ABSTRACT

Several previous reviews have described different ways to enhance the flavor and texture of cheese, including use of live cells and nonviable attenuated cells as adjunct cultures. However, comparisons between viable and nonviable cultures were never discussed in these reviews. In addition, recent publications on adjunct cultures have not been covered in previous reviews. This article will survey the more recent work on adjunct cultures—with particular attention to whether the adjuncts contained viable or nonviable cells—and propose areas where additional research is needed.

(Key words: adjunct lactobacilli, attenuation, autolysis, cheese ripening)

Abbreviation key: LAB = lactic acid bacteria, NSLAB = nonstarter lactic acid bacteria.

INTRODUCTION

At the end of many cheese-making processes, a bland rubbery mass of curd is obtained. It is during ripening that many cheese types develop their characteristic flavors through the gradual breakdown of carbohydrates, fats, and proteins. Several agents are involved in the ripening of cheese, and the impact of the contribution of each of these agents varies according to the type of cheese. The coagulants are principally responsible for the first steps in protein degradation, but play a minor role in small peptide formation (98). Indigenous milk lipases and proteinases play a limited role during ripening; however, plasmin hydrolyzes β-casein (48, 76). This action is more intense in highly cooked cheese varieties (e.g., Swiss), which is probably caused by increased conversion of plasminogen to plasmin under these conditions. This conversion is promoted by the absence of the plasmin inhibitors, which are largely removed with cheese whey or are already denatured during cheese milk pasteurization (36, 97, 98).

It is now well established (28) that in most ripened cheese varieties, enzymes from cheese-related microorganisms and more particularly lactic acid bacteria (LAB) are the major contributors to flavor development through the action of their intracellular enzyme systems. This was clearly demonstrated in studies of aseptic cheese making where microorganism-free cheese developed little flavor (97). These organisms can originate from the milk, or they can be intentionally or unintentionally added during the cheese-making process.

MICROORGANISMS INVOLVED IN CHEESE RIPENING

The microorganisms involved in cheese making and cheese ripening can be divided into two major groups: 1) microorganisms that are added to the cheese milk after being carefully selected by the starter manufacturer or the cheese-making company, and 2) nonstarter lactic acid bacteria (NSLAB).

Group 1 can be further subdivided into two subgroups: the primary starter and the secondary starter. The roles of these two groups have been well documented in several reviews (42, 54). The role of the primary starter culture is to ensure consistent acid development during cheese making. This group is also involved in the degradation of protein and fat during ripening. Cultures in this group also play an important role in the biological protection of the product (e.g., low pH, bacteriocin production). The secondary starter represents cultures that are added to a limited number of cheese varieties to provide well-defined functions, e.g., gas production in Swiss type cheese is ensured by Propionibacterium shermanii ssp. freudenreichii, while Brevibacterium linens is one of the major contributors to surface coloration in surface ripened cheese. Penicillium roqueforti and Penicillium camemberti can also be included in this group. In addition, the secondary
