Flavour compounds in fermented milks

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Fermented milk foods can be numbered among the most important contributors to human welfare. Lactating ruminants have always had economic importance and these animals were the first to be domesticated. Use of bacteria to induce souring and to enhance flavour of milk has (without the knowledge of the existence of bacteria) been practiced for centuries, but it is only in modern times that soured milks have been investigated and the souring attributed to bacterial growth. In 1890 Storch found that different milk-souring bacteria produced different aromas and flavours and Weigmann in 1896 suggested combining acid-producing bacteria with aroma-producing bacteria for use in ripening cream.[1,2] Fine-flavoured butter with good keeping qualities resulted from cream ripened in this way. This work was later supported by Conn in 1900 who also attached importance to bacterial growth in the development of aroma in cultured products.[3] He concluded that cream ripening involved more than souring, since addition of acid did not accomplish the same results as bacterial growth. He suggested that acid and flavour were the results of different fermentations and that aroma was separate from flavour.

Fermentation of milk is carried out by microorganisms which ferment the milk sugar, lactose, to lactic acid. This compound is responsible for the sharp refreshing taste of all fermented milks and although it is nonvolatile it serves as an excellent background for the more distinctive flavours and aromas characteristic of each fermented milk. These flavours and aromas are, as Weigmann suggested, a consequence of multistrain fermentations.

It is now generally accepted that a selection of a multistrain inoculum is necessary to obtain good flavour and aroma production in a fermented product. Also important is a cultured milk of good consistency and a bacterium producing polymers of protein is often included in the multistrain inoculum to improve the texture—these are known as ropy strains.

Fermented milks were used by the people of Eastern Europe and Asia Minor long before the discovery of bacteria. Although it is often said that fermented milks originated in the Middle East, there is a long tradition of milk fermentation in Asia and Northern Europe. Indeed, today Finland and Sweden are the largest consumers per capita of fermented milks (International Dairy Federation, 1977). There is also a correlation between the flora and the type of cultured milk and the geographic region. The milks can be divided into four types which are dependent on the culture(s) used and each, therefore, has its own characteristic flavour (Table I).

Each type of fermented milk is discussed at some length in the subsequent sections and examples of each are given. Table II lists or-

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Table I. Relationship Between Type of Cultured Milk and Geographical Area

Type	Culture/s used	Region
I	Streptococcal and Leuconostoc species	Norway, Sweden, Finland, Iceland
II	Lactobacillus species	Bulgaria, Japan
III	Streptococcal and Lactobacillus species	Egypt, Iraq, Lebanon, Syria, Turkey, India
IV	Streptococcal and Lactobacillus species plus yeasts	USSR

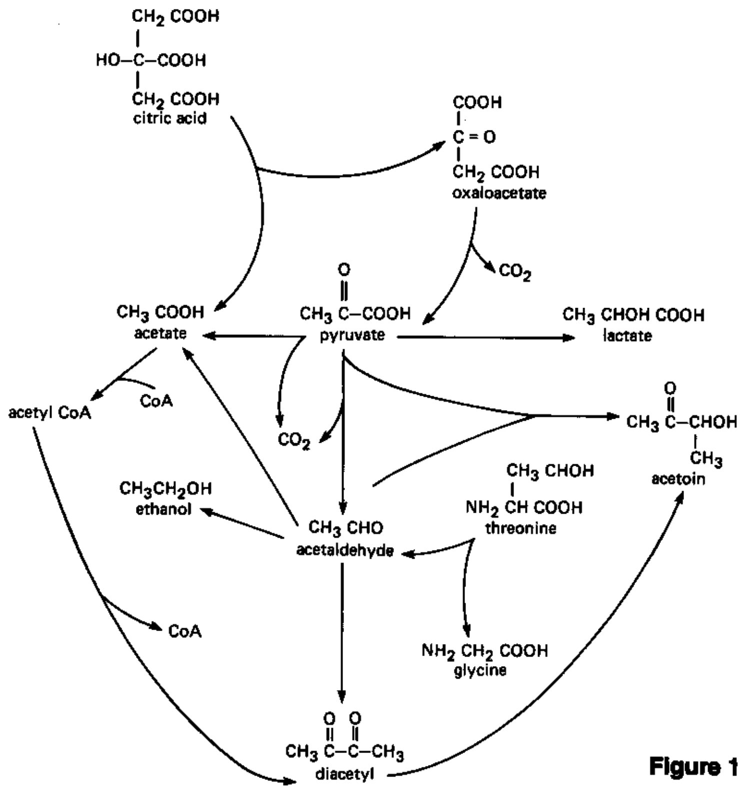

Figure 1

ganisms which are used to ferment milk; also shown are the principal metabolic products which contribute to flavour and aroma. The pathways leading to the production of these flavour compounds are known. Most of them are outlined in figure 1.

