# THE USE OF LACTOBACILLUS BULGARICUS IN THE MANUFACTURE OF CHEDDAR CHEESE FROM PASTEURIZED MILK

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TABLE OF CONTENTS

# INTRODUCTION

In recent years, the use of pasteurized milk for cheese making has made necessary the use of starters of lactic acid producing organisms for the purpose of producing lactic acid during the manufacturing process. This has been accomplished by the addition of commercial cheese starters usually considered to be composed of Streptococcus lactis with the associated organisms Streptococcus citrovorous and Streptococcus paracitrovorous.

Difficulty has been encountered in many instances with poor acid production in the cheese during the manufacture. This condition usually resulted in a poor vat of cheese, which when sold on the market, was usually penalized in price for its inferior quality. This slowness in acid production may have been caused by poor handling of the starter or contamination of the starter with bacteriophage which caused lysis of the bacteria.

Besides acid production the problem of the proper development flavor in cheese made from pasteurized milk is important. Difficulty was encountered in developing characteristic cheddar cheese flavor in cheese made from pasteurized milk. This was thought to be caused by the killing by pasteurization of the Lactobacillus organisms, which have been considered by many to be responsible for the characteristic cheddar cheese flavor.

The possible answer to both the problem of acid production and the development of flavor during curing theoretically might be the use of a strong lactic acid producing Lactobacillus organism which probably would not be subject to attack by bacteriophage as easily as ordinary starter organisms.

## REVIEW OF LITERATURE

Pasteurization of milk intended for cheese manufacture has become a desirable practice in recent years. Freidel (9) reviewed the post-war picture of cheese making and found that the cheese industry was gradually changing from raw to pasteurized milk for cheese making. He pointed out that the war brought about an entirely new aspect in the program of pasteurizing milk for cheese, owing to the occurence of outbreaks of diseases attributed to the comsumption of fresh, raw milk cheese. He called attention to the fact, however, that the objection to pasteurized milk cheese was that it did not cure as quickly and did not develop the characteristic flavor of raw milk cheese.

Wilster (20) listed several advantages of the manufacture of cheddar cheese from pasteurized milk. The advantages are:

1. Pathogenic bacteria causing disease in man, if present in the milk, are destroyed by proper pasteurization.
2. Gas producing bacteria and other undesirable bacteria are either destroyed or greatly reduced in number.
3. There is much better control of the manufacturing procedure, especially with respect to the control of acid and moisture.
4. The cured cheese is generally of a better flavor than when raw milk is used.
5. The cheese can generally be ripened at a higher temperature than can raw milk cheese.
6. The cheese is uniform from day to day.
7. The yield of cheese is slightly increased because less fat is lost in the whey and slightly more moisture is retained in the cheese. (About 0.1 pound cheese additional

from 100 pounds of four per cent milk.)

8. The financial loss resulting from a decrease in the quality of cheese when kept in storage is reduced to a minimum.
9. The amount of second grade and undergrade cheese is greatly reduced when the cheese is made from pasteurized milk instead of from raw milk.
10. If milk of good quality is used, if an active starter is added, and if the manufacturing methods are correct, the cheese obtained will regularly score 92 to 93.

The disadvantages listed by Wilster are:

1. The cost of manufacture is slightly increased. This may amount to as much as 0.2 to 0.3 cent a pound of cheese. However, an increased yield may offset this increase in the cost of manufacture.
2. As the cheese from pasteurized milk ripens more slowly than that made from raw milk, it must be held longer before marketing.
3. A sharp flavor does not develop in cheese from pasteurized milk if the manufacturing methods are correct.

In investigating the manufacture of cheddar cheese from pasteurized milk in commercial plants, Walter and Lochry (19) found that the proportion of No. 1 cheese was much less when the milling acidity was less than 0.35 per cent or higher than 0.55 per cent. When the milling acidity was comparatively low or comparatively high, most of the cheese usually graded No.2 or undergrade, because of defects in body and flavor.

Walter and Lochry (19) showed data on the grades of cheese made from raw and from pasteurized milk in plants that had changed from

raw to pasteurized milk. While the cheese was being made from raw milk the percentage of No. 1 cheese made in these plants ranged from 0% to 42%. Shortly after pasteurization of the milk was introduced, the percentage of No.1 cheese increased in nearly all of the plants to more than 80 per cent, and after pasteurization had been in use for several months these factories produced at least 96 per cent of No. 1 cheese. Two of them produced 100 per cent No. 1 cheese.

With the introduction of pasteurization of milk for cheese making, the problem of offsetting the longer curing period necessary was encountered. The United States Department of Agriculture Bureau of Dairy Industry research workers (22) found that cheese held in a curing room at 60°F. was as fully ripened in from 3 to 4 months as cheese held at 50°F. or lower from 6 to 8 months, provided the cheese was made from milk that was of good quality, and that it had been pasteurized. They also noted that the cheeses held at the higher temperature developed more and better flavor than the cheeses held at lower temperatures.

Freeman (8) studied factors influencing the rate of ripening of cheddar cheese and suggested that the use of higher temperatures of storage for accelerating the ripening process offered possibilities. In his experiments, he employed temperatures of 45°F. and 63°F. for ripening. He concluded that the rate of proteolysis in cheddar cheese ripening can be increased 40 to 100 per cent by raising the ripening temperature from 45°F. to 63°F.

As early as 1914, investigators were concerned with the factor or factors responsible for the flavor and body breakdown of cheddar

cheese. Evans, Hastings and Hart (7), in their investigation of bacteria concerned in the production of characteristic flavor of cheddar type cheese, indicated that at the beginning of the ripening period the ratio between "Bacterium lactis acidi" and "lactic bacilli", in a general way, be expressed as 99 to 1. This ratio gradually changes as the ripening progresses until, in cheese 3 to 6 months old, the ratio is reversed. They considered it apparent that the larger part of the growth of the "Bacterium casei" group must take place after the fermentation of the sugar in the cheese by "Bacterium lactis acidi". They showed, in effect, that two major fermentations took place and were responsible for the biological part of flavor development in cheddar cheese:

1. Fermentation of lactose to lactic acid by the Streptococcus group of lactic organisms.
2. Fermentation of casein to simple amino acids by the Lactobacillus group, and probably some breakdown of both lactic acid and remaining traces of lactose by these same organisms.

It was noted in this work that in raw milk cheddar cheese and in pasteurized milk cheese the evidence pointed to the fact that the "Bacterium casei" group common to both types of cheeses was apparently responsible for the biting characteristic taste.

In 1934, Davies, Davis, Dearden, and Mattick (5) made some studies on cheddar cheese in which one of the experiments consisted of adding either Lactobacillus casei or Lactobacillus plantarum to the starter used in making the cheese. They noticed that, at the end of one month, there was a decided difference in the amount of protein breakdown in the cheeses to which Lactobacillus had been added as compared to the control cheeses. However, after five

months, no apparent difference could be detected.

In determining the types of bacteria found in commercial cheddar cheese, Hucker (12) examined 37 samples from approximately 25 factories. He found that the better grades of cheese contained a distinctly different flora from that of poorer grades. He concluded that in the better types of cheese Streptococcus lactis and lactobacilli predominated, while in the poorer grades, the spore forming and gram-negative rods were present in the largest numbers.

Lane and Hammer (13), in studying the effect of Lactobacillus casei on the nitrogenous decomposition in cheddar cheese made from pasteurized milk, made nine series of cheese using eight strains of the organism combined with commercial cheese starter. Control cheese was made using only commercial cheese starter. They found that the cheeses made with inoculations of Lactobacillus casei had a more rapid breakdown of the protein than those made without the addition of the organisms.

In examining the bacterial flora of New Zealand cheddar cheese, Sherwood (16) isolated 720 strains of lactic acid bacteria from thirty-six typical cheddar cheeses. Of these 720 strains, the predominant organism was shown to be "Streptobacterium plantarum".

Dahlberg and Kosikowsky (3), in discussing flavor development in cheddar cheese, maintained that the bacteria in starters used in cheese making died rather rapidly during the ripening of cheese. However, some bacteria such as lactobacilli are present in small numbers in milk, and develop to large numbers in cheese. They attack casein and other proteins and might produce flavor compounds.

McDonald (14) studied the rate of ripening of cheddar cheese.

