature within the range studied the more rapidly the organoleptic quality deteriorated.

Medium quality cream (grade A or 92 score)

This sample of cream was secured from a producer who regularly sold to a commercial butter plant and had a reputation for good cream and good sanitary practices. The portion secured represented a 2-day accumulation of cream. The cream was routinely kept in a household refrigerator; however, the warm cream separated at each milking was mixed with the cooled cream. At the time the cream was secured it had a slight acid flavor but no other defects. The organism populations during the incubation period are shown in table 5, and the values of the other factors determined are shown

Table 5

Microbial populations of medium quality cream sample

	Holding temperature		
	10° C.	20° C.	30° C.
	0 day		
Total count	116,000,000		
Coliforms	360,000		
Lipolytics	440,000		
Proteolytics	110,000		
Gram-negatives	3,400,000		
Lactobacilli	1,300		
Yeasts	690		
Molds	30		
	1 day		
Total count	340,000,000	320,000,000	2,400,000,000
Coliforms	2,200,000	300,000	None
Lipolytics	1,400,000	1,800,000	14,000,000
Proteolytics	60,000,000	170,000,000	190,000,000
Gram-negatives	5,500,000	900,000	Not detectable[a]
Lactobacilli	6,200	9,000	13,000
Yeasts	640	580	1,300
Molds	9	90	2,700
	3 days		
Total count	640,000,000	2,700,000,000	240,000,000
Coliforms	500,000	9,900	None
Lipolytics	260,000	280,000[b]	1,400,000[b]
Proteolytics	Not detectable[a]	130,000	22,000,000
Gram-negatives	2,600,000	36,000	Not detectable[c]
Lactobacilli	2,900	Not detectable[c]	Not detectable[c]
Yeasts	1,100	26,000	320,000
Molds	18	190,000	1,100,000

[a]No growth at high dilutions and overgrown with mold at low dilutions
[b]Appear to be all mold
[c]Heavy mold growth

Table 5 (continued)

	Holding temperature		
	10° C.	20° C.	30° C.
	5 days		
Total count	380,000,000	430,000,000	22,000,000[c]
Coliforms	50,000	1,370	None
Lipolytics	Not detectable[c]	480,000[d]	5,000,000[d]
Proteolytics	Not detectable[a]	Not detectable[c]	Not detectable[c]
Gram-negatives	3,300,000	Not detectable[c]	Not detectable[c]
Lactobacilli	2,800	Not detectable[c]	Not detectable[c]
Yeasts	Unsatisfactory	Unsatisfactory	1,000,000
Molds	plates	plates	
	7 days		
Total count	490,000,000	380,000,000	Sample
Coliforms	34,000	2,200	discarded
Lipolytics	32,000,000	6,000,000[c]	(N.G.)
Proteolytics	120,000,000	8,000,000[c]	
Gram-negatives	125,000,000	27,000[c]	
Lactobacilli	3,200	Not detectable[c]	
Yeasts	2,900	1,200,000	
Molds	600	6,900,000	
	10 days		
Total count	330,000,000	Sample	
Coliforms	5,700	discarded	
Lipolytics	Not detectable[c]	(N.G.)	
Proteolytics	210,000,000		
Gram-negatives	Not detectable[c]		
Lactobacilli	Not detectable[c]		
Yeasts	24,000		
Molds	150,000		

[a]No growth at high dilutions and overgrown with mold at low dilutions
[c]Heavy mold growth
[d]All mold

in figure 37.

Incubation at 10° C. The initial total count shown in table 5 seems high; however, it probably is consistent for this type of cream on which there are no bacterial standards. Maximum population was attained on the third day and remained high throughout the incubation period. The coliform count was initially high, reaching a maximum on the first day and then gradually declining. The lipolytic and proteolytic populations increased on the first day, decreased on the third day and could not be determined beyond the third day because of mold interference. The Gram-negatives continued to increase during the incubation period and were not detectable after the seventh day because of mold interference. The lactobacilli remained comparatively constant throughout the incubation period. The yeast and mold population did not show any pronounced increase until after the seventh day.

Titratable acidity and pH curves (figure 37) are normal. The extreme fluctuations in tryptophan values during incubation were confusing but representative of similar variations which occurred in other samples.

The W.I.A. value decreased the first day, remained below the initial value throughout the first 5 days of incubation and then increased slightly. It is probable that the pH decrease minimized the activity of the lipases and also the early decrease suggests organism utilization of W.I.A. The