Acetaldehyde could be considered at the centre of flavour production as acetoin, diacetyl and ethanol can all come from this compound. Some bacteria have a number of the pathways shown in figure 1, others only one or two. *Lb.acidophilus*, for example, converts pyruvate almost exclusively to lactate whereas *Lb.brevis* produces CO_2, ethanol, acetate, acetoin and diacetyl. *Str.thermophilus* and *Lb.bulgaricus* produce large quantities of acetaldehyde and appear to lack or have low levels of the enzymes leading to its metabolism to other flavour compounds,[4] while *Str.lactis* and *Str. cremoris* accumulate little acetaldehyde and are used mainly for acid production. When these latter organisms are used to ferment milk, the presence of acetaldehyde is considered a defect, whereas its production is obligatory in the manufacture of yoghurt.

Fermented Milks of Type I

• *Buttermilk*. Cultured buttermilk is consumed in quantity in the USA and the Irish Republic. Traditionally buttermilk was the by-product of farmhouse buttermaking after churning cream ripened with *Str. cremoris* or *lactis* plus a species of *Leuconostoc: Leuconostoc cremoris* and/or *Leuconostoc dextranicum* (these used to be known as *L. citrovorum* and *L. paracitrovorum*).

Owing to variations in the churning process and differences in the quality of cream during the year, a uniformly high quality genuine buttermilk was not always available. To supply the demand for the beverage, cultured buttermilk became available. This is made from skim milk. Pasteurised skim milk is inoculated with *Str.cremoris* and/or *Str.lactis* and incubated at 22°C to an acidity of 0.7-0.9% lactic acid (pH 4.6-4.5). The flavour is sharp and "buttery." The buttery flavour is due to diacetyl production and is the principal aroma compound of cultured buttermilk. Diacetyl is derived from citric acid via pyruvate and acetyl CoA but this component of milk shows quite a seasonal variation.[5]

Fresh milk from cows on pasture land contains approximately 0.17% citrate but this declines during winter feeding. Thus fortification of milk with citric acid or sodium citrate (0.1%) will improve flavour. Another type of bacterium, the Leuconostocs, will produce diacetyl from citrate, but citrate cannot be utilised by these bacteria until the pH of the milk falls to below pH 5.0, so utilisation of citrate, when these organisms are included in the inoculum, only occurs as a result of associative growth between the two types of bacteria. *Str.lactis* subsp. *diacetylactis*, however, grows better in milk than does the Leuconostoc and, as its name suggests, produces high concentrations of diacetyl (up to 5 ppm); it therefore can be used to produce both acid and aroma. This organism unfortunately produces acetaldehyde, an undesirable flavour in buttermilk (described as a "green", off-flavour). Leuconostocs do not have this disadvantage, indeed they scavenge acetaldehyde.

It is clear that the flavour of cultured buttermilk differs depending on the selection of the organisms for the multistrain inoculum. Microbiological properties of milks are not constant. Quite marked differences are found, not only between countries, but also between products prepared in the same country!

• *Scandinavian Fermented Milks*. Some of these milks are named *filmjölk, lättfil* and *langfil*. Sales of cultured milk products in Sweden are rising by about 5% annually. Per capita consumption in 1976-7 was 21 kg, the second highest in the world. (Finland is top of the league table with consumption approaching 35 kg.) The largest sales of Swedish cultured milks were of *filmjölk*, taking 68% of the market.[6] Most of this is consumed at breakfast where it is used in place of fresh milk on cereals. *Filmjölk* contains 3% fat and *lättfil* only 0.5%. Both are fermented with multiple strains of bacteria which include *Str.cremoris* and/or *lactis* and *Str.lactis* subsp. *diacetylacis*. Leuconostocs are often added to improve flavour. *Filmjölk* with its 5% fat has a sharp, creamy, full flavour. Acetaldehyde and diacetyl both contribute to the aroma and the pH is around 4.6. *Lättfil* with only 0.5% is not so "full" and the texture is not so consistent. Both products are pourable.