There were three groups of experiments run. Both raw and pasteurized milk cheese was made with additions of Lactobacillus casei in the following manners:

1. Milk in the cheese vat was inoculated with cultures containing varying proportions of Lactobacillus casei and Streptococcus cremoris.
2. Just prior to the hooping, the curd was inoculated with cultures of Lactobacillus casei, using whey as the basic medium for growth.
3. Immediately after removal of the whey from the vat the curd was inoculated with cultures of Lactobacillus casei grown in milk.

It was concluded that:

1. Development of mature flavor in cheddar cheese could be hastened to a considerable extent by the addition of cultures of Lactobacillus casei during manufacture.
2. The increase in rate of flavor development was most marked when whole milk cultures were added to the cheese in the latter stages of manufacture, but the flavors which developed as a result of the technique were not uniform and undesirable off-flavors might occur.
3. The most controlled improvement in the rate of development of mature flavor took place when whey was used as a basic medium for growth of lactobacilli cultures, but at present no means have been devised to make use of whey base cultures as commercial propositions.
4. Extreme caution would be necessary in approaching the

subject of commercial application of lactobacilli cultures to increase the rate of ripening of cheddar cheese.

Deane, Anderson, Warren, and Dahle (6) experimented with various pure cultures added to regular starters in the manufacture of cheddar cheese from raw milk. The cultures used were Lactobacillus bulgaricus, Streptococcus citrovorous, Streptococcus paracitrovorous, Streptococcus lactis and an acidoproteolytic coccus which could not be identified. Their results showed that cheese made with regular commercial mixed starter supplemented with Lactobacillus bulgaricus was lower in flavor score than the control cheese made with regular starter. Among the other cultures used in a similar manner, the Streptococcus citrovorous produced an unclean flavor when the cheese was cured at 63°F. and a bitter flavor when it was cured at 43°F. Streptococcus paracitrovorous produced a slightly better flavored cheese at a ripening temperature of 43°F. than did the control cheese made with regular commercial cheese culture, and a slightly inferior cheese as compared to the control at 63°F. Streptococcus lactis gave good results throughout and produced cheese higher in score than the control in all cases. The acidoproteolytic coccus, which could not be identified, was isolated from cheddar cheese and gave higher scoring cheese than the control and the other cheeses in all cases; it usually reached full flavor in eight weeks at 63°F.

Hansen, Bendixen and Theophilus (11) compared cheeses made with Streptococcus lactis, Streptococcus citrovorous, Streptococcus paracitrovorous and two commercial cultures. Their results were similar to those of Deane, Anderson, Warren, and Dahle (6). The

commercial cultures gave cheeses that were good in body, texture and flavor. Streptococcus lactis produced a cheese slightly inferior in flavor but better in body and texture. When less than four per cent Streptococcus paracitrovorous was used, the cheese produced was slightly bitter in flavor with an open body. Four to ten per cent Streptococcus paracitrovorous produced a cheese decidedly bitter in flavor. Streptococcus citrovorous in all percentages used, which was from one to ten per cent, gave cheese with a bitter flavor and an open body.

In a recent work, Dahlberg and Kosikowsky (4) report excellent results in flavor production and proper body and texture development through the use of commercial starter supplemented by Streptococcus faecalis. They claim that a well-ripened cheese of medium flavor intensity was produced in 2.5 months at 60°F. when Streptococcus faecalis starter was used with the usual lactic starter.

In previous work, Dahlberg and Kosikowsky (2) attempted to find a correlation between the flavor, volatile acidity, and soluble protein in cheddar cheeses and concluded that there was no direct parallel between flavor and volatile acidity. In only two cases did they notice a direct relationship between the soluble protein and flavor. However, the association of soluble protein in the cheeses and the age of the cheeses followed the general trend of increased soluble protein with increased age.

Work in which moisture content was shown to influence the breakdown of the cheese was done by Van Slyke and Hart (18) in which one lot of cheese was paraffined and another lot unparaffined. These cheeses were cured at the same temperature, and at the end of 12 months were analyzed. The amount of protein breakdown

in the paraffined cheeses was decidedly greater and the moisture loss less than in the unparaffined cheeses. Freeman (8), however, concluded that changing the moisture content of the cheese through slight modifications in the curd making process could not be expected to influence materially the rate of ripening in the cheese.

The majority of investigators seem to think that lactobacilli play a major part in the curing process of cheddar cheese. Slatter and Halvorson (17) found that the majority of lactobacilli present in raw milk cheese can be killed by pasteurization at 143°F. for 30 minutes or 160°F. for 15 seconds. Based on their findings, they suggested that the ripening of cheddar cheese made from pasteurized milk may possibly be accelerated by adding the proper Lactobacillus culture, or by raising the ripening temperature of the cheese to supply favorable growing temperature for the reduced number of lactobacilli that survive pasteurization.

The three better known species of the genus Lactobacillus are given by Hammer (10) as Lactobacillus bulgaricus, Lactobacillus acidolphilus, and Lactobacillus casei.

A comparatively high temperature, in relation to other lactic acid producing organisms, greatly favors the growth of these Lactobacillus organisms. The growth temperature is one differentiating characteristic between them. Lactobacillus acidolphilus and Lactobacillus bulgaricus grow at 113°F. but not below 60°F., while Lactobacillus casei does not grow at 113°F. but does grow at 59°F. and also at lower temperatures. The organisms are facultative. Hammer further explains that the close relationship of Lactobacillus casei, Lactobacillus acidolphilus, and Lactobacillus bulgaricus is soon evident when these organisms are compared. There appears to be no clearly differentiating characteristics,

and the differences that occur are quantative rather than qualitative. Lactobacillus bulgaricus seems to be the strongest lactic acid producer with Lactobacillus acidolophilus second while Lactobacillus casei produces the least acid. All three seem to possess the ability to breakdown milk proteins.

## STATEMENT OF PROBLEM

Using a starter in the manufacture of cheddar cheese is a normal and accepted practice. The principal use of a starter has been for rapid development of lactic acid in the cheese making process, as lactic acid is necessary for the proper coagulation of the milk with rennet and for the restriction of undesirable bacteria. It also aids in expelling whey and in controlling the moisture content of the cheese.

In the past, the principal starters employed, excluding those in experimental work, have been those of Streptococcus lactis with its associated organisms. The difficulties encountered with these starters were their vulnerability to bacteriophage or other conditions causing slow acid development, and their inability when used alone to produce a characteristic cheddar cheese flavor. It has been thought by many, that organisms of the genus Lactobacillus have much to do with the proper flavor and body development owing to their outstanding predominance in cured cheddar cheese.

Raw milk cheddar cheese normally contains Lactobacillus organisms which were present in the milk delivered to the cheese plants. The majority of these Lactobacillus organisms are killed during the pasteurization of cheese milk. It seemed desirable, therefore, to add lactobacilli as part of the cheese culture.

In view of the information available, the problem presented itself as being to attempt to manufacture cheddar cheese from pasteurized milk with the aid of a pure culture of Lactobacillus bulgaricus organisms alone or in combination with commercial cheese starters, so as to give proper acid development and pro-

duction of a characteristic cheddar cheese flavor.

The problem involves five requirements:

1. Pasteurization of the milk for making the cheese.
2. Use of a controlled time method of manufacture, in so far as possible, in order to have a minimum of variables in manufacturing procedure.
3. The use of a pure strain of Lactobacillus bulgaricus alone or combined with regular lactic cheese culture.
4. Ripening of the cheese at the highest recommended temperature.
5. Examination of the cheeses after four months curing to obtain information as to the results. The examination consisting of scoring the cheeses for flavor, color, body and texture and analyzing them for moisture content and water soluble protein content.

## METHODS

### A. Culture selection and propagation

From a practical standpoint, no attempt was made to procure an outstanding culture of the organisms used. The cultures used were obtained from commercial laboratories and were started simultaneously, approximately one week after being received. One was a regular lactic cheese culture, presumably containing the usual Streptococcus lactis with the associated organisms, Streptococcus citrovorous and Streptococcus paracitrovorous; the other was a pure culture of Lactobacillus bulgaricus.

No attempt was made to verify the identity of the organisms in the mixed lactic culture, but the pure culture of Lactobacillus bulgaricus was stained and observed microscopically. The organisms were gram-positive, and the appearance of the organisms agreed with the usual morphological description given for them.