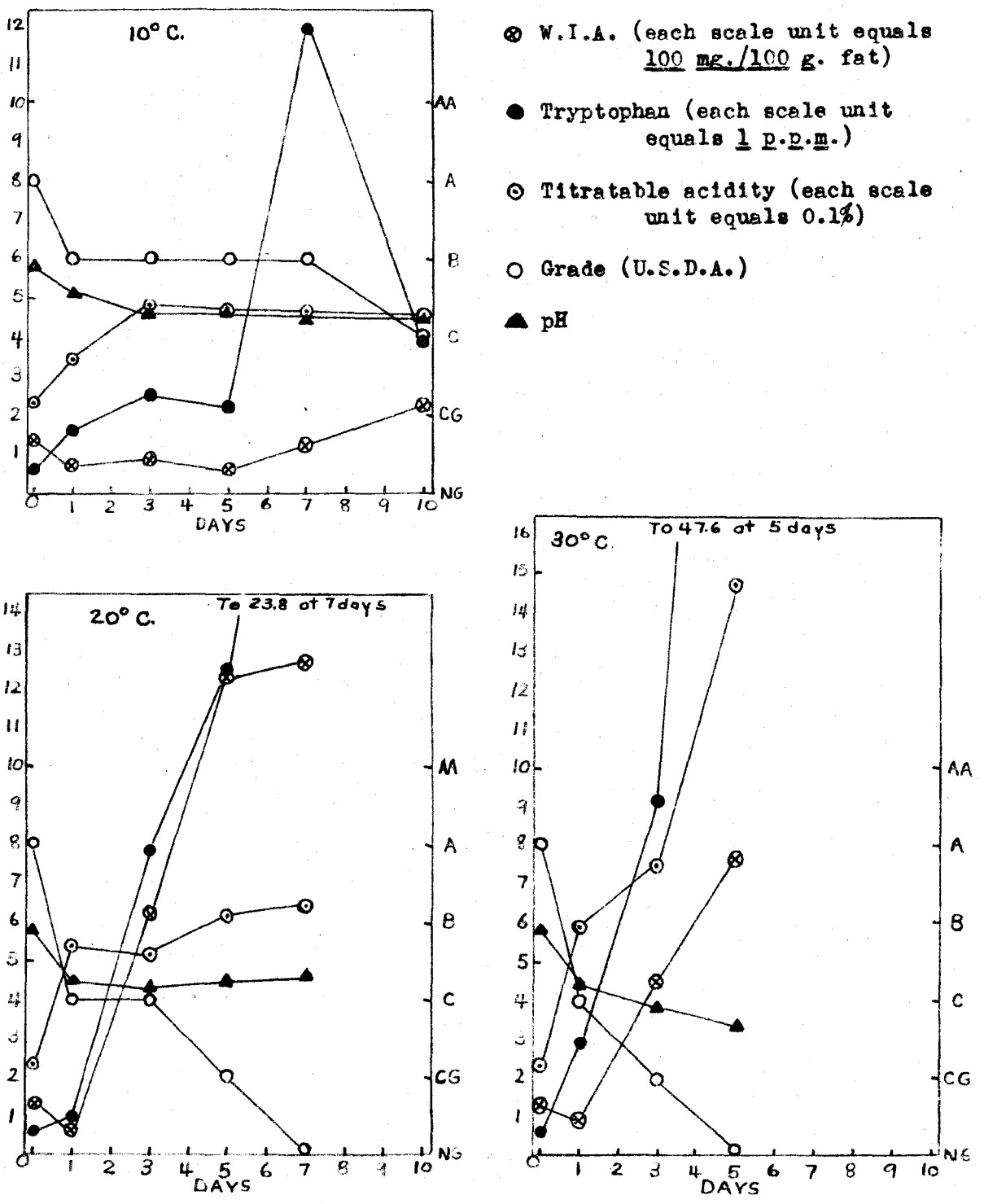


Fig. 37. W.I.A., tryptophan, titratable acidity, grade and pH values on uninoculated medium quality commercial raw cream.

organoleptic quality deteriorated at a moderate rate and was related to increase in acidity and decrease in pH.

Incubation at 20° C. The maximum total count as shown in table 5, was attained on the third day and the population subsequently decreased. The coliform count was lower after 1 day than on the original sample and the decline continued throughout the duration of the incubation period. The lipolytic count increased after 1 day and these organisms could not be enumerated in subsequent samples because of mold interference. The proteolytic count reached a maximum after 1 day, decreased markedly on the third day and subsequently could not be enumerated because of mold interference. The Gram-negative count decreased to the third day and thereafter these organisms could not be enumerated because of mold interference. The lactobacilli increased on the first day after which the plates were dominated by mold. The yeast and mold population showed no significant change on the first day and then progressively increased to extremely high counts on the seventh day.

In figure 37 the titratable acidity showed a substantial increase on the first day and then only a slight subsequent increase. Also the pH declined on the first day and then leveled off. The tryptophan content began increasing after the first day and reached a maximum of 23.8 p.p.m. on the seventh day, showing a considerable degree of proteolysis.

The W.I.A. value decreased on the first day, then increased abruptly to 1,236 mg./100 g. fat on the fifth day and leveled off. The organoleptic quality deteriorated rapidly from grade A to grade C, with an acid and unclean flavor apparent on the first day. On the fifth day a musty flavor appeared and on the seventh day the sample was extremely moldy and was discarded.

Incubation at 30° C. The total count, shown in table 5, was the maximum on the first day. Yeast and mold growth interfered with subsequent counts. There were no coliforms detectable on the first day or on any analysis thereafter. All other counts showed substantial increases on the first day. The lipolytic, proteolytic and total counts declined on the third day. Yeasts and molds continued to increase until the sample was discarded.

Figure 37 shows the titratable acidity increased to 1.47 per cent on the fifth day, suggesting the presence of extremely acid-tolerant organisms such as the lactobacilli. The pH decreased to 3.43 on the fifth day. Tryptophan increased after the first day to a value of 9.2 on the third day and 47.6 on the fifth day, indicating pronounced proteolytic deterioration. The W.I.A. value was above the legal tolerance on the third day and was 767 mg./100 g. fat on the fifth day. The organoleptic quality was grade C with a sour, unclean flavor on the first day and was discarded as

N.G. with a yeasty and musty flavor on the fifth day. The results of incubating medium quality cream indicate that such cream will retain market acceptability as sour cream for 7 to 10 days if kept refrigerated at 10° C. If kept at 20° C. such cream would be acceptable up to 3 or 4 days of age, and when kept at 30° C. the holding time should be limited to 1 or 2 days.

Within the temperature range used, the organism population increased more rapidly as the temperature increased. It is noteworthy that the coliform population decreased at all three temperatures, with rate of decrease being greater as the temperature increased. High yeast and mold populations made it impossible to follow most organism counts beyond the third day. Increase in titratable acidity and decrease in pH was much greater at 30° C.; also organoleptic deterioration and proteolytic activity were much greater at 30° C. Lipolytic activity was greatest at 20° C., suggesting that the lower pH at 30° C. inhibited the lipases more than the proteases, or perhaps 20° C. was a less favorable temperature for the proteolytic organisms and their proteases. Obviously there was some condition existing at 30° C. which interfered with lipase activity because the mold population was sufficient to cause the W.I.A. value to be much greater than actually attained.

Poor quality cream (grade B or 90 score)

This sample of cream represented a 2-day accumulation from a producer who had a reputation for maintaining poor sanitary conditions. The cream had a sour, weedy flavor and was grade B when secured.

Incubation at 10° C. Table 6 indicates the total count reached a maximum on the third day and then gradually decreased. The coliform count increased on the third day and then gradually diminished. The lipolytic, proteolytic and Gram-negative populations reached maximums on the first day and then decreased. The lactobacillus count continued to increase throughout the 10-day interval. The yeast and mold count followed an unusual pattern, reaching a maximum on the third day, dropping on the fifth day, and again increasing and levelling off on the seventh day.

Figure 38 shows that the titratable acidity increased and the pH decreased throughout the incubation interval except for an unexplained drop in titratable acidity and increase in pH on the fifth day. The tryptophan content was comparatively high (6.9 p.p.m.) when the cream was secured and continued to increase to a maximum of 34.0 p.p.m. on the tenth day, indicating substantial proteolysis. The W.I.A. value was 386 mg./100 g. fat initially, remained nearly constant through the third day, increased to 639 on the fifth day, was unchanged on the seventh day and dropped to

Table 6

Microbial populations of poor quality cream sample

	Holding temperature		
	10° C.	20° C.	30° C.
	0 day		
Total count	460,000,000		
Coliforms	5,500,000		
Lipolytics	3,100,000		
Proteolytics	60,000		
Gram-negatives	24,000,000		
Lactobacilli	4,600,000		
Yeasts	310,000		
Molds	42,000		
	1 day		
Total count	1,140,000,000	840,000,000	41,000,000
Coliforms	36,000,000	31,000,000	None
Lipolytics	44,000,000	5,000,000	Not detectable[a]
Proteolytics	20,000,000	90,000,000	6,000,000
Gram-negatives	210,000,000	65,000,000	Not detectable[a]
Lactobacilli	10,500,000	19,000,000	20,600,000
Yeasts	650,000	7,400,000	4,100,000
Molds	180,000	640,000	410,000
	3 days		
Total count	2,700,000,000	780,000,000	Sample
Coliforms	63,000,000	12,000,000	discarded
Lipolytics	17,000,000	41,000,000	(N.G.)
Proteolytics	4,200,000	3,100,000	
Gram-negatives	64,000,000	24,000,000	
Lactobacilli	28,000,000	36,000,000	
Yeasts	4,600,000	11,400,000	
Molds	700,000	7,000,000	