Langfil is also made with a multistrain inoculum of Streptococcus including a strain which produces ropiness. Before becoming commercially available *langfil* was made locally on farms and tradition has it that the inoculum was enriched by rubbing the interior of milk pails with leaves of the butterwort plant (*Pinguicula vulgaris*). This, it was thought, introduced to the milk a slime-producing bacterium whose general habitat was the leaf of this bog weed. Indeed it has been shown that a slime-producing bacterium (*alcaligenes viscosum*) is associated with this plant although it is not used today to produce the Swedish long milk.[7] (In passing it is interesting to note that in Old English folklore milk from a cow that has eaten *Pinguicula vulgaris* could protect a new-born child.)

Commercially, *langfil* is made using *Str.cremoris*, a ropy variant of *Str.lactis* and *Str.lactis* subsp. *Diacetylactis*. The milk is incubated at 18-20°C for 24 h and the final pH is about 4.4. Leuconostocs also may be included in the inoculum to increase the diacetyl flavour. The product is sour and extremely ropy. It is because of this ropiness that the product is fermented in the carton in which it is sold: it cannot be piped. Although Leuconostocs may be present, the flavour is flat in comparison with that of *filmjölk*.

• *Ymer* (Danish equivalent of *filmjölk*). It contains 3% fat, 11% solids-not-fat, has a pH 4.4-4.6 and is made using *Str.cremoris* and *Str. lactis* subsp. *diacetylactis*.[8] The flavour is full, creamy, sharp and buttery. *Ymer* is also becoming popular in Iceland where there are 17 dairies producing butter and cheese.[9] There is considerable milk surplus in summer so these dairies are now turning the excess into *ymer*. This product may also be thickened by allowing the whey to drain off and adding cream to 5%. It is a new product in Sweden and not widely known. *Laktofil* is its suggested name.[10]

• *Skyr* (traditional fermented milk of Iceland dating before the tenth century). Ewe's milk was used and rennet added with the culture.[11] The whey was drained off in linen bags and the flavour was more like that of cottage cheese.

• *Villi* (Vilia). This fermented milk is from Finland, and the multistrain inoculum is similar to that used for Swedish *filmjölk*, but a mould *Geotrichum candidum* is included. Incubation is carried out as for *filmjölk* until the lactic acid reaches 0.9%. The mould is said to be of great importance as it promotes flavour formation by preventing autoxidation.[12] This may be disputed, however, as it is known that diacetyl formation is stimulated when aeration is increased.[13]

Fermented Milks of Type II

• *Acidophilus milk* (well known in the USA). Claims about its therapeutic value are outside the scope of this article. *Lb.acidophilus* isolated from the intestinal tract grows slowly in milk, multiplying only five-fold in 18-24 h. The flavour of the product is poor. Sweet *acidophilus milk* is the discovery of Dr. Marvin Speck; a culture of *Lb.acidophilus* is simply stirred into milk, chilled and sold immediately so that the flavour is not changed. This milk, therefore, is not a fermented product.

• *Bulgarian buttermilk* (popular only in Bulgaria). *Lb.bulgaricus* is used to ferment pasteurised whole milk. The product is highly acid and has a flavour and aroma similar to that of *yoghurt*, due to acetaldehyde accumulation. There is no diacetyl production and the flavour is very sharp and clean.

• *Yakult*. A milk fermented in Japan using a special variant of *Lb.casei* and delivered door to door by some 50,000 saleswomen on bicycles.[12] Skim milk plus sugar, or starch syrup is fermented to the desired acidity and flavoured with fruit and essences. The culturing produces minor constituents of citric, succinic, malic and acetic acids and acetaldehyde, diacetyl and acetone.[14]

Fermented Milks of Type III

• *Yoghurt* (probably the most well known and most widely used of all the fermented milks). Originating in the Middle East it is known under names such as *yahourth, mast, zabady, matzoon, katyk*. Early investigations into the original *yoghurt* flora reported controversial data which may be explained by the infancy of the science of microbiology at that time. *Yoghurt* samples from original sources showed the presence of various lactic acid bacteria, yeasts and moulds in addition to the predominant proportions of *Str.thermophilus* and *Lb.bulgaricus*.

Yoghurt has become the best studied of the fermented milks and is now almost always made commercially using only the two organisms *Str.thermophilus* and *Lb.bulgaricus*. These have a symbiotic relationship in which the streptococcus supplies formate to stimulate the lactobacillus. This in turn breaks down casein to small, manageable peptides for the streptococcus. Both organisms like higher temperatures in which to grow (40-45°C).