The cultures were propagated daily, except Sunday, in normal mixed herd skimmilk, following recommended procedures. The regular lactic culture was carried in 1000 ml. Erlenmeyer flasks containing 500 ml. skimmilk. The flasks were stoppered with non-ansorbent cotton plugs and capped with parchment paper, held in place by a rubber band. The flasks containing the milk were heat treated in an autoclave in flowing steam at 210°F. for 30 minutes. They were then cooled to 70°F. (plus or minus 2°F.) and inoculated with one per cent of culture using aseptic technic. Transfers were made with sterile glass pipettes. The flasks were then incubated in a thermostatically controlled incubator at 70°F. (plus or minus 2°F.) for a period of 14 to 16 hours. Upon removal from the incubator at the end of the incubation

period, the cultures were observed for firmness of coagulation and then cooled in a refrigerator in an iced water-bath until again transferred.

The pure culture of Lactobacillus bulgaricus required different handling than the regular lactic culture due to the growth temperature requirements of the organisms. This culture was propagated in the same type of containers, containing the same amount of milk, and stoppered in the same manner as the regular lactic cultures. However, the milk used was sterilized at 15 pounds steam pressure for 15 minutes because the incubation temperature necessary for growth of these organisms is very favorable for the growth of organisms which might be present in the milk that withstand ordinary pasteurization exposures. After sterilization, the flasks were cooled to between 80°F. and 100°F. and inoculated with one per cent of culture. The flasks were then incubated at 100°F. (plus or minus 2°F.) for a period of 14 to 16 hours. After incubation the flasks were placed in a refrigerator in an iced water-bath until the next transfer.

When bulk starter was needed, it was made in the same manner as the cultures, except that the Erlenmeyer flasks used were of 2000 ml. capacity and the amount of milk used was 1500 ml. per flasks. The length of heat treatment was extended to 45 minutes for the milk used for the regular lactic starter and to 20 minutes for the Lactobacillus bulgaricus milk as a safety measure because of the additional quantity of milk used.

B. Pasteurization of the cheese milk.

The milk used for making the cheese was regular mixed milk

from the Texas A. & M. college dairy herd, and no attempt was made to select any especially good milk. The butter fat content of the milk varied from 3.6% to 4.1%. All milk used was pasteurized in a batch pasteurizer at 143°F. to 145°F. for a period of 30 minutes and then cooled from 80°F. to 68°F. over a surface cooler employing sweet water as the coolant. It was drawn off in ten gallon cans and poured immediately into a previously sanitized cheese vat.

C. Manufacturing process.

As soon as the cheese milk was placed in the vat, the temperature was adjusted to 86°F. and the starter, usually 1%, was added. The method of manufacture was carried out as closely as possible to the method recommended by Wilson as given by Wilster (21), and also advocated by Price (15). A schematic outline of this method taken from Wilster (21) is reproduced on page 17.

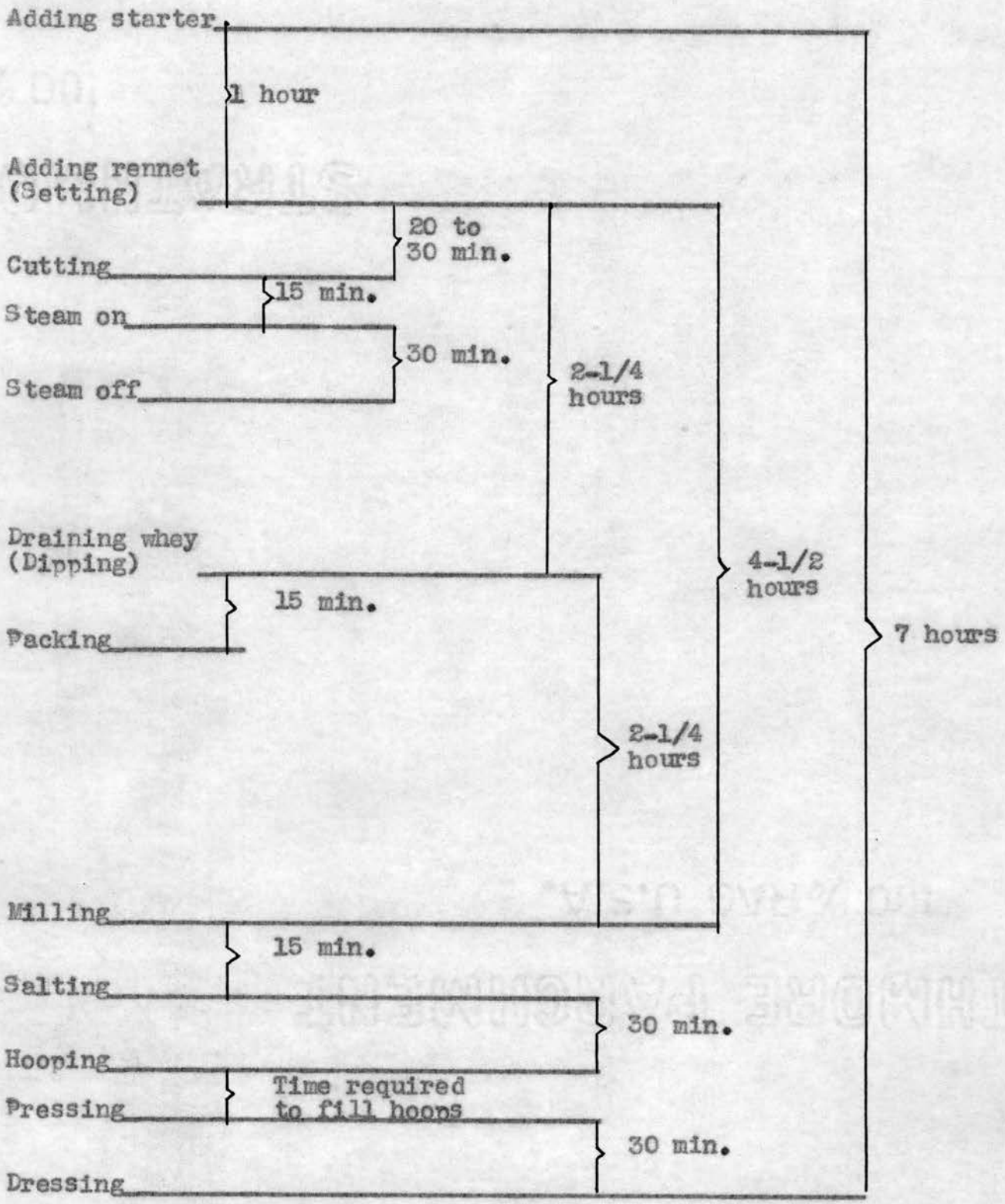
The cheese making procedure was as uniform as possible from day to day so that variations involved only the proportions of the two starters used. The starters used consisted of pure Lactobacillus bulgaricus starter and combinations of Lactobacillus bulgaricus with commercial cheese starter.

The lowest ratio of Lactobacillus bulgaricus to commercial cheese starter used as starters in the cheese making was 40% Lactobacillus bulgaricus to 60% commercial cheese starter. Inasmuch as a preponderance of lactobacilli were desired in the cheese, cheeses with lower percentages of Lactobacillus bulgaricus starter were not made.

The acidities of the cultures as well as the acidity development during the making process were measured by titration of a

PLATE I

TIME METHOD OF MANUFACTURE.



9 ml. sample with 0.1N sodium hydroxide solution, using phenolphthalein as the indicator. The ml. of 0.1N NaOH used divided by 10 was recorded as the per cent lactic acid in the sample.

All lots of milk were set at temperatures from 86°F. to 88°F. The rate of rennet addition varied from 3 to 3½ ounces per 1000 pounds of milk. Color was added at the rate of 1 to 1½ ounces per 1000 pounds of milk.

The cheese was cut after the coagulation was sufficient to cause a clean break of the curd by the finger test. The cutting of the cheese was done with ½ inch wire knives, following the customary method of using the horizontal knife lengthwise and then the vertical knife lengthwise and crosswise.

After cutting, the curd was stirred gently for 15 minutes without heat in order to expel whey and to slightly firm the curd.