[a]Plates overgrown with mold

Table 6 (continued)

	Holding temperature		
	10° C.	20° C.	30° C.
	5 days		
Total count	1,410,000,000	340,000,000	
Coliforms	35,000,000	2,900,000	
Lipolytics	4,700,000	7,000,000[b]	
Proteolytics	60,000[b]	6,000,000[b]	
Gram-negatives	44,000,000	24,000,000	
Lactobacilli	27,000,000	66,000,000	
Yeasts	1,300,000[c]	16,000,000	
Molds		8,000,000	
	7 days		
Total count	460,000,000	38,000,000	
Coliforms	8,700,000	25,000	
Lipolytics	4,000,000	4,800,000[d]	
Proteolytics	4,000,000	4,200,000[d]	
Gram-negatives	14,200,000	170,000	
Lactobacilli	12,000,000	31,000,000	
Yeasts	4,900,000	7,100,000	
Molds	500,000	6,800,000	
	10 days		
Total count	580,000,000	Sample	
Coliforms	1,300,000	discarded	
Lipolytics	2,000,000	(N.G.)	
Proteolytics	Not detectable[a]		
Gram-negatives	11,000,000		
Lactobacilli	66,000,000		
Yeasts	5,900,000[c]		
Molds			

[a]Plates overgrown with mold
[b]All mold
[c]Could not differentiate
[d]Mostly mold

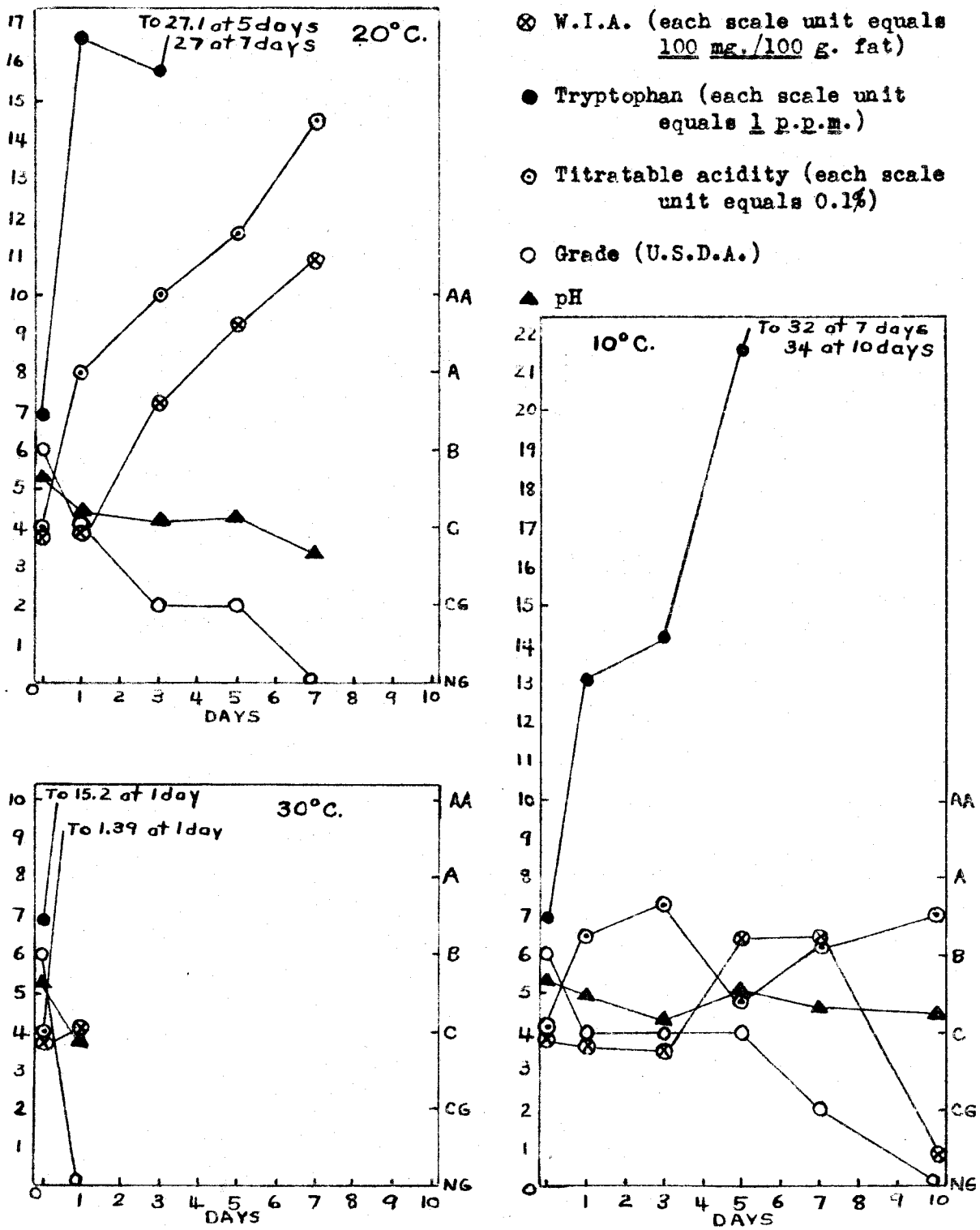


Fig. 38. W.I.A., tryptophan, titratable acidity, grade and pH values on uninoculated poor quality commercial raw cream.

91 on the tenth day. Organism utilization is the most plausible explanation for such an abrupt disappearance of W.I.A. Considering the original quality, organoleptic deterioration was moderate, with grade C prevailing from the first through the fifth day.

Incubation at 20° C. The data from table 6 suggest that the maximum total count occurred between the 0 and 1 day analyses, for the total count subsequently decreased. Coliforms, proteolytics and Gram-negatives reached a maximum population at 1 day and then decreased. Lipolytics reached a maximum on the third day and then apparently decreased. Beginning with the fifth day, mold colonies interfered with enumeration of lipolytic and proteolytic organisms. There was no mold growth on the lactobacillus and Gram-negative plates, but the media used for enumerating these two groups of organisms frequently supported mold growth. The yeast and mold population increased rapidly and remained at a high level.

