DAIRY FOODS

Role of Micrococcus and Pediococcus Species in Cheese Ripening: A Review

TARUN BHOWMIK and ELMER H. MARTH
Department of Food Science
and
The Food Research Institute
University of Wisconsin
Madison 53706

INTRODUCTION

The cheese ripening process is long, complicated, and costly. The characteristic body, texture, and flavor develop during the ripening process through various chemical changes such as breakdown of fat, protein, and carbohydrate. Several enzymes derived from the lactic starter culture and from the secondary microflora of cheese are responsible for those chemical changes. Proteolysis is important during cheese ripening and it helps to give desired body and texture, short-chain peptides, and amino acids that contribute to flavor development (also off-flavor, i.e., bitterness) and nutrients for growth of the cheese microflora. Protein breakdown products also serve as substrates for production of sapid compounds. Proteolysis occurs through action of cheese microflora, coagulants, and natural milk proteases. Several approaches, including addition of adjunct starter cultures or exogenous enzymes or using modified manufacturing conditions to reduce the ripening time through accelerated proteolysis have been suggested (4, 47, 72, 78).

During ripening, limited lipolysis also occurs, depending on type of cheese, and contributes to development of flavor. In Cheddar cheese, lipolysis results through action of lipases from the starter culture, the secondary flora, and naturally present milk lipases. Several reviews (30, 46) are available on accelerated ripening of cheese with major emphasis on starter lactic acid bacteria and on nonstarter lactobacilli either added or present as adventitious bacteria. However, only limited reports (45, 52) deal with the importance of the secondary flora of cheese during its maturation. This paper discusses the occurrence and significance of micrococci and pediococci, their role in cheese ripening, and their possible use for accelerated ripening of cheese.

MICROCOCCI

Description of Micrococci

The genus Micrococcus is taxonomically in the family Micrococcaceae, which also includes the genera Staphylococcus and Planococcus (76). The bacteria are gram-positive, catalase-positive, spherical, and occur as regular or irregular clusters or packets. There are several discrepancies in differentiation between Micrococcus and Staphylococcus, and the nomenclature is being changed. Because staphylococci...
are important food-poisoning organisms it is necessary to characterize a culture carefully and to confirm it as *Micrococcus* sp. before using it in dairy or other food processing. In addition to determining DNA homology, several rapid and reliable biochemical tests such as growth in thioglycolate medium (27), sensitivity to erythromycin and lysostaphin (28, 77), and other physiological and biochemical characteristics (5) have been suggested to differentiate between staphylococci and micrococci.

**Occurrence of Micrococci in Milk and Cheese**

Micrococci enter milk from such sources as the udder of cows, dairy utensils, milking machines, air, and dust. Several reports indicate that the primary source of micrococci in milk and cheese is the udder of animals. Micrococci predominate in the microflora of raw milk drawn aseptically from the udder (1, 38, 43, 44). It has been estimated that, with some seasonal variation, micrococci account for 67 to 77% of the total bacteria in raw milk produced under sanitary conditions (43). Occurrence of micrococci in ewe's milk also has been reported (7).

Although micrococci occur in large numbers in raw milk and cheese made from raw milk, their occurrence in pasteurized milk and cheese made from pasteurized milk also has been reported. In the past, attention has been given to thermal resistance of micrococci because their presence in large numbers in pasteurized milk has been related to the keeping and bacteriological quality of the milk. There are some contradictory reports regarding thermal resistance and occurrence of micrococci in pasteurized milk. Myhr and Olson (60) reported complete destruction of micrococci by laboratory pasteurization [61.7°C (143°F) for 30 min] although micrococci predominated in the raw milk flora before heat treatment. These authors (60) also observed that only 5 of 30 cultures of micrococci survived pasteurization following subculture in milk. However, it has been reported that certain micrococci are thermolabile in nature and survive pasteurization although this characteristic is variable even in the same isolates (36, 61, 79, 80).