The cheese was cooked by raising the temperature of the curd in the whey to 100°F. to 102°F. in a period of 30 minutes, and holding it at that temperature for 45 minutes to an hour in order to attain the proper firmness of the curd. This was determined by pressing a handful of curd particles lightly and then opening the hand quickly. When the firmness of the curd was such that the particles fell apart rather easily, the whey was drained.

The curd was matted in two piles, one on each side of the vat. When matting was sufficient, the piles were cut into six inch blocks and packed by inverting the blocks. The cheddaring process was begun as soon as blocks were firm enough to handle easily. Cheddaring was done by turning the blocks of curd approximately every 10 minutes. When whey ceased to drain with blocks only one high, they were piled two high and then three and four

high as was required. Cheddaring in all cases was complete in 1 hour and 45 minutes to 2 hours.

Milling of the curd was done with a hand operated curd mill of the usual type. After milling, the curd was forked slightly to heal the cut edges and then salted. The rate of salting was 2.5 pounds per thousand pounds of milk used.

All cheeses were hooped in daisy style hoops, pressed for 30 minutes, and then dressed. After dressing, the cheeses were returned to the press and pressed overnight at full pressure.

The cheeses were removed from the press the following morning and placed in the curing room where they remained for 24 hours to dry the surface. At the end of this period, the cheeses were paraffined by dipping in paraffin at 240°F. to 250°F.

D. Curing of the cheeses.

The cheeses were cured in a cheese curing room at temperatures ranging from 60°F. to 65°F.

E. Analysis and examination of the cheese.

The data collected consisted of the results of three separate analyses, namely, scoring the cheeses for flavor, body and texture, and color; moisture content; and water soluble protein content. These determinations were made when the cheeses had reached an age of four months.

1. Scoring.

The cheeses were scored organolentically by two, and in some instances three judges. The identity of the cheeses was unknown to the judges. Finish was considered to be perfect in all cheese.

2. Moisture

The cheeses were analyzed for moisture content because it

is the opinion of some investigators that moisture content directly influences the ripening. The procedure for determining the moisture was the 24 hour method given by Wilster (21), consisting of heating the weighed sample (approximately 2 grams of cheese) for a 24 hour period in an oven at 212°F. (100°C.), after which, the sample was cooled in a desiccator to room temperature and reweighed. The difference between the original weight of sample and the weight after drying represented the amount of moisture lost. All samples were weighed to four decimal places. The moisture percentage was calculated to the nearest one hundredth of one per cent.

3. Water soluble protein.

Water soluble protein content has been considered one of the constituents in cheese indicating the degree of proteolysis which has taken place during curing.

The method of extracting the water soluble protein followed a technic based upon a method by Sharp as reported by Dahlberg and Kosikowsky (2). The principle of the method is to maintain the pH (approximately 5) and the salt content comparable to that of cheddar cheese by a buffer salt solution while the soluble protein is being dissolved in the water.

The soluble protein extraction solution was prepared as follows:

A. Stock solution

57.5 ml. glacial acetic acid
136.1 g. sodium acetate ($3H_2O$)
47.0 g. sodium chloride
8.9 g. calcium chloride (anhydrous)
Add water to make 1 liter.

B. Extraction solution
Make 250 ml. stock solution up to 1 liter with water.

The soluble protein was extracted as follows: Three grams

of cheese were weighed to within 0.01 gram and placed in a porcelain mortar. A small amount of extracting solution at 122°F. (50°C.) was added and the cheese was ground to a thick paste. Additional solution was added to dilute the paste. The flask was placed in a water bath at 122°F. (50°C.), filled to the mark with extracting solution, and, with occasional shaking, maintained at this temperature for one hour.

The solution was filtered through a fluted filter, and a 50 ml. sample of the filtrate was analyzed for nitrogen content by the Gunning method (1). After digestion, the nitrogen in the form of ammonia was distilled into hydrochloric acid of a known normality (approximately 0.2N), and the excess acid titrated with sodium hydroxide solution of known normality (approximately 0.2N). From the milliequivalents of acid unaccounted for by titration with the standard base, the per cent of soluble protein was calculated.

## EXPERIMENTAL

A. The influence of various combinations of Lactobacillus bulgaricus and commercial cheese starters on the rate of acid development during cheese manufacture.

Sixteen lots of cheese were made using various combinations of Lactobacillus bulgaricus and commercial cheese starter as shown in Table No. I.

In Lots 1, 2, and 3 Lactobacillus bulgaricus culture only was used, in Lots 4 to 15 inclusive Lactobacillus bulgaricus was mixed with commercial cheese starter ranging from 5% to 60% of the total starter used and in Lot 16 commercial cheese starter only was used.

Acid development at the various stages of the manufacturing process in each of the lots is shown in Table II. Detailed manufacturing reports are shown in Appendix I.

1. Cheeses made with Lactobacillus bulgaricus starter.

Lots No. 1, 2, and 3, which were made using Lactobacillus bulgaricus starter entirely, showed unsatisfactory acid development. A definite increase in acidity of the cheese milk was noted immediately after the addition of the starter. This was probably due to the high acidity of the starters used which ranged from 1.86% to 2.20% calculated as lactic acid. The cheeses showed slow acid development during the ripening period in these three lots. With ordinary cheese culture the increase in acidity during the cooking period is usually 0.04% to 0.05% while Lots 1, 2, and 3 showed an average increase of only 0.01% acidity.

The most decided lack of acid development in the cheeses made with Lactobacillus bulgaricus starter was observed during the cheddaring period. The results in Table II show that the milling

acidity in the three cheeses made with Lactobacillus bulgaricus starter was 0.20%, while in commercial cheese the acidity normally develops up to 0.45% to 0.55% in the whey draining from the cheese at milling.

Lots 1, 2, and 3, made from Lactobacillus bulgaricus starters, also did not firm up as well as was desired during the cooking period, but no attempt was made to increase the firmness of the curd by using cooking temperatures above 102°F. Neither was a longer cooking period employed than the regular 1 hour and 15 minutes to 1 hour and 30 minutes in order to make the curd more firm. Although the cheese closed up well during cheddaring and at milling was normal in appearance, the curd lacked the tough, rubbery feel of normal curd.

The results in Table II indicate that pure Lactobacillus bulgaricus starters give unsatisfactory acid development for cheddar cheese making.

2. Cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starters.

Lots 4 to 15 inclusive were made using various combinations of Lactobacillus bulgaricus starter and commercial cheese starter. The additions of commercial cheese starter ranged from 5% to 60% of the total starter used.

Lots 4 to 7 inclusive, in which the starter used contained from 5% to 20% commercial cheese starter, showed in general, that as the proportion of commercial cheese starter increased the acid development in the cheese increased. The acid development during cooking was not particularly dignificant, but the milling acidity in general was higher than in the cheeses made using Lactobacillus

bulgaricus only. The milling acidities ranged from 0.19% to 0.28% in Lots 4 to 7 inclusive, which is generally considered too low for practical commercial manufacture.

The curd in Lots 4 to 7 appeared normal but it lacked the characteristic tough, rubbery feel of normal curd at milling.

Lot No. 8, which was made with a starter combination of 25% commercial cheese starter and 75% Lactobacillus bulgaricus, showed a distinct improvement in acid production over the cheeses inoculated with smaller amounts of commercial culture. The development of acidity during cooking of Lot 8 was not significant, but the final milling acidity of 0.41% was much higher than the milling acidities of the previous seven cheeses in which the highest milling acidity developed was 0.28%.

Lot No. 8 produced a curd more characteristic of good cheddar cheese in appearance and feel than any of the preceding lots.

Lots No. 9 to 15 inclusive were made with combinations of Lactobacillus bulgaricus starter and from 30% to 60% of commercial cheese starter. The results in Table II show that acid development progressed normally in these lots, although there was some variations in the rate of acid development among the various lots. The acidity at milling in Lots 9 to 15 inclusive ranged from 0.50% to 0.54% which is within the desirable range for commercial cheese manufacture.

The curd in Lots 9 to 15 had the tough, rubbery character desired in cheddar curd at milling.

Lot No. 16 was made with commercial cheese starter entirely. Acid development was normal throughout the manufacturing process and the final milling acidity was 0.50%.

The results in Table II indicate that Lactobacillus bulgaricus alone was unsatisfactory as a starter for the manufacture of cheddar cheese. When regular commercial cheese starter was used in conjunction with Lactobacillus bulgaricus starter, the rate of acid development increased as the proportions of commercial starter used was increased, and when 30% or more of commercial cheese starter was used in conjunction with 70% or less of Lactobacillus bulgaricus starter the acid development during the manufacture was satisfactory.