Figure 38 shows that the titratable acidity increased extremely rapidly on the first day and attained a maximum of 1.45 per cent on the seventh day. The increase presumably was due to a combination of acid-producing bacteria, gas production by yeasts and coliform bacteria and free fatty acid formation by lipases. The pH dropped in accord with the acidity increase. The tryptophan value increased

abruptly, reaching 27.1 p.p.m. on the fifth day and then leveled off. The W.I.A. content increased progressively to 1,089 mg./100 g. fat on the seventh day. The organoleptic flavor deteriorated rapidly, being grade C with an acid and unclean flavor on the first day. A slightly yeasty and musty flavor was apparent on the third day and the sample was discarded due to mold on the seventh day.

Incubation at 30° C. Figure 38 shows this sample was graded N.G. when examined after 1 day. It possessed a foamy body, a bitter, high acid and yeasty flavor and was discarded after analysis. Table 6 indicates that the total count had declined considerably. No coliform bacteria were present and no lipolytic or Gram-negative organisms were detectable. The proteolytic and lactobacillus counts had increased considerably.

The titratable acidity, as shown in figure 38, was 1.39 per cent, the pH 3.93 and tryptophan 15.2 p.p.m. W.I.A. had increased only slightly to 404 mg./100 g. fat.

The results of incubating this series of cream samples suggest that cream produced under very poor sanitary conditions would remain marketable for about 5 days if kept refrigerated at 10° C. When held at 20° C. such cream will keep only 1 or 2 days, and cream produced under such conditions cannot be kept at 30° C. Considerable proteolysis occurred at all temperatures and lipolysis was greater at 20° C.

Tryptophan Content of Representative Producers' Cream Delivered to a Churning Plant

In order to determine the free tryptophan content of representative producers' cream being delivered to churning plants, 14 samples were secured at the time patrons made deliveries to the receiving room of a local commercial plant. Each producer was questioned concerning the age of the cream, separation method, storage method and temperature and whether fresh cream was cooled before mixing with the bulk quantity being accumulated. The information as reported by the producers is recorded in table 7, which also includes the analytical results. These producers milked from one to four cows. (All owners of large dairy herds in the Southwest are producing grade A milk.) The age of the cream ranged from 4 to 14 days, the quantity delivered varied from 12 to 38 lb., the titratable acidity ranged from 0.23 to 0.77 per cent, the grade varied from A to C.G. and the free tryptophan content ranged from 1.5 to 13.0 p.p.m. A consistent correlation between age, grade, titratable acidity and tryptophan content of serum does not exist. The correlation might be better among producers supplying a higher quality of cream; however, the receipts of this plant are representative of sour cream butter production.

The relationship between organoleptic grade and

Table 7

Tryptophan content of representative producers' cream delivered to a churning plant

Sample no.	Age of cream (days)	Separation method	Storage method	Wt. of cream (lb.)	Fat test (%)	Titratable acidity (%)	pH	Criticism	Organoleptic grade	Tryptophan in serum (p.p.m.)
1	7	skimmed	rfgr.	23	20	0.23	5.28	slt. sour	A	1.5
2	14	water dil.	rfgr.	12	38	0.26	5.09	sour	B	2.7
3	7	centrifugal	rfgr.	19	31	0.55	4.53	sour, feed	B	3.0
4	4	water dil.	rfgr.	20	38	0.35	4.78	sour	B	6.1
5	9	centrifugal	rfgr.	20	37	0.44	4.29	sour	B	6.7
6	7	centrifugal	rfgr.	15	38	0.32	4.72	sour	B	7.3
7	14	water dil.	rfgr.	12	38	0.68	4.40	sour	C	4.5
8	14	water dil.	rfgr.	31	39	0.58	4.69	sour	C	6.8
9	4	centrifugal	wet sack	39	28	0.63	4.56	sour, rancid	C.G.	2.0
10	10	skimmed	rfgr.	17	25	0.68	4.28	sour, rancid	C.G.	2.9
11	5	centrifugal	rfgr.	15	25	0.69	4.44	sour, stale	C.G.	6.1
12	7	skimmed	water vat	27	28	0.77	4.22	sour	C.G.	6.3
13	7	centrifugal	rfgr.	38	29	0.71	4.42	sour, unclean	C.G.	6.8
14	8	skimmed	rfgr.	18	25	0.65	4.64	sour, rancid	C.G.	13.0

tryptophan content is shown as follows:

Grade	No. of samples	Range of p.p.m. free tryptophan
A	1	1.5
B	5	2.7 to 7.3
C	2	4.5 to 6.8
C.G.	6	2.0 to 13.0

It appears unrealistic to suggest a correlation between organoleptic grade and p.p.m. of free tryptophan. Obviously the tryptophan value alone would not be an accurate index of the quality of this cream. This conclusion is supported by other data presented in this research in which pure culture inoculations into sterile cream sometimes produced high free tryptophan values while good organoleptic quality was retained. Also low free tryptophan values frequently were encountered in samples of poor organoleptic quality.

Tryptophan Content of Commercial Cream and the Resulting Butter

Six representative churnings of cream were selected for determination of the tryptophan content of the cream and the resulting butter. Tryptophan also was determined after the butter had been stored 90 days at 40° F., which was considered to be a severe storage test. Information concerning the quality of the cream and finished butter, titratable acidity of the cream before and after neutralization, pH of

the butter and tryptophan content of the cream and butter is shown in table 8. Four of the churnings were made in the college creamery and two were processed in a local churning plant. Churning no. 227 was neutralized, pasteurized and held over night at churning temperature. Churning no. 228 was held over night in cans on the receiving floor of the plant, prior to processing.

In calculating the free tryptophan content of butter, a serum value of 17 per cent was used rather than 20 per cent recommended in the formula of Duggan (32). As described in the section on procedure, this alteration of calculation was found to yield much more accurate results.

The four churnings processed in the college creamery represented average quality for the grade designated. The tryptophan values of the two commercial samples are much higher. This particular commercial plant makes only 89 score or grade C butter and blends all the cream purchased. Good correlation exists between the tryptophan content of the cream and the finished butter. Some increase in tryptophan occurred in all cases following 90 days storage of the butter at 40° F. The increases were substantially greater in the grade A and grade B butter than in the grade C. The two commercial samples and one of the T.T.C. samples declined one point in score while being held at 40° F. The four T.T.C. churnings were neutralized with "Kurdex", a

Table 8

Grade and tryptophan content of cream and the resulting butter

Sample source	Cream			Butter				
	Grade and criticism	Acidity before and after neutralization (%)	Tryptophan in serum (p.p.m.)	When churned: Grade and criticism	pH	Tryptophan in serum (p.p.m.)	After 90 days at 40° F.: Grade and criticism	Tryptophan in serum (p.p.m.)
T.T.C. 1[a]	A (92) coarse slt. acid	0.18	0.12	2.1	A (92) coarse	7.18	2.0	A (92) coarse	5.1
T.T.C. 2[a]	A (92) coarse	0.13	0.13	1.3	A (92) coarse	6.89	1.2	A (92) coarse	3.3
T.T.C. 3[a]	B (90) old cream	0.35	0.12	3.0	B (90) old cream	7.11	2.6	B (90) old cream	5.8
T.T.C. 4[a]	B (90) old cream	0.39	0.13	2.9	B (90) old cream	6.97	2.6	C (89) old cream storage	11.2
Commercial 227[b]	C (89) sour stale	0.52	0.15	12.7	C (89) stale neutralizer	6.82	10.3	C.G. (88) stale neutralizer	12.7
Commercial 228[b]	C (89) sour stale	0.57	0.13	16.2	C (89) stale neutralizer	7.00	16.1	C.G. (88) stale neutralizer	16.6

[a]Butter manufactured in the Texas Technological College creamery
[b]Butter manufactured in a commercial creamery

neutralizer composed of sodium hydroxide and sodium carbonate, and pasteurized at 170° F. for 30 minutes. The two commercial samples were double neutralized with lime and soda and pasteurized at 165° F. for 30 minutes.