Yoghurt has a sharp refreshing acid taste; the pH is usually between 4.0 and 4.4. The symbiotic relationship makes the flavour quite unlike that encountered in any other dairy food. Acetaldehyde, diacetyl, acetoin, acetone and butan-2-one are all present in *yoghurt*, but acetaldehyde is recognised as the principal flavour component.[15-17]

The flavour has been described as similar to that of walnuts.[18] Optimum flavour and aroma is obtained at acetaldehyde values between 23 and 41 ppm. The rate of its production is highly dependent on acidity level, beginning at pH 5.0 and rapidly increasing as the pH drops to 4.4.[19] Diacetyl and acetoin result from metabolic activity of *Str.thermophilus* and are very low, rarely reaching 0.5 ppm. Its presence contributes to the delicate and full flavour and aroma and is especially important if acetaldehyde is low because it can enhance the *yoghurt* flavour.[20] Acetone and butan-2-one are of limited importance and usually originate from the milk itself. Volatile fatty acids increase during fermentation: these include acetic, formic, caproic, caprylic, butyric and propionic acids. They are also present in milk but their proportions change.[21,21]

Physically, *yoghurt* is produced as a smooth viscous liquid or a soft curd. Lumpiness and graininess are undesirable as are whey pockets in the coagulum. Texture influences the organoleptic qualities and contributes to appreciation of both flavour and aroma in the mouth.

• *Dahi* (Indian equivalent of *yoghurt*). It is not made commercially and therefore its microbiology is extremely variable. It follows that taste and appearance are also variable. Made in mudpots from cow or buffalo milk, the bacteria used are *Lb.bulgaricus* and/or *plantarum* and *Str.thermophilus* and/or *Str.lactis*. The inoculum is much higher than is usual (approximately 20%) and incubation is for 1-3 h only. As a consequence the pH is only 5.5. In addition *dahi* is normally sweetened. About 12% sugar is added before the fermentation. Total solids can therefore reach 30% and sweetness is the overriding taste.[23]

Fermented Milks of Type IV

• *Kefir.* (the most famous of the truly alcoholic fermented milks). *Kefir* is generally reported to have come from the Caucasus mountains and is consumed at a rate of 4.5 kg/cap/yr in the USSR. Fermentation is started by addition of *kefir* grains. These grains, which resemble small cauliflower heads when wet and brownish seeds when dry, are particles of clotted milk plus the *kefir* organisms: *Lb.brevis* (earlier known as *Lb. caucasicus), Str.lactis* and a number of lactose fermenting yeasts (Table II).

Production of *kefir* involves two fermentation steps: first, fermentation of lactose to lactic acid by the bacteria during an incubation at 18-20°C for 20 h; second, fermentation of lactose to alcohol and CO_2 by the yeasts. This latter fermentation proceeds at much lower temperatures, often as low as 6-8°C for 6 days.[24]

After ripening a good *kefir* foams like beer, has a creamy consistency and a refreshing acidic taste due to the lactic acid and acetic acid present. The pH is 4.3-4.4, acetaldehyde is at 1-2 ppm and diacetyl at approximately 3 ppm. Alcohol from yeast fermentation is very variable, but rarely exceeds 0.5-1%. A yeasty flavour is as undesirable as overacidification. A great deal of work has recently centered upon improving the consistency (XX International Dairy Congress Communications 840-844).

• *Laban.* The organisms which ferment milk to *laban,* the popular alcoholic fermented milk of Lebanon, have been investigated by Baroudi and Collins.[25] Five organisms have been identified: *Str.thermophilus* and *Lb.bulgaricus* were responsible for acid production (the pH = 4.25), *Kluyveromyces fragilis* produced the majority of the acetaldehyde (4.2 ppm), *Saccharomyces cerevisiae* produced the alcohol (1.25%), and *Str.thermophilus* the acetoin (34 ppm). *Leuconostoc lactis,* although present, was considered non-essential for flavour. The aroma compound diacetyl was not detected organoleptically in the finished *laban.* Its flavour was said to be tart and slightly yeasty.