TABLE I

LACTIC CULTURES USED IN MAKING THE EXPERIMENTAL CHEESES

| Lot No. | Comm. of Culture Used | | Amount of starter used to inoculate cheese milk % | Acidity of Lactobacillus bulgaricus starter % | Acidity of commercial cheese starter % |
|---|---|---|---|---|---|
| | Lactobacillus bulgaricus % | Commercial cheese starter % | | | |
| 1 | 100 | 0 | 1 | 1.86 | --- |
| 2 | 100 | 0 | 2 | 2.05 | --- |
| 3 | 100 | 0 | 1 | 1.93 | --- |
| 4 | 95 | 5 | 1 | 2.12 | 0.81 |
| 5 | 90 | 10 | 1 | 2.01 | 0.80 |
| 6 | 85 | 15 | 1 | 2.18 | 0.83 |
| 7 | 80 | 20 | 1 | 2.06 | 0.88 |
| 8 | 75 | 25 | 1 | 2.06 | 0.89 |
| 9 | 70 | 30 | 1 | 1.94 | 0.82 |
| 10 | 65 | 35 | 1 | 1.91 | 0.82 |
| 11 | 60 | 40 | 1 | 2.16 | 0.91 |
| 12 | 55 | 45 | 1 | 2.05 | 0.89 |
| 13 | 50 | 50 | 1 | 2.15 | 0.88 |
| 14 | 45 | 55 | 1 | 2.20 | 0.85 |
| 15 | 40 | 60 | 1 | 1.98 | 0.83 |
| *16 | 0 | 100 | 1 | ---- | 0.85 |

*Control cheese - No Lactobacillus bulgaricus used

TABLE II

Acid Development During the Cheese Making Process in Cheese Made from Pasteurized Milk Inoculated with Various Proportions of *Lactobacillus bulgaricus* and Commercial Cheese Culture.

| Stage of Process | Lot Number | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | *16 |
| | Per Cent of *Lactobacillus bulgaricus* Used in Each Lot | | | | | | | | | | | | | | | |
| | 100 | 100 | 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 0 |
| | % Acidity Calculated as Lactic Acid | | | | | | | | | | | | | | | |
| Original acidity of milk | .15 | .15 | .15 | .14 | .14 | .15 | .15 | .15 | .15 | .17 | .15 | .16 | .17 | .16 | .15 | .16 |
| After adding starter | .16 | .17 | .17 | .16 | .15 | .16 | .16 | .17 | .17 | .18 | .16 | .17 | .185 | .165 | .16 | .17 |
| Adding coagulant | .17 | .18 | .18 | .16 | .16 | .16 | .17 | .18 | .18 | .18 | .165 | .17 | .185 | .18 | .17 | .17 |
| Cutting | .10 | .10 | .10 | .10 | .10 | .09 | .10 | .10 | .10 | .11 | .10 | .11 | .10 | .11 | .11 | .10 |
| Steam on | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .11 | .10 | .11 | .10 | .11 | .11 | .10 |
| Steam off | .11 | .10 | .11 | .10 | .10 | .10 | .11 | .10 | .11 | .11 | .12 | .12 | .11 | .12 | .11 | .11 |
| Draining | .11 | .11 | .11 | .11 | .14 | .11 | .11 | .11 | .14 | .14 | .14 | .15 | .145 | .16 | .14 | .13 |
| Packing | .14 | .11 | .12 | .11 | .15 | .11 | .14 | .13 | .16 | .16 | .17 | .16 | .18 | .20 | .15 | .14 |
| Milling | .20 | .20 | .20 | .19 | .28 | .22 | .27 | .41 | .51 | .50 | .54 | .50 | .51 | .54 | .52 | .50 |

*Control cheese - No *Lactobacillus bulgaricus* used

B. The influence of various combinations of Lactobacillus bulgaricus and commercial cheese starters on the score of cheddar cheese.

The influence of the use of starters composed of pure cultures of Lactobacillus bulgaricus and of various combinations of Lactobacillus bulgaricus and commercial cheese starter on the score of cheddar cheese after ripening for four months is shown in Table III.

1. Cheese made with Lactobacillus bulgaricus starter.

The data in Table III show that Lots 1, 2, and 3, made with Lactobacillus bulgaricus as the sole cheese starter, scored lower in flavor than the control cheese made with commercial cheese starter. The flavor on these lots ranged from 36.8 to 37.5 and averaged 37.1 while the flavor score of the control cheese was 40. The highest scoring cheese was Lot 2, made with 2% Lactobacillus bulgaricus, which scored 37.5.

The principal flavor criticism of the cheese made with Lactobacillus bulgaricus starter was acidy with a secondary criticism of heated in Lots 1 and 2, and unclean in Lot 3. The control cheese was not criticized for any off-flavor.

The body and texture score of the lots made with Lactobacillus bulgaricus starter was slightly lower than that of the control cheese. They ranged from 28 to 29 and averaged 28.6 while the control cheese scored 29.5. All of these lots were criticized for having a slightly crumbly body except the control cheese which was not criticized.

The color score of the lots made with Lactobacillus bulgaricus starter ranged from 8.0 to 10 and averaged 9.1, while the control cheese scored 9.5. These lots were criticized for being acid cut and slightly mottled except Lot 3 which was not criticized. The

control cheese was criticized for being slightly seamy.

2. Cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter.

The data in Table III show that the cheese in Lots 4 to 15 inclusive, made with combinations of Lactobacillus bulgaricus and commercial cheese starter were, in general, lower in flavor score than the control cheese made with commercial cheese starter only. The flavor scores of these lots ranged from 34.5 to 41 and averaged 37.9 while the control cheese scored 40. The majority of these cheeses ranged from 36.5 to 39 with two exceptions. These exceptions were Lot 4 which scored 34.5 and Lot 6 which scored 41. The reason for these variations could not be explained. In general, as the amount of commercial cheese starter increased and the amount of Lactobacillus bulgaricus starter decreased, the flavor score of the cheese increased; however, there was the exception of Lot 6 which scored 41.

There was a general relationship between the milling acidity of the cheese and the score of the cheese, in that Lots 4 to 7 inclusive, in which the highest milling acidity obtained was 0.28%, had an average flavor score of 37.3, while Lots 8 to 15 inclusive, in which the lowest milling acidity was 0.41%, scored an average of 38.1.

All of the lots made with combinations of Lactobacillus bulgaricus and commercial cheese starter were criticized for heated flavor except Lot 4 which was criticized for being unclean and slightly bitter. It is possible that the heated flavor was present in this lot also but was masked by the unclean flavor to such an extent that it was missed. Lot 7 was also criticized for being unclean in flavor, but it was not present strongly enough to mask

the heated flavor. Three of the lots, namely, Lots 4, 5, and 10 were criticized for being bitter or slightly bitter. In Lots 5 and 10, however, this flavor was accompanied by the heated flavor and in Lot 4 by the unclean flavor. The degree of the heated flavor in the cheese seemed to decrease as the amount of Lactobacillus bulgaricus starter used in the manufacture decreased. This is illustrated by Lots 5 to 9 inclusive, in which Lactobacillus bulgaricus was never less than 70% of the total starter, being criticized for the full heated flavor, while the heated criticism was only slight in Lots 10 to 15 inclusive in which the per cent Lactobacillus bulgaricus ranged from 65% of the total starter down to 40%. The heated flavor was much the same as the flavor of the ripened Lactobacillus bulgaricus starter which had been propagated in sterilized milk. These observations suggest that the heated flavor was caused by some by product of the growth of Lactobacillus bulgaricus. It is also interesting that none of the cheese made with starter containing Lactobacillus bulgaricus organisms produced a characteristic cheddar cheese flavor, while the control cheese made with commercial cheese starter only, produced a good cheddar flavor.