Grade and Tryptophan Content of Commercial Butter Samples

In order to determine the tryptophan content of representative samples of butter and the effect of holding butter at 40° F. for 90 days, six samples from different commercial concerns were secured and analyzed. The results are shown in table 9. Reasonably good correlation exists between grade and tryptophan content. Sample 6 was sufficiently objectionable to be rejected as a marketable sample. The other samples show varying amounts of tryptophan increase during the 90-day storage. Samples 1 and 2 were endorsed with a U.S.D.A. grade AA certificate of quality but sample 2 was only grade A when secured.

The results of the two series of analyses on butter shown in tables 8 and 9 are not based on a sufficient number of samples to warrant definite conclusions on correlation between grade and free tryptophan content but are sufficient to indicate that a correlation may exist when miscellaneous quantities of producers' cream are blended. The results suggest that tryptophan values for blended sweet cream butter (grades AA and A) generally do not exceed 2.0 to

Table 9

Grade and tryptophan content of commercial butter samples

Sample number	Condition when secured: Grade and criticism	Tryptophan in serum (p.p.m.)	Condition after 90 days at 40° F. Grade and criticism	Tryptophan in serum (p.p.m.)
1	AA (93) (U.S.D.A. certified AA)	0.9	A (92) coarse	1.1
2	A (92) (U.S.D.A. certified AA)	2.0	B (90) coarse storage	4.7
3	C (89) stale cream	2.7	N.G. moldy	7.5
4	C (89) stale cream	5.4	N.G. putrid fruity	17.0
5	C (89) neutralizer old cream	4.1	C.G. (88) neutralizer old cream storage	10.3
6	C.G. (88) putrid surface taint	17.0	C.G. (88) putrid surface taint sl. cheesy	16.3

2.5 p.p.m. Only two grade B samples were available and their tryptophan contents were 3.0 and 2.9. The tryptophan content of five grade A samples ranged from 2.7 to 16.2 p.p.m.

The limitations of projecting a recommended maximum tryptophan value for each grade should not be overlooked. For example it should be recalled that sterile cream inoculated with a pure culture of B. subtilis and incubated 1 day at 30° C. retained an AA flavor but had a tryptophan content of 24.2 p.p.m. Such a situation definitely is abnormal, being induced under laboratory rather than commercial conditions, but emphasizes the conclusion that a single quality test is insufficient to establish quality standards.

DISCUSSION

Inoculated Cream

Lipolytic activity and W.I.A. content

G. candidum and Ps. fragi were the organisms studied which caused significant lipolysis as indicated by high W.I.A. values. When pure cultures of these organisms were grown in sterile cream, flavor defects were not apparent at W.I.A. values up to 400 mg./100 g. fat, which is the maximum tolerated by the Federal Food and Drug Administration. The lowest W.I.A. values which were associated with rancid flavor were 840 mg./100 g. fat with G. candidum and 3,277 mg./100 g. fat with Ps. fragi. These samples contained no other off-flavors. Inasmuch as both of these values occurred in cream which was organoleptically acceptable as grade C, it must be recognized that cream which is objectionable by Federal Food and Drug Administration standards may escape detection by the customary organoleptic grading procedure. The lowest W.I.A. value at which G. candidum produced a moldy flavor was 2,337 mg./100 g. fat. Moldy flavors did not appear until after the presence of mold could be visibly detected by careful scrutiny; however, the mold would have been less apparent if the sample had been stirred more frequently. Production of

moldy flavor and W.I.A. were closely related to G. candidum population. The W.I.A. values produced by G. candidum in sterile cream compare favorably with results reported by Purko et al. (99).

When G. candidum and Ps. fragi were each combined with S. lactis and inoculated into sterile cream, a rancid flavor appeared at substantially lower W.I.A. values than prevailed when the lipolytic organisms were not associated with S. lactis. Numerous instances in the data indicate that the presence of S. lactis is related to the occurrence of a rancid flavor at W.I.A. values below 400 mg./100 g. fat. G. candidum also produced a moldy flavor at W.I.A. values as low as 868 and 1,067 mg./100 g. fat when S. lactis was present. Decreases in W.I.A. attributable to S. lactis may be related to the appearance of the rancid flavor at low W.I.A. values. Decreases in W.I.A. in the presence of S. lactis occurred more rapidly with the more vigorous culture of S. lactis. The occurrence of the initial decrease was coincident with the attainment of maximum population. Utilization of long-chain fatty acids by S. lactis is the most logical explanation for the fluctuating W.I.A. values which were encountered during the incubation periods. Addition of oleic acid to cream caused no reduction in the activity of S. lactis. The availability of oleic acid in abundant amount resulted in a W.I.A. decrease which was quantitatively greater than normal,

substantiating the conclusion that W.I.A. is utilized by S. lactis. Peters et al. (93) also encountered decreases in the presence of S. lactis and suggested the possibility of fatty acid utilization by the organism. The rancid flavor occurring at low W.I.A. values in the presence of S. lactis combined with lipolytic organisms may be due to utilization of the long-chain fatty acids by S. lactis, while the short-chain fatty acids continue to accumulate as fat hydrolysis progresses. This opinion is supported by the work of Tarassuk and Smith (112) who observed an increase in surface tension when S. lactis was grown in milk. They attributed this increase to utilization by S. lactis of fatty acids which contribute to reduced surface tension. Costilow and Speck (27) found that short-chain fatty acids inhibited S. lactis but fatty acids of 14 carbons and longer showed no inhibition.

A significant difference was encountered in the optimum temperatures for Ps. fragi cells and the lipase elaborated from these cells. When Ps. fragi was inoculated into sterile cream, the W.I.A. value increased more rapidly and reached a greater maximum at 30° C., but the greatest population occurred at 20° C. and the Ps. fragi count at 10° C. was greater than at 30° C. With this particular strain of Ps. fragi the optimum temperature of the lipase enzyme was substantially higher than the optimum growth temperature of

the organism. These results are in agreement with those of Nashif and Nelson (87), who reported a temperature of 15° C. or below, depending upon the individual strain, to be most favorable for production of lipase by Ps. fragi. These same workers (86) also reported that the maximum activity of this lipase occurred at 40° C. when a coconut oil substrate was used.