As just mentioned, micrococci constitute a major portion of the secondary flora of Cheddar cheese made from raw or pasteurized milk. Feagan et al. (29) noted that micrococci comprised from 16 to 68% of the nonstarter population in cheese. Alford and Frazier (2) found $6 \times 10^5$ to $10 \times 10^6$ nonlactic organisms/g of cheese made from pasteurized milk. Those authors (2) also isolated large numbers of micrococci from cheese made from raw as well as from pasteurized milk and indicated that these organisms, because of their lipolytic activity, are important for flavor development in cheese. They also reported that certain micrococci such as *Micrococcus freudenreichii* can grow at pH below 5.5 in the presence of *Streptococcus lactis* (*Lactococcus lactis* ssp. *lactis*) and may be important for cheese quality if added to pasteurized cheese milk. Evans et al. (26) observed the occurrence of large numbers of micrococci in bitter cheese and related the defect to presence of the bacteria. Occurrence of micrococci in cheese also has been reported by other investigators (14, 34, 39, 41, 68). Micrococci in considerable numbers have been found in different types of soft cheese as well as some hard cheeses made from sheep milk; it has been suggested that micrococci contribute to the ripening process through lipolytic and proteolytic activities (23, 53, 71, 85).

**Significance and Use of Micrococci in Cheese Ripening**

It has already been established that micrococci occur in cheese made from raw or pasteurized milk. However, there are several contradictory reports regarding their use and their possible role during the cheese ripening process. Alford and Frazier (3) reported improvement of flavor of Cheddar cheese by addition of a strain of *M. freudenreichii* isolated from raw milk cheese. Those investigators (3) inoculated cheese milk with $1 \times 10^6$ of the bacterium/ml and observed an increase of up to $2 \times 10^6$/g of cheese by the time of pressing. They (3) also suggested that micrococci are important for rapid development of flavor in cheese made from pasteurized milk. Harris and Hammer (37) noted improvement of Cheddar cheese made from pasteurized milk fortified with certain strains of micrococci, and they observed that other strains had no effect or reduced flavor quality. Thus, they suggested that micrococci may be important in cheese maturation but the proper strain should be selected for use.
Deane and Anderson (20) improved the flavor score of Cheddar cheese from .5 to 2.0 points over control cheese at 3 wk of age by using a starter culture supplemented with a strain of *Micrococcus*. Robertson and Perry (75) reported an improvement in flavor of Cheddar cheese made from milk fortified with additional milk that contained micrococci and that was incubated for 4 d before use. However, Deane (19) also reported that two unidentified acidoproteolytic micrococci had no effect on flavor development or protein breakdown although the micrococci were proteolytic in nature. Several patents have been issued on use of such micrococci as *Micrococcus caseolyticus*, *M. freudenreichii*, *M. conglomeratus*, and *M. cohn* for rapid production of intense flavor in cheese and cheese-related products (42, 51, 74). Bitterness in Edam cheese made with *M. caseolyticus* has been reported (66), but *M. caseolyticus* did not affect the increase of NPN during ripening of Edam cheese (67). It has been suggested that micrococci exhibit their effect through proteolytic, lipolytic, and esterolytic activities as well as through their ability to produce methanethiol in cheese during the ripening process. The following sections will describe the different enzyme systems in micrococci as well as their possible role in the cheese ripening process.

**Proteolytic Enzymes of Micrococci**

Several investigators have found that certain species of micrococci can produce extracellular proteolytic enzymes. McDonald (55) noted that about 90% of the proteinase appeared in culture supernatant liquid when cells of *M. freudenreichii* ATCC 407 grew in the presence of 2% sodium chloride. He (55) also observed that proteinase can be bound to the cell membrane or wall and very little appeared in the cytoplasmic content of cells. Extracellular proteinase also has been described for *M. caseolyticus* (22), *Micrococcus sodonensis* (57), *Micrococcus spp.* MCC 315 (69), and *Micrococcus lysodeikticus* (35). Membrane-associated proteinase also has been reported for *M. lysodeikticus* (73). There is no convincing report that demonstrates secretion of extracellular proteinase by micrococci in cheese during the ripening process. It is noteworthy that all purified extracellular proteinases from different strains of micrococci had optimal activity in an alkaline pH range and lost activity markedly in a lower pH range of 5.0 to 5.5 (22, 40, 69, 73). However, Paird et al. (64) reported increased proteolysis using encapsulated proteinase from *M. caseolyticus* in Saint Paulin cheese. Several reports (21, 54, 56, 70, 87) are available regarding the nutrient requirements for production of extracellular proteolytic enzymes.