The body and texture scores of the cheeses made with combinations of Lactobacillus bulgaricus and commercial cheese starter were lower than the body and texture score of the control cheese which was made using commercial cheese starter only. The scores ranged from 25.5 to 28 and averaged 26.7 while the control lot scored 29.5. However, it appeared that the body and texture score of the cheese decreased, in general, with decreases in the amount of Lactobacillus bulgaricus and increases in the amount of commercial

cheese starter used. Lots 4 to 7 inclusive, in which the amount of Lactobacillus bulgaricus starter used was from 80% to 95% of the total starter, had an average body and texture score of 27.2 while in Lots 8 to 15 inclusive, in which the amount of Lactobacillus bulgaricus used dropped from 75% to 40%, the body and texture score average was 26.5.

The body and texture score of the cheese made using Lactobacillus bulgaricus combined with commercial cheese starter appeared to decrease with increasing milling acidity. Lots 4 to 7 inclusive, in which the highest milling acidity was 0.28%, had an average body and texture score of 27.2 while Lots 8 to 15 inclusive, in which the lowest milling acidity was 0.41%, scored an average of 26.5 on body and texture.

All the cheeses made with combinations of Lactobacillus bulgaricus and commercial cheese starters were criticized as being crumbly. Three of the cheeses, namely, Lots 4, 7, and 8 were also criticized for being corky, while Lots 9, 10, 11, 12, and 13 were criticized for being mealy in addition to crumbly. It was noted that all of the lots criticized for being corky had milling acidities of less than 0.50% while the lots criticized for being mealy were those having a milling acidity of 0.50% or higher. It maybe also noted that Lot 8 which had less corkiness than Lots 4 and 7 also had a higher milling acidity. This fact might substantiate an observation that corkiness appeared to decrease with an increase in milling acidity.

The color score of the cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter ranged from 9 to 10 and averaged 9.45 in comparison to a score of 9.5 for the control

cheese. Three lots, namely, Lots 9, 10, and 13 were criticized for seamy color. Lots 4, 11, and 15 were criticized for being slightly mottled in color, while Lots 6 and 14 were given no criticism. Lots having individual criticisms were Lot 5 criticized for being slightly acid cut, Lot 7 for being slightly bleached, Lot 8 for having white specks and Lot 12 for being wavy.

The data in Table III show that cheese made with pure Lactobacillus bulgaricus rated a lower average flavor score than cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter as well as the control cheese. Although rating a lower flavor score, the cheese made with pure Lactobacillus bulgaricus starter averaged a higher body and texture score than the cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter, and averaged only 0.9 of a point lower than the control. The flavor score of the cheese, in general, was higher as the amount of commercial cheese starter used in conjunction with Lactobacillus bulgaricus starter was increased.

TABLE III

Influence of Various Combinations of Lactobacillus bulgaricus and Commercial Cheese Starters on the Score of Cheddar Cheese at Four Months of Age.

| Lot No. | Total amount of starter % | Comm. of starter used | | Score after 4 months of storage | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Lactobacillus bulgaricus % | Commercial cheese starter % | Flavor | Criticism | Body and texture | Criticism | Color | Criticism |
| 1 | 1 | 100 | 0 | 37 | Heated acidy | 28 | Sl. crumbly | 8.0 | Acid cut |
| 2 | 2 | 100 | 0 | 37.5 | Sl. acidy Sl. heated | 29 | Sl. crumbly | 9.5 | Sl. mottled |
| 3 | 1 | 100 | 0 | 36.8 | Acidy Sl. unclean | 29 | Sl. crumbly | 10 | ----- |
| 4 | 1 | 95 | 5 | 34.5 | Unclean Sl. bitter | 27.5 | Corky Crumbly | 9.5 | Sl. mottled |
| 5 | 1 | 90 | 10 | 37.5 | Heated Sl. bitter | 27.5 | Crumbly | 9 | Sl. acid cut |
| 6 | 1 | 85 | 15 | 41 | ----- | 27.5 | Crumbly | 10 | ----- |
| 7 | 1 | 80 | 20 | 36.5 | Heated unclean | 26.5 | Corky Crumbly | 9.5 | Sl. bleached |
| 8 | 1 | 75 | 25 | 37.5 | Heated | 26.5 | Sl. corky crumbly | 9.5 | White specks |

TABLE III (Continued)

| Lot No. | Total amount of starter % | Comm. of starter used: Lactobacillus bulgaricus % | Commercial cheese starter % | Score after 4 months of storage: Flavor | Criticism | Body and texture | Criticism | Color | Criticism |
|---|---|---|---|---|---|---|---|---|---|
| 9 | 1 | 70 | 30 | 37 | Sl. acidy Heated | 26 | Crumbly Mealy | 9 | Seamy |
| 10 | 1 | 65 | 35 | 38 | Sl. heated Sl. bitter | 25.5 | Very crumbly Mealy | 9 | Seamy |
| 11 | 1 | 60 | 40 | 38.5 | Sl. heated | 27 | Sl. crumbly Mealy | 9.5 | Sl. mottled |
| 12 | 1 | 55 | 45 | 38.5 | Sl. heated | 26 | Crumbly Mealy | 9.5 | Wavy |
| 13 | 1 | 50 | 50 | 38.5 | Sl. heated | 26.5 | Crumbly Sl. mealy | 9.5 | Seamy |
| 14 | 1 | 45 | 55 | 39 | V. slight heated | 28 | Sl. crumbly | 10 | ----- |
| 15 | 1 | 40 | 60 | 38.5 | Sl. heated | 27 | Crumbly | 9.5 | Sl. mottled |
| *16 | 1 | 0 | 100 | 40 | ----- | 29.5 | ----- | 9.5 | Sl. seamy |

*Control cheese - No Lactobacillus bulgaricus added

C. The influence of various combinations of Lactobacillus bulgaricus and commercial cheese starter on the moisture content of cheddar cheese.

The influence of the use of starters composed of pure cultures of Lactobacillus bulgaricus and of combinations of Lactobacillus bulgaricus and commercial cheese starters on the moisture content of cheese is shown in Table IV.

1. Cheese made with Lactobacillus bulgaricus starter.

The data in Table IV show that Lots 1, 2, and 3, made with Lactobacillus bulgaricus starter, had a higher moisture content than the control cheese made with commercial cheese starter only. The moisture content of these lots ranged from 38.95% to 40.28% and averaged 39.46% as compared to a moisture content of 36.49% in the control cheese. The cheese having the highest moisture content of these lots was Lot 2 which contained 40.28% moisture. It was also noted that the average moisture content of these three lots was approximately 3% higher than the moisture content of the control cheese.

2. Cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter.

The data in Table IV show that the cheese in Lots 4 to 15 inclusive, made with combinations of Lactobacillus bulgaricus and commercial cheese starter averaged lower in moisture content than the control cheese made with commercial cheese starter exclusively. The moisture content of these lots ranged from 32.00% to 36.32% and averaged 33.86%, while the control cheese had a moisture content of 36.49%. There appeared to be a relationship between the amount of Lactobacillus bulgaricus starter used and the moisture content of the cheese in Lots 4 to 7 inclusive. As

the amount of Lactobacillus bulgaricus decreased from 95% of the total starter used to 80%, there was a corresponding decrease in the moisture content from 35.91% to 32.00%. However, this relationship ceased at Lot 7 and the cheeses from Lot 8 to Lot 15 inclusive varied in moisture content from 32.24% to 36.32% with no particular sequence in the variations. In Lots 4 to 8 inclusive, of the lots made with combinations of Lactobacillus bulgaricus and commercial cheese starter, there was noted an excretion of a sticky, clear fluid during the curing period, while in the remaining lots, namely, Lots 9, 10, 11, 12, 13, 14, and 15, this condition was not observed. This may account for the average moisture content of Lots 4 to 8 inclusive for being lower (33.52%) than the average moisture content of Lots 9 to 15 inclusive (34.12%). The excretion of sticky, clear fluid which appeared in Lots 4 to 8 inclusive could not be explained.

The data in Table IV show that cheese made with pure Lactobacillus bulgaricus starter contained a higher percentage of moisture than those made with combinations of Lactobacillus bulgaricus and commercial cheese starter, as well as the control cheese. This was probably due to poor expulsion of whey from the curd caused by poor acid development by the pure Lactobacillus bulgaricus starter during the manufacture.