Organoleptic deterioration induced by Ps. fragi occurred in response to enzyme action and was not directly related to organism population.

Proteolytic activity and free tryptophan content

Among the organisms studied, B. subtilis and Ps. fragi were the most proteolytic, as indicated by the high free tryptophan values produced when pure cultures were inoculated into sterile cream. High free tryptophan values were encountered when A. aerogenes, L. casei and B. subtilis were individually combined with S. lactis. Some of these organisms or combinations resulted in the production of high acidity, but it is improbable that any tryptophan was liberated by acid hydrolysis of protein due to lactic acid accumulation. Hammer and Patil (55) and van der Zant (113) have reported that 1 per cent lactic acid added to milk did not produce any free tyrosine or tryptophan. Under no circumstances should the serum acidity of marketable cream exceed 1 per cent.

Fluctuations in the free tryptophan content during incubation of the cream were encountered frequently with several different organisms and combinations. Increases in free tryptophan probably were due primarily to protein hydrolysis. The decreases are more difficult to explain. Reduction due to decomposition of free tryptophan at low pH represents a possible cause but was not investigated. A number of tryptophan analyses were performed on a single sample with portions of the acetone extract being adjusted to 0.2 intervals between pH 5.0 and 6.0, in order to determine the optimum pH for filtration. In this series, no quantitative difference in free tryptophan was obtained. Braz and Allen (18) observed that an alternate hydrolysis and synthesis of milk protein occurred in the presence of streptococci and lactobacilli, with synthesis occurring after incubation for a few days. Decreases result if some of the tryptophan in peptide form and in position to react with the color reagent were built into protein. Fluctuations in free tryptophan may also be due to a lack of uniformity between the amount liberated by the protease and utilized by the organisms. This seems to be the most logical explanation for variations, especially when various environmental influences such as pH, temperature, air supply and the presence of various products of organism activity are considered.

B. subtilis was the most proteolytic of any of the organisms or combinations studied. Within the range studied, organism population and protease activity increased as the temperature increased, indicating both the organism and its protease preferred the higher temperature.

The maximum free tryptophan produced by the protease of Ps. fragi was approximately the same at 10 and 20° C. and somewhat lower at 30° C. Amount of proteolysis was closely related to cell population which also was lower at 30° C. The above quantitative correlation suggests that the protease of this strain of Ps. fragi was fairly equally active over the temperature range of 10 to 30° C.

The maximum free tryptophan values produced by the protease of L. casei were quantitatively similar, despite the fact that there was much difference in organism population at the three temperatures. The following explanation is offered. Within the temperature range studied, as the temperature increased the population increased and the pH decreased. It is probable that in the samples containing the greater organism population and accumulation of lactic acid, the low pH inhibited either the production or the activity of the protease.

Bitter flavors suggestive of proteolysis were detected at free tryptophan values as low as 13.5 p.p.m. and were uniformly present in cream containing 18 to 25 or more

p.p.m. The presence of S. lactis did not alter the free tryptophan level at which bitter or putrid flavors were apparent.

Effect of pH on enzymes

Within the temperature range studied lipolysis by G. candidum increased substantially as the temperature increased, although the population was only slightly greater at 30 than at 20° C. This indicates that the lipase preferred the higher temperature. When S. lactis was combined with G. candidum the lipase was greatly inhibited. The inhibition was attributed to the reduced pH resulting from the accumulation of lactic acid. This conclusion was substantiated by the fact that when G. candidum was combined with a weak strain of S. lactis, the pH reduction was less and the amount of lipolysis, as measured by W.I.A. values, was much greater. These results agree with the work of Nelson (90), who found that the lipase of G. candidum was retarded as the acidity increased, and became completely inactive at pH 4.0.

When S. lactis was combined with Ps. fragi the lipase and protease of Ps. fragi may have been inhibited by the low pH induced by lactic acid. However, in the 20 and 30° C. samples there is a fallacy in this conclusion because S. lactis inhibited Ps. fragi cell population. It is probable

that at 20 and 30° C. the cell population was insufficient to elaborate a functional quantity of enzymes. In the 10° C. sample containing S. lactis and Ps. fragi there is sufficient reason to conclude that low pH partially inhibited the lipase and protease enzymes, although substantial fat hydrolysis occurred at pH 4.22 to 4.46. Ps. fragi attained sufficient population to provide a functional quantity of enzymes, but the activity of both enzymes at this temperature was much less than when Ps. fragi was grown alone.

Low pH induced by S. lactis exhibited little influence on the protease of B. subtilis when S. lactis and B. subtilis were inoculated into sterile cream and incubated at 10 and 20° C. At 30° C. the pH was 4.62 and 4.31 on the first and third days, respectively, and the protease activity, as measured by free tryptophan production, was inhibited. The data suggest that with this combination of organisms there was little decline in rate of proteolysis until the pH decreased to about 4.65. Proteolysis did not cease until the pH reached 4.50 to 4.55. It is noteworthy that increases in free tryptophan continued after the population of both organisms began to decline, indicating that the protease functioned independent of cell growth.

When A. aerogenes and S. lactis were combined the protease activity at 30° C. was interrupted after the first day, probably by the rapid pH decline. At 20° C. the rate of decline in pH was slower and vigorous protease activity

continued until the third day, before leveling off or decreasing.

With most of the organisms studied individually and in combination with S. lactis, the free tryptophan value began to level off or decrease simultaneously with the occurrence of near minimum pH. It has been mentioned previously that decreases in free tryptophan probably are attributable to organism utilization exceeding protease production. It is noteworthy that these decreases began at pH values ranging from 4.5 to 5.25. The protease elaborated from the combination of S. lactis and L. casei displayed considerable acid tolerance, for increases in free tryptophan continued to occur, but at a decreased rate, after the pH declined below 4.5.

When pure cultures of S. lactis were inoculated into cream, increases in titratable acidity continued after the population began to decline, indicating that the carbohydrate fermenting enzyme functioned independent of cell growth and apparently was less sensitive to decreasing pH.

Changes in organism population and effect of interrelationship of organisms on population and deterioration in cream

The decrease which occurs in S. lactis population when growing in milk and cream is commonly attributed to declining pH or increasing titratable acidity. There may be another unidentified factor because these two characteristics were

approximately the same in cultures of S. lactis growing in sterile cream at 20 and 30° C. and the rate of population decline was much greater at 30° C. Since the decrease also occurs in pure cultures, competition from other organisms cannot be solely responsible.