Barbo and Foster (6) found the intracellular proteinase from *M. freudenreichii* 325 was most active at 30°C at a near neutral pH and suggested it contributed to proteolysis during ripening of cheese. Nath and Ledford (62) reported the preferential degradation of αs-casein by extracellular proteinases from certain strains of micrococci, whereas β-casein was preferentially degraded by extracts containing intracellular components. Reports (58, 59) also are available regarding degradation of β-casein into short peptides and amino acids by micrococcal cell-free extracts. Hence, it was suggested that micrococci produce flavorful compounds during cheese maturation. Intracellular proteinase, endopeptidase, aminopeptidase, and dipeptidase activity occurs in cells of different strains of micrococci (8). The same authors (8) also reported preferential degradation of β-casein during growth of micrococci in skim milk and also by proteinases in cell-free extracts of micrococci. Bhowmik and Marth (9) described characteristics of an aminopeptidase purified from *M. freudenreichii* ATCC 407 and observed its broad substrate specificity and its sensitivity to sodium chloride and pH of the environment.

**Lipolytic Enzymes of Micrococci**

It is generally though that lipase from micrococci is important during cheese ripening and contributes to flavor development in the product. Peterson and Johnson (65) observed lipolytic activity of *M. caseolyticus*, *M. freudenreichii*, and *M. conglomeratus* and concluded that upon autolysis of cells, liberated intracellular lipase was active at pH 5.0 to 6.0 and took part in the cheese ripening process. Extracellular lipase from *M. freudenreichii* NCDO 1223 has been partially purified and characterized by Lawrence et al. (49). The enzyme preparation...
was most active at pH 8.0 to 8.5 (49). Stadhouders and Mulder (81) observed that certain strains of micrococci hydrolyzed milk fat in cream under laboratory conditions but the same strains failed to hydrolyze fat in cheese. These authors (81) concluded that lipase from micrococci is not responsible for fat hydrolysis in cheese, which probably is caused by milk lipase.

Esterolytic Enzymes of Micrococci

In addition to lipolytic enzymes, esterases are important for liberation of short-chain fatty acids, which contribute to flavor of ripened cheese. Only a few reports are available regarding esterases of micrococci. Brandl and Zizer (13) noted esterolytic activities of several strains of micrococci and observed a higher rate of hydrolysis of o-nitrophenyl butyrate than of p-nitrophenyl butyrate when both were used as substrates. Lawrence et al. (49) reported esterase activity of *M. freudenreichii* NCDO 1223 was equal against the substrates p-nitrophenyl acetate and o-nitrophenyl butyrate. These authors (49) suggested that lipase and esterase activity in this strain was the result of a single enzyme. Recently, Bhowmik and Marth (12) investigated the esterase activity of five different strains of micrococci and with histochemical staining they identified multiple forms of active esterase bands in all the strains. They (12) observed further that crude esterase in cell-free extracts of *Micrococcus* sp. ATCC 8459 had an optimum pH at 8.0 and was severely inactivated at pH 5.0 and in the presence of 5% sodium chloride.

PEDIOCOCCI

Description of Pediococci

The genus *Pediococcus* is in the family Streptococaceae. Bacteria in this genus occur singly or in tetrads, pairs or short chains, are gram-positive, generally are catalase-negative, are microaerophilic, homofermentative, and produce DL-lactate from carbohydrates (33). This genus consists of seven species as recognized by the International Committee on Systematic Bacteriology: *Pediococcus pentosaceus, Pediococcus acidilactici, Pediococcus dam- nosus, Pediococcus halophiles, Pediococcus parvulus, Pediococcus urinaee-equi*, and the unnamed Gunther and White group III. The species once designated as *Pediococcus cerevisiae* and used as a starter culture especially for meat fermentations has been reclassified as *P. acidilactici* (33).

Occurrence of Pediococci in Milk and Cheese

Presence of pediococci in cheese was first observed by Dacre (17, 18), who reported a gradual decrease in their number during ripening until they constituted about one-fourth of the total bacterial population in 6-mo-old cheese (18). Dacre (18) also claimed that flavor formation in Cheddar cheese was related to development of pediococci rather than lactobacilli. Occurrence of pediococci in Cheddar cheese also has been reported by other investigators (31, 48). Fryer and Sharpe (32) found pediococci to be the dominant nonstarter flora in a series of Cheddar cheeses and identified all of the pediococci as *P. cerevisiae* [no longer valid; see reference (33)]. Elliott and Mulligan (25) observed that pediococci constituted about 1% of the nonstarter flora of young Canadian Cheddar cheese and also identified them as *P. cerevisiae*. Recently, occurrence of pediococci in American Cheddar cheese has been reported by Litopoulou-Tzanetaki et al. (50); the bacteria were found in four of the 17 samples examined.