TABLE IV

The Influence of Various Combinations of *Lactobacillus bulgaricus* and Commercial Cheese Starters on the Moisture Content of the Cheese.

| Lot No. | Per cent total amount of starter | Composition of starter | | Per cent moisture in cheese | Average per cent moisture in groups of cheese | |
|---|---|---|---|---|---|---|
| | | Per cent *L. bulgaricus* starter | Per cent commercial cheese starter | | | |
| 1 | 1 | 100 | 0 | 39.16 | | |
| 2 | 2 | 100 | 0 | 40.28 | 39.46 | Cheese made with *Lactobacillus bulgaricus* only |
| 3 | 1 | 100 | 0 | 38.95 | | |
| 4 | 1 | 95 | 5 | 35.91 | | |
| 5 | 1 | 90 | 10 | 34.11 | | |
| 6 | 1 | 85 | 15 | 33.05 | | |
| 7 | 1 | 80 | 20 | 32.00 | | |
| 8 | 1 | 75 | 25 | 32.53 | | |
| 9 | 1 | 70 | 30 | 33.47 | | |
| 10 | 1 | 65 | 35 | 34.05 | 33.86 | Cheese made with combinations of *Lactobacillus bulgaricus* and commercial cheese starter |
| 11 | 1 | 60 | 40 | 34.37 | | |
| 12 | 1 | 55 | 45 | 32.24 | | |
| 13 | 1 | 50 | 50 | 36.32 | | |
| 14 | 1 | 45 | 55 | 35.03 | | |
| 15 | 1 | 40 | 60 | 33.30 | | |
| *16 | 1 | 0 | 100 | 36.49 | | |

*Control cheese - No *Lactobacillus bulgaricus* used

D. The influence of various combinations of Lactobacillus bulgaricus and commercial cheese starter on the water soluble protein content of cheddar cheese at four months of age.

The influence of the use of starters composed of pure cultures of Lactobacillus bulgaricus and of combinations of Lactobacillus bulgaricus and commercial cheese starter on the water soluble protein content of the cheese at four months of age is shown in Table V.

1. Cheese made with Lactobacillus bulgaricus starter.

The data in Table V show that Lots 1, 2, and 3, made using Lactobacillus bulgaricus starter exclusively, had a higher average water soluble protein content than the control cheese made with commercial cheese starter only. The water soluble protein content of these lots ranged from 6.88% to 8.53% and averaged 7.51%, while the control cheese had a water soluble protein content of 6.91%. Lot 1 was lower in water soluble protein than the control cheese, but both Lots 2 and 3 were higher in water soluble protein than the control cheese. Lot 2, made with 2% Lactobacillus bulgaricus starter, was found to contain the highest amount of water soluble protein (8.53%) of any of the lots made in the entire experiment. The cheeses made with pure Lactobacillus bulgaricus starter showed no significant relationship between the amount of water soluble protein and the score of the cheese. Lot 2, which had the highest water soluble protein content of these three lots, scored only 0.5 of a point higher than Lot 1 which contained the least amount of water soluble protein. Lot 3, which was higher in water soluble protein than Lot 1, was 0.2 of a point lower in score.

2. Cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter.

The data in Table V show that Lots 4 to 15 inclusive, made with combinations of Lactobacillus bulgaricus and commercial cheese starter, had a higher average water soluble protein content than the control cheese made using commercial cheese starter exclusively. The water soluble content of these lots ranged from 6.64% to 8.37% and averaged 7.62%, while the control cheese had a water soluble protein content of 6.91%. There appeared to be a general relationship between the amount of Lactobacillus bulgaricus starter used and the amount of water soluble protein present in Lots 4 to 8 inclusive. As the amount of Lactobacillus bulgaricus starter used decreased in these lots from 95% of the total culture to 75%, the amount of water soluble protein increased from 7.66% to 8.37%. However, at this point the relationship ceased and increases in the amounts of water soluble protein in the remaining lots were not consistent with decreases in the amount of Lactobacillus bulgaricus used. On the other hand, Lots 4 to 8 inclusive, which were made with starter variations containing from 75% to 95% Lactobacillus bulgaricus, had a higher average water soluble protein content (8.08%) than Lots 9 to 15 inclusive, in which starter variations containing from 40% to 70% Lactobacillus bulgaricus were used, which had an average water soluble protein content of 7.30%. Lots 9 and 13, of the cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter, were the only cheeses of this group having less water soluble protein content than the control cheese. There was no relationship between the amount of water soluble protein present in the cheese and the

flavor score of the cheese. The control cheese, which was the second highest scoring cheese of the entire group, had a water soluble protein content of 6.91%. Although, the water soluble protein content of the control cheese was exceeded by 12 other cheeses, only one of the twelve cheeses exceeded the control cheese in flavor score.

White specks, which are considered by some investigators, as being crystals of the amino acid tyramine and an indication of considerable protein breakdown as well as considerable age, were noted to be in evidence in all of the cheeses made with Lactobacillus bulgaricus present in the starter. No white specks were observed in the control cheese which was made with commercial cheese starter.

The results in Table V show that there appeared to be no significant relationship between the amount of Lactobacillus bulgaricus starter used in the manufacture of the cheese, and the per cent of water soluble protein present in the cheese at four months of age. However, in general, the cheese made with Lactobacillus bulgaricus alone or in combination with commercial cheese starter contained a higher percentage of water soluble protein than the control cheese. Although, the cheeses made with pure Lactobacillus bulgaricus alone or in combination with commercial cheese starter had a higher average water soluble protein content than the control cheese, only one of these cheeses had a higher flavor score. In general, the water soluble protein content of the cheeses had no relationship to the flavor score given the cheeses.

TABLE V

The Influence of Various Combinations of *Lactobacillus bulgaricus* and Commercial Cheese Starters on the Water Soluble Protein Content of Cheddar Cheese at Four Months of Age.

| Lot No. | Total amount of starter % | Comm. of starter used: *Lactobacillus bulgaricus* starter % | Comm. of starter used: Commercial cheese starter % | Water soluble protein % | Average water soluble protein in groups of cheese. % | |
|---|---|---|---|---|---|---|
| 1 | 1 | 100 | 0 | 6.88 | | |
| 2 | 2 | 100 | 0 | 8.53 | 7.51 | Cheeses made with *Lactobacillus bulgaricus* only |
| 3 | 1 | 100 | 0 | 7.13 | | |
| 4 | 1 | 95 | 5 | 7.66 | | |
| 5 | 1 | 90 | 10 | 7.86 | | |
| 6 | 1 | 85 | 15 | 8.29 | | |
| 7 | 1 | 80 | 20 | 8.23 | | |
| 8 | 1 | 75 | 25 | 8.37 | | |
| 9 | 1 | 70 | 30 | 6.80 | 7.62 | Cheeses made with combinations of *Lactobacillus bulgaricus* and commercial cheese starter. |
| 10 | 1 | 65 | 35 | 7.86 | | |
| 11 | 1 | 60 | 40 | 8.10 | | |
| 12 | 1 | 55 | 45 | 6.64 | | |
| 13 | 1 | 50 | 50 | 7.56 | | |
| 14 | 1 | 45 | 55 | 7.15 | | |
| 15 | 1 | 40 | 60 | 7.01 | | |
| *16 | 1 | 0 | 100 | 6.91 | | |

*Control cheese - No *Lactobacillus bulgaricus* used.

## SUMMARY

Fifteen lots of experimental cheese and one lot of control cheese were made using a time controlled method of manufacture. Lactobacillus bulgaricus starter and combinations of Lactobacillus bulgaricus and commercial cheese starter were used in the experimental cheeses. The control cheese was made using only commercial cheese starter. The acid development during the manufacturing process was checked and recorded. At four months of age the cheese was scored for flavor, body and texture, and color, and analyzed for moisture content, and water soluble protein content.

The results show that when Lactobacillus bulgaricus is used as the sole starter the acid development during the manufacturing process was unsatisfactory. Combinations of Lactobacillus bulgaricus starter and commercial cheese starter containing less than 30% commercial cheese starter also gave unsatisfactory acid development. However, when 30% to 60% of the total amount of starter used was commercial cheese starter, and the remainder Lactobacillus bulgaricus, the acid development progressed normally.

As shown by the results, the use of Lactobacillus bulgaricus alone or in combination with commercial cheese starter appeared to be detrimental to the flavor score of the cheese. The average flavor score of the lots made with pure Lactobacillus bulgaricus starter was lower than the average flavor score of the lots made with combinations of Lactobacillus bulgaricus and commercial cheese starter, and also lower than the flavor score of the control cheese. The flavor score of the cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter also had a lower average flavor score than the control cheese made with

commercial cheese starter only. The flavor score of the cheese appeared to become higher as the amount of Lactobacillus bulgaricus starter used became less, and the amount of commercial cheese starter became greater.