Combining S. lactis with G. candidum caused a numerical stimulation of G. candidum population at all three incubation temperatures. This may be due to preference of the mold for low pH. In most instances mold counts on acidulated potato dextrose agar were slightly higher than on T.G.E.M. agar; however, this variation was not consistent. The utilization of W.I.A. by S. lactis was less apparent in the presence of G. candidum, probably because W.I.A. was being produced continuously by G. candidum. Rancid and moldy flavors occurred at lower W.I.A. values when S. lactis was combined with G. candidum; this has been explained previously. Despite the fact that S. lactis inhibited the lipase of G. candidum, the rate of organoleptic deterioration was increased when S. lactis was combined with G. candidum.

When S. lactis was combined with T. cremoris, a pronounced inhibition of the cell population of T. cremoris was apparent. Although the yeasts are considered to be acid tolerant, the most logical reason for the inhibition of T. cremoris seems to be the low pH caused by S. lactis action. This assumption is supported by the fact that plate counts

of T. cremoris were substantially lower on acidulated potato dextrose agar than on T.G.E.M. agar. In association with T. cremoris there was no noticeable inclination for S. lactis to utilize W.I.A. or produce free tryptophan. The presence of S. lactis in combination with T. cremoris reduced the rate of organoleptic deterioration at 10° C. but exhibited no influence at 20 and 30° C.

The inhibitory effect of S. lactis on the lipase, protease and population of Ps. fragi was much greater at 20 and 30° C. and has been previously attributed to low pH caused by lactic acid accumulation. However, when S. lactis was combined with Ps. fragi, the acidity produced by S. lactis increased the rate of organoleptic deterioration at all temperatures.

Low temperature seems to be the only factor responsible for the abrupt decrease in A. aerogenes population at 10° C., although many coliform bacteria tend to increase at this temperature. The extremely high free tryptophan values which occurred at 20° C. when S. lactis and A. aerogenes were combined seem to result from the synergistic action of the two organisms, because the quantity greatly exceeds the combined tryptophan production of the organisms when grown individually. The data also indicate that an extremely high free tryptophan value would have occurred at 30° C. if proteolysis had not been arrested by a more rapid decline in pH. Inoculation of

S. lactis with A. aerogenes had no apparent effect upon the rate at which A. aerogenes caused organoleptic deterioration in cream.

When S. lactis was combined with B. subtilis there was a pronounced decrease in B. subtilis population which began when the pH decreased to about 5.5. The B. subtilis count ultimately diminished to less than 500/ml. at all temperatures, with the rate being related to pH decline. Marshall (83) and Cox and Whitehead (29) reported that B. subtilis in small numbers stimulated the population and acid production of S. lactis. No stimulating influence was evident in this work in which the initial inoculation averaged about 250,000 S. lactis and 500 B. subtilis per milliliter. The addition of S. lactis to B. subtilis increased the amount of proteolysis and the rate of organoleptic deterioration at 10 and 20° C. At 30° C. the presence of S. lactis retarded proteolysis but did not materially influence the rate of organoleptic deterioration by B. subtilis.

When S. lactis and L. casei were combined, the resulting pH was lower than with any other combination of organisms and an extremely rapid decrease in S. lactis population occurred. At 20 and 30° C. this combination of organisms was able to produce free tryptophan values which were greater than the sum of the quantities produced by the organisms individually. Also with this combination of

organisms, titratable acidity increase and pH reduction at 20 and 30° C. was greater than occurred when either organism was grown alone, indicating that these factors are partially accumulative. Except for greater maximum acid production, the addition of S. lactis did not influence the rate of organoleptic deterioration caused by L. casei.

Commercial Samples

Decreases in, and in some cases the total disappearance of, coliform bacteria occurred to a greater extent than anticipated. There was only one instance in which the commercial samples deteriorated to N.G. and retained any surviving coliform bacteria. There was one instance in which the coliform bacteria had disappeared from grade C cream. At the lower temperatures coliform bacteria showed initial increases and lesser subsequent decreases. Their decline occurred simultaneously with decreases in pH below 5.0 and development of high yeast and mold populations; however, these conditions may be only coincidental. The conditions prevailing in souring cream which influence the death rate of coliform bacteria should be investigated.

In the commercial samples of cream no positive correlation exists between the incubation temperatures and the W.I.A. values. The greatest W.I.A. values occurred at 10° C. in the excellent quality cream and at 20° C. in the

medium and poor quality cream. The maximum W.I.A. values did not coincide with degree of organoleptic deterioration, which was most rapid at 30° C. in all cases. In each of the commercial samples incubated at 30° C. the pH was below 4.5 on the first day. The greater lipolytic action at the lower temperatures probably was due to the higher pH at these temperatures. W.I.A. values were not directly related to lipolytic counts in any of the commercial samples at any of the incubation temperatures.

In the excellent and medium quality commercial cream, proteolysis was much greater at 30° C. than at the other temperatures, despite the low pH. At 30° C. the poor quality cream did not remain salable long enough to establish a pattern, but proteolysis was quite pronounced at 10 and 20° C. Low pH seemed to exert less inhibitory influence on the proteases than on the lipases encountered in commercial cream. The extreme acid tolerance of proteases naturally occurring in commercial cream is noteworthy. In two instances the free tryptophan increased from 9 to 47 and from 7 to 51 p.p.m. after the pH had declined below 4.0. In the samples in which the pH decreased below 4.0, lactobacilli probably were responsible for most of the acid development, but the evidence is not conclusive because mold interfered with lactobacillus counts. The yeast and mold counts were high in all samples with a pH below 4.0. Gas production by

the yeasts and fat hydrolysis by the molds probably contributed to the low pH. The W.I.A. content of samples with a pH below 4.0 varied from 404 to 1,067 mg./100 g. fat, which does not suggest a great pH reduction by lipolysis. There was good correlation between increases in free tryptophan and organoleptic deterioration; however, the latter also was related to increases in acidity. There appears to be some agreement between increases in proteolytic population and in free tryptophan but the relation cannot be substantiated because mold interfered with proteolytic counts.

The data on commercial samples are insufficient to merit extensive conclusions; however, it seems appropriate to indicate the ranges in W.I.A. and free tryptophan values that were encountered with the various organoleptic grades of cream. All of the commercial samples on which analyses were performed are included. There were seven samples which graded AA or A. The free tryptophan content ranged from 0.4 to 2.5 p.p.m. The W.I.A. values ranged from 128 to 444 mg./100 g. fat. Fourteen samples were grade B. The free tryptophan ranged from 1.6 to 11.9 p.p.m. and W.I.A. ranged from 59 to 819 mg./100 g. fat. There were ten grade C samples. The free tryptophan ranged from 1.0 to 21.6 p.p.m. The W.I.A. ranged from 61 to 639 mg./100 g. fat. The Federal Food and Drug Administration has been rejecting as unfit for food cream and butter containing in excess of 400

mg. of W.I.A./100 g. fat. In tables 7, 8 and 9 there are five samples of cream which graded C or above organoleptically but exceeded the W.I.A. standard. There are also five samples of similar quality cream which contained in excess of 10 p.p.m. of free tryptophan. However, 10 p.p.m. is only an arbitrarily suggested value which seems appropriate if a maximum for acceptable quality is to be proposed. Neither the W.I.A. standard of 400 mg./100 g. of fat used by the Federal Food and Drug Administration, nor the above suggested free tryptophan standard of 10 p.p.m. can be detected organoleptically.