• *Koumiss* (a variety of *kefir* made from mares' milk). Its fermentation is carried out using *Lb.bulgaricus* and a Torula yeast. There

Table II. Microorganisms Used in Milk Fermentations and Their Metabolic Products (All ferment lactose to lactic acid)

Organism	Metabolic products
Str.cremoris	mainly lactic acid
Str.lactis	mainly lactic acid
Str.lactis subsp. diacetylactis	diacetyl, CO_2, acetaldehyde, acetoin
Str.thermophilus	acetyladehyde, (diacetyl, acetoin)
Lb.bulgaricus	acetaldehyde
Lb.casei	mainly lactic acid
Lb.acidophilus	mainly lactic acid
Lb.plantarum	mainly lactic acid
Lb.brevis	CO_2, ethanol, acetic acid, acetoin, diacetyl
L.dextranicum	diacetyl, acetoin
L.cremoris	diacetyl, acetoin
L.lactis	mainly lactic acid
Saccharomyces cerevisiae	all produce CO_2, ethanol
Saccharomyces kefir	
Kluyveromyces fragilis	
Kluyveromyces lactis	
Torula kefir	
Candida kefir	
Bettanomyces anomalus	

are more than 150 horse farms in Russia which supply the milk for making 420,000 kg *koumiss* per year. Each mare is hand-milked four times daily. After fermentation the product is a white liquid with a greyish cast and there is no tendency to "whey off."[18] This uniformity may be due to the higher whey protein content of mares' milk (1.2 g/100 g compared with 0.7 g/100 g for cows' milk). Mares' milk also has higher lactose (6.5%), consequently larger quantities of alcohol are produced. It may have up to 3% ethanol. A further refinement is the distillation of *koumiss* to produce a highly intoxicating beverage. Mongolian *koumiss* is milder and has been described as "prickling" due to high CO_2 content.[12]

Table III. The Different Composition of Milks

Animal	Total Solids	Fat	Lactose
Cow	12.79	3.85	4.72
Mare	11.10	1.90	6.20
Goat	12.95	3.93	4.65
Buffalo	17.76	7.96	4.86
Yak	17.80	6.50	4.60
Ewe	18.66	6.80	4.91
Reindeer	34.60	10.90	2.80

Other Lesser-Known Fermented Milks

Leben is a *yoghurt*-type product, prepared from the milk of the cow, goat, sheep or buffalo, which has been known in Egypt for centuries. It is apparently drunk diluted 2-3 times with water and 0.1% salt added.[12] *Urda* and *skuta* are wheys in which an alcoholic fermentation has been induced. They are prepared from sheep's milk. *Kaeldermaelk* (cellar milk) is made in Norway again using an alcoholic fermentation of whole milk.

Flavour Differences in Milks

Table III shows the different composition of milks of several different species, all of which can be fermented. Fat content makes a large difference to both texture and flavour, although rancidity can be a problem. There is a very marked difference in the flavour of *yoghurt* made with cows' milk and *yoghurt* made with goats' milk due to lack of acetaldehyde in the latter. Goats' milk may be oxidising and acetaldehyde may be converted to acetate, or bisulphite ions may be present which form an addition compound with acetaldehyde thus preventing development of full *yoghurt* flavour. Milks with high total solids may be less acid due to the higher buffering capacity of the milk proteins.

Milks can also bring off-flavours to the finished products. A bitter taste is present if colostrum is fermented, traces of metal will produce a tallow taste, and rancidity occurs during bull-rut.[26] Lack of flavour can only be ascribed to changes in the metabolism of the organisms. The natural flavour of soured skim milk may be improved by addition of citrates, cream, lecithin and sodium chloride.

Natural *yoghurt* occupies a small place in the USA and UK marketplace. The greatest sales are of fruit and flavoured *yoghurts* which are consumed as desserts or between-meal snacks. *Quality of fruit and flavoured yoghurts* has been reviewed by Brown and a digest of publications has been published by Mann.[27,28]

We tend to think that addition of fruits and flavours to fermented milks is a new innovation. It is not. Fermented milks have been used to augment foods from the beginning. In Persia, herbs and spices are added to the *yoghurt* and the mix dried. In this form the *yoghurt*, known as *kashk*, resembles cobbles or stones which are grated to flavour soups and stews. In this way fermented milk is rendered into an easily carried, pleasantly flavoured, high quality protein package, providing first class food to the countless nomads and remote villages in the area. *Yoghurt* may also be mixed with cereals and dried, so providing an almost complete food. This is done by the Syrians and in Lebanon a blend of *yoghurt* with wheat flour is known as *kishk*.