The average body and texture score of the cheese made with pure Lactobacillus bulgaricus starter was higher than the average body and texture score of the cheese made with combinations of Lactobacillus bulgaricus and commercial cheese starter. However, the average body and texture score of all lots, whether made with Lactobacillus bulgaricus alone or in combination with commercial cheese starter, was lower than that of the control cheese. All of the cheeses were criticized for having crumbly body, and this defect was more noticeable in the cheeses made with combinations of the two starters than in those made with individual starters of Lactobacillus bulgaricus or commercial cheese starter.

The average moisture content in the lots made using pure Lactobacillus bulgaricus starter was approximately 5.5% higher than in the lots made with combinations of Lactobacillus bulgaricus and commercial cheese starters, and approximately 3% higher than the control lot. None of the lots made using a combination of Lactobacillus bulgaricus and commercial cheese starter had a commercially desirable moisture content.

The water soluble protein content of the cheese was not parallel to the amount of Lactobacillus bulgaricus starter used in making the cheeses. However, all but three of the cheeses made with starter containing Lactobacillus organisms had a higher water soluble protein content than the control cheese made with commercial cheese

starter. There was no correlation between the amount of water soluble protein and the flavor score.

## DISCUSSION

The data show that pure Lactobacillus bulgaricus starter does not give satisfactory acid production during the manufacturing process of cheddar cheese. The possible explanation for this is that the setting temperature of cheddar cheese is from 86°F. to 88°F. which is below the optimum growth temperature of Lactobacillus bulgaricus. The milling acidity of the cheese made with pure Lactobacillus bulgaricus starter was too low for commercial cheese manufacture. A low milling acidity is undesirable in cheese because of a development of an acidy flavor during curing as well as the fact that the pH is not low enough to inhibit flavor and body damaging organisms which might be present in the cheese.

An improvement in acid development during the manufacturing process was experienced when Lactobacillus bulgaricus starter and commercial cheese starter were combined in the cheese milk, however, acid development in these cheeses did not become normal until a combination of 70% Lactobacillus bulgaricus and 30% commercial cheese starter was used. It is believed that this improvement in acid development was due principally to the presence of the commercial cheese starter.

Lactobacillus bulgaricus starter alone or in combination with commercial cheese starter produced cheese which scored lower in flavor than the control cheese made with commercial cheese starter exclusively, except in one case. The main criticism common to all of the cheese made in which the starter contained Lactobacillus bulgaricus was heated. This flavor was very similar to the flavor produced by the Lactobacillus bulgaricus starter propagated in sterile milk. It is believed that the heated flavor noted in the

cheese is produced normally by the growth of Lactobacillus bulgaricus organisms in milk and its products, if the organisms are in abundance.

All of the cheeses except the lots made with pure Lactobacillus bulgaricus starter were low in moisture content as compared to the desired moisture content of commercial cheese. This was probably due to the cheese being made in small quantities and in small vats in which the drainage of whey during cheddaring is more complete than in commercial manufacture. The reason for the high moisture content of the cheese made with pure Lactobacillus bulgaricus starter was probably due to poor whey expulsion during cheddaring. Acid development aids in expulsion of the whey during this period and in these cheeses it was less than normal which would cause more moisture to be retained in the curd.

The water soluble protein content of the cheeses did not coincide with the amount of Lactobacillus bulgaricus starter used in making the cheese. It has been thought that the proteolysis in cheese was due principally to the action of lactobacilli. Assuming this to be correct, it might be considered, theoretically, that the more lactobacilli present the more extensive would be the proteolysis. This was not substantiated by the results obtained in this work. However, it was found that of the 15 lots of cheese made with starters containing Lactobacillus organisms, only three lots had less water soluble protein than the control cheese made with commercial cheese starter. This indicates that the proteolysis was probably increased in the cheese by the additions of Lactobacillus bulgaricus in the starter. It should be noted,

however, that the majority of investigators consider the Lactobacillus organisms most commonly found in cheddar cheese to be *Lactobacillus casei* or some other lactobacilli than *Lactobacillus bulgaricus*.

# CONCLUSIONS

1. Pure Lactobacillus bulgaricus starters give unsatisfactory acid production during the manufacturing process of cheddar cheese.
2. When Lactobacillus bulgaricus and commercial cheese starter are combined in the cheese milk, combinations containing 30% to 60% commercial cheese starter produce acid satisfactorily during the manufacturing process.
3. Pure Lactobacillus bulgaricus starter or combinations of Lactobacillus bulgaricus and commercial cheese starter produce cheese scoring lower in flavor than cheese made with commercial cheese starter only.
4. A true cheddar cheese flavor is not produced in cheese made with starter containing Lactobacillus bulgaricus organisms.
5. A heated flavor is produced in the cheese when starter containing Lactobacillus bulgaricus organisms is used.
6. A crumbly body is produced in cheese when starter containing Lactobacillus bulgaricus organisms is used.
7. There is no direct correlation between the water soluble protein content of cheese made with starter containing Lactobacillus bulgaricus organisms and the amount of Lactobacillus bulgaricus starter used.
8. The water soluble protein content of cheese does not affect the score of the cheese directly in proportion to the amount present.
9. It is further concluded that the manufacture of cheddar cheese using Lactobacillus bulgaricus as the starter or part of the starter is commercially impractical as no appreciable benefit would be derived from it.

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# Appendix I

SUMMARY of PERTINENT DETAILED MANUFACTURING DATA

| | LOT NUMBER | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Pounds of milk | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 210 | 220 | 220 | 220 | 190 | 210 | 220 | 220 |
| Fat test | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.6 | 3.7 | 4.1 | 3.6 | 3.8 | 3.6 | 3.7 |
| Original acidity of milk | .15 | .15 | .15 | .14 | .14 | .15 | .15 | .15 | .15 | .18 | .15 | .16 | .18 | .16 | .15 | .16 |
| Per cent commercial cheese starter added | 0 | 0 | 0 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 | 0.45 | 0.50 | 0.55 | 0.60 | 1.0 |
| Per cent Lacto-bacillus bulgaricus added | 1.0 | 2.0 | 1.0 | 0.95 | 0.90 | 0.85 | 0.80 | 0.75 | 0.70 | 0.65 | 0.60 | 0.55 | 0.50 | 0.45 | 0.40 | 0 |
| Per cent total starter added | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Acidity of milk in vat | .15 | .15 | .15 | .14 | .14 | .15 | .15 | .15 | .15 | .17 | .15 | .16 | .17 | .16 | .15 | .16 |
| Acidity after adding starter | .16 | .17 | .17 | .16 | .15 | .16 | .16 | .17 | .17 | .18 | .16 | .17 | .185 | .165 | .16 | .17 |
| Adding coagulant | .17 | .18 | .18 | .16 | .16 | .16 | .17 | .18 | .18 | .18 | .165 | .17 | .185 | .18 | .17 | .17 |

SUMMARY of PRODUCTION DETAILED MANUFACTURING DATA
(Continued)

| | 1 | 2 | 3 | 4 | 5 | Lot No. 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cutting | .10 | .10 | .10 | .10 | .10 | .09 | .10 | .10 | .10 | .11 | .10 | .11 | .10 | .11 | .12 | .10 |
| Steam on | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .10 | .11 | .10 | .11 | .10 | .11 | .11 | .10 |
| Steam off | .11 | .10 | .11 | .10 | .10 | .10 | .11 | .10 | .11 | .11 | .12 | .12 | .11 | .13 | .11 | .11 |
| Draining | .11 | .11 | .11 | .11 | .14 | .11 | .11 | .11 | .14 | .14 | .14 | .15 | .145 | .16 | .14 | .13 |
| Pack and cheese | .14 | .11 | .12 | .11 | .15 | .11 | .14 | .13 | .16 | .16 | .17 | .16 | .15 | .20 | .15 | .14 |
| Filling | .20 | .20 | .20 | .19 | .23 | .23 | .27 | .41 | .51 | .50 | .54 | .50 | .51 | .54 | .52 | .50 |

Typists:

Helen Taylor
and
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