Significance and Use of Pediococci in Cheese Ripening

Although it has been recognized that pediococci constitute a major fraction of the nonstarter flora, contributions of these bacteria during cheese ripening have not been well-studied. Improvement of Cheddar flavor during the early stage of ripening by addition of a *Pediococcus* species along with starter streptococci (strains HP and K of *S. cremoris*) has been reported by Dacre (16). Development of flavor in cheese through addition of pediococci to cheese milk and thus to cheese also has been reported by other investigators (24, 75). However, Law et al. (48) observed that pediococci had no effect when they were used separately; however, flavor development in cheese occurred when they were combined with other
nonstarter bacteria. The mechanism of flavor development by pediococci is not well-studied. It has been reported that *P. pentosaceus* oxidizes lactose, peptides, and L(+)- and D(−)-lactate (82). Reports (83, 84) also are available that show that *P. pentosaceus* isolated from cheese can produce acetate from lactate and in that way may contribute to flavor development. Production of diacetyl from glucose by pediococci as a possible means of flavor improvement also has been reported (63).

**Enzymes of Pediococci**

There is only one report (63) regarding proteolytic enzymes in pediococci. Tzanetakis and Litopoulos-Tzanetaki (86) used the API ZYM system and found leucine and valine aminopeptidases present in 49 strains of *P. pentosaceus*. They suggested these enzymes may be important during cheese ripening if sufficient numbers of cells are present. They (86) also noted presence of weak lipase or esterase activity in all strains studied together with leucine aminopeptidase, valine aminopeptidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosaminidase. Bhowmik and Marth (10) found intracellular aminopeptidase, protease, dipeptidase, and dipeptidyl aminopeptidase in six strains of *P. pentosaceus* and two of *P. acidilactici*. They (10) also noted that both purified αs1- and β-casein fractions as well as skim milk were hydrolyzed by the crude cell-free extracts of pediococci, but no proteolysis occurred during growth of the bacteria in sterilized skim milk (10). Active esterases have been quantified and identified by histochemical staining in all the strains of *P. pentosaceus*, but no esterase activity was found in any of the strains of *P. acidilactici* examined (11).

**CONCLUSIONS**

It is well-recognized that micrococci and pediococci constitute a major fraction of the nonstarter flora of cheese. Certain strains of micrococci and pediococci have a positive effect on flavor development in cheese, and this may be linked to their proteolytic, peptidolytic, and esterolytic activities as well as to some of their metabolites such as diacetyl and acetate. Micrococci are highly proteolytic but the enzymes are most active at alkaline pH, and their actual contribution to proteolysis during cheese ripening needs to be determined through suitable studies. Similarly, esterase and aminopeptidase of micrococci are very sensitive to low pH and salt. However, long exposure of substrates to enzymes during cheese ripening should be considered. Furthermore, other metabolic effects such as production of methanethiol by micrococci (15) may be important during the cheese ripening process. Acid production is a problem if an adjunct starter culture of lactic streptococci or lactobacilli is added to milk for ultimate accelerated ripening of cheese; this is unlikely to be a problem if micrococci are used.

The role of pediococci in cheese ripening has not been well-studied, although it has received recent attention. Activity by protease, peptidase, and esterase has been observed for different strains of pediococci. Furthermore, the ability of cell-free extracts of pediococci to hydrolyze milk protein has been reported. In addition, acetate and diacetyl production by pediococci may contribute to flavor development in ripening cheese. Poor growth and limited acid production may be added advantages for use of pediococci to accelerate the cheese ripening process. However, production of D(−)-lactate and subsequent formation of calcium lactate, which can produce white crystals (haziness) on cheese during the ripening process may limit use of pediococci (Bhowmik and Marth, unpublished data). Further research is needed to avoid this problem and to improve strains of pediococci for use in cheese production.

**ACKNOWLEDGMENTS**

A contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison. Preparation of this review paper was supported by the W. V. Price Cheese Research Institute of the University of Wisconsin-Madison.

**REFERENCES**


Journal of Dairy Science Vol. 73, No. 4, 1990