The fallacy of using only chemical methods of measuring quality is reflected by numerous instances of extreme fluctuations in the quantity of W.I.A. and free tryptophan present in cream during incubation at various temperatures. Deteriorating cream conceivably could change from an illegal to a legal category as organisms or enzymes effected a reduction in W.I.A. or free tryptophan. Chemical tests should be used as a supplement to, and not in lieu of organoleptic grading.

SUMMARY AND CONCLUSIONS

1. Excellent quality 35 per cent cream was sterilized by intermittent steaming for 1 hour on each of 3 successive days. Groups of samples were inoculated with an organism commonly encountered in dairy products and incubated at 10, 20 and 30° C. for a maximum of 10 days or until the cream deteriorated below the level of commercial acceptability. The individual organisms also were paired with Streptococcus lactis and both cultures inoculated into similarly sterilized cream. The organisms used were Geotrichum candidum, Torula cremoris, Pseudomonas fragi, Aerobacter aerogenes, Lactobacillus casei, Bacillus subtilis and Streptococcus lactis. The cream samples were analyzed at 0, 1, 3, 5, 7 and 10 days for organism population, titratable acidity, pH, W.I.A., free tryptophan and commercial grade. Samples of excellent, medium and poor quality raw uninoculated cream were incubated and analyzed in the same manner.

2. Geotrichum candidum and Pseudomonas fragi were the most lipolytic organisms examined. Geotrichum candidum produced maximum lipolysis and population at 30° C., with 8,344 mg. of W.I.A./100 g. fat formed in 10 days. Pseudomonas fragi produced maximum population at 20° C. and

maximum W.I.A. at 30° C., with a value of 6,855 mg./100 g. fat occuring in 10 days, indicating that the optimum temperature for the lipase of Pseudomonas fragi was above the optimum temperature for organism reproduction. Organoleptic deterioration was greater at 30° C., indicating that quality deterioration was more intimately associated with enzyme activity than organism population. In cream containing only Geotrichum candidum a rancid flavor was noticeable at W.I.A. values as low as 840 and 1,027 mg./100 g. fat and a moldy flavor was present at 2,337 and 3,074 mg./100 g. fat. When Geotrichum candidum and Streptococcus lactis were both inoculated into sterile cream, rancid flavors were evident as low as 329 and 402 mg./100 g. fat and a moldy flavor was apparent at 868 and 1,067 mg./100 g. fat. Decreases in W.I.A. in the presence of Streptococcus lactis were encountered in several instances. Utilization of W.I.A. by Streptococcus lactis was demonstrated by adding known quantities of oleic acid to Streptococcus lactis cultures in sterile cream and quantitatively measuring the amount of decrease.

3. Bacillus subtilis, Pseudomonas fragi and Lactobacillus casei produced substantial quantities of free tryptophan in sterile cream. Bacillus subtilis was the most proteolytic and produced 46 p.p.m. of free tryptophan on the third day at 30° C. Bitter flavors suggestive of proteolysis

were detectable at free tryptophan values as low as 13.5 p.p.m. and were uniformly present in cream containing 18 to 25 p.p.m. Among all of the proteolytic organisms studied, fluctuations in the free tryptophan content occurred during the incubation period. These fluctuations were attributed to a lack of coordination between organism utilization and enzyme liberation of free tryptophan, which seemed to be influenced by pH. Streptococcus lactis and Aerobacter aerogenes combined produced a quantity of free tryptophan greater than the sum of the production of the two organisms individually.

4. The presence of Streptococcus lactis was inhibitory to the population development of Torula cremoris, Pseudomonas fragi, Bacillus subtilis and Aerobacter aerogenes. The beginnings of population declines were generally associated with maximum Streptococcus lactis population and minimum pH. The presence of Streptococcus lactis stimulated the population development of Geotrichum candidum. The normal population development of Streptoccus lactis was inhibited by the presence of Aerobacter aerogenes and Lactobacillus casei. The population of Aerobacter aerogenes, both when alone and when associated with Streptococcus lactis, decreased continuously throughout the incubation period at 10° C. In the commercial samples incubated at 30° C. coliform bacteria counts decreased as the quality decreased.

The most rapid organoleptic deterioration, in the inoculated samples was caused by Torula cremoris and Aerobacter aerogenes at 20 and 30° C., but neither organism produced significant quantities of W.I.A. or free tryptophan.

5. The presence of Streptococcus lactis and the accompanying reduction of pH inhibited the lipase activity and the W.I.A. production of Geotrichum candidum and Pseudomonas fragi; however, in one instance, the lipase of Pseudomonas fragi was active at pH 4.22. In commercial samples containing high mold and high lipolytic bacteria populations, low W.I.A. values prevailed in the presence of low pH. The low pH induced by Streptococcus lactis materially reduced the protease activities of Bacillus subtilis and Pseudomonas fragi. Protease activity continued after Bacillus subtilis population began to decline, indicating that the enzyme functioned independent of the cells.

6. In commercial samples of cream no correlation existed between W.I.A. values and incubation temperature, but greater free tryptophan values uniformly occurred at 30° C. In seven samples of cream which graded AA or A, the free tryptophan content ranged from 0.4 to 2.5 p.p.m. and the W.I.A. ranged from 128 to 444 mg./100 g. fat. In fourteen samples of grade B cream the free tryptophan ranged from 1.6 to 11.9 p.p.m. and W.I.A. ranged from 59 to 819 mg./100 g. fat. In ten grade C samples the free tryptophan ranged

from 1.0 to 21.6 p.p.m. and the W.I.A. ranged from 61 to 639 mg./100 g. fat. Fourteen samples of cream, randomly selected, delivered to a commercial plant ranged from A to C.G. in grade and contained from 1.5 to 13.0 p.p.m. of free tryptophan. The maximum free tryptophan contained in cream grading C or above was 7.3 p.p.m. Fourteen samples of commercial butter, randomly selected, ranged from AA to C.G. in grade and contained from 0.9 to 17.0 p.p.m. of free tryptophan with the lower values being uniformly associated with the higher grades. The maximum contained in butter grading C or above was 16.2 p.p.m. Increases in free tryptophan occurred in butter stored at 40° F. for 90 days. A standard of not more than 10 p.p.m. of free tryptophan is suggested for acceptable products.

7. The considerable fluctuations which may occur in quantities of W.I.A. and free tryptophan during holding of cream limit the ability of these chemical tests to accurately assess quality. Organism activity could cause cream to change from an illegal to a legal category during progressive deterioration. Chemical tests should be used as a supplement to, and not in lieu of, organoleptic grading.

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