Modern Methods for the Assessment of Quality

Asperger related titratable acidity, acetaldehyde and tyrosine equivalents to results of flavour assessment in official Austrian tests of 165 yoghurt samples in 1972.[29] Low acidity values generally resulted in assessments such as "mild" or "insipid" and high values such as "acid" or "too acid." Optimum flavour was about 0.9% lactic acid. This is difficult to equate with pH unless the total solids are known. It would probably correspond to pH 4.3 if total solids were about 13%.[30] Acetaldehyde of less than 10 ppm was associated with "little or no yoghurt flavour" and according to Görner, Palo and Bertan optimum values ranged between 23 and 41 ppm.[17] Tyrosine content had little correlation with flavour. More than 100 μg/ml tended to have a slightly bitter assessment.

Microbiological analysis can now ensure a product of uniform quality. Cultures can be dried with nutrients and energisers and distributed commercially. More recently cultures are available in a frozen state for direct addition to vats. This means that should anything go wrong with the fermentation a new culture can be bought to replace the weak or contaminated one. Biochemical studies of the fermentations has led to elucidation of the pathways leading to flavour production and so to improvements, e.g., addition of citrate to encourage diacetyl production.

The future of fermented milks and their cultures lies in genetic studies with further improvement of flavour production and strain vitality. There is no doubt that food shortages on a global basis will persist and the fermented milk industries of all developed countries will be asked to bring their expertise to the aid of those countries where the tradition of fermentation is strong but in dire need of technological advance.

References

1. W. Storch, Milch-Zeit **19,** 304 (1890); cited by Sandine et al. J. Milk and Food Technol. **35,** 176 (1972)
2. H. Weigmann, Cent. fur Bakteriol (II) **2,** 150 (1896)
3. H. W. Conn, Ann. Rep. of the Storrs School (1900); cited by Sandine et al. J. Milk and Food Technol. **35,** 176 (1972)
4. G. J. Less and G. R. Jago, J. Dairy Res. **43,** 63 (1976)
5. F. Z. Hegazi and I. G. Abo-Elnaga, Zeit. Lebensmittel unter. Forschung **171,** 367 (1980)
6. E. Bertelsen and U. Jonsson, Svenska Mejeritid **68,** 9 (1976)
7. R. Nilsson and G. Nilsson, Archiv. fur Mikrobiol. **31,** 191 (1958)
8. A-G.O. Delaney, Milchwissenschaft **32,** 651 (1977)
9. S. Magnusson, Dairy Sci. Abstracts **40,** 3448 (1978)
10. F. V. Kosikowski, Cultured Dairy Products J. **13,** 5 (1978)
11. G. Simonarson, Dairy Sci. Abstracts **40,** 7082 (1978)
12. F. Lang, Milk Industries **82,** 22 (1980)
13. E. B. Collins, J. Dairy Sci. **55,** 1022 (1972)
14. L. J. Manus, Cultured Dairy Products J. **14,** 11 (1979)
15. J. W. Pette and H. Lolkema, Neth. Milk and Dairy J. **4,** 261 (1950)
16. M. E. Schulz and G. Hingst, Milchwissenschaft **9,** 330 (1954)
17. F. Görner, V. Palo and M. Bertan, Milchwissenschaft **23,** 94 (1968)
18. F. V. Kosikowski, in Cheese and Fermented Milk Foods. Edwards Bros Inc., Ann Arbor, Michigan (1977)
19. V. Bottazzi, B. Battistotti and A. Montescani, Le Lait **53,** 295 (1973)
20. M. Groux, Le Lait **53,** 146 (1973)
21. M. S. Kondratenko and B. Gulosheva, XX Intl. Dairy Congress **E,** 297 (1978)
22. M. Turcić, J. Rasić and V. Canić, Milchwissenschaft **24,** 277 (1969)
23. D. C. Bhattacharya, D. Raj and B. Tiwari, Ind. J. Dairy Sci. **33,** 38 (1980)
24. E. J. Mann, Dairy Ind. Intl. **44,** 39 (1979)
25. A.A.G. Baroudi and E. B. Collins, J. Dairy Sci. **59,** 200 (1975)
26. J. L. Rasić and J. A. Kurmann, in Yoghurt. Technical Publishing House, Copenhagen, Denmark (1978)
27. L. D. Brown, Cultured Dairy Products J. **14,** 7 (1979)
28. E. J. Mann, Dairy Ind. Intl. **41,** 128 (1976)
29. H. Asperger, Osterreichische Milchwirtschaft **28,** 125 (1973)
30. V. M. Marshall, W. M. Cole and L. A. Mabbitt, J. Soc. Dairy Technol. **33,** 129 (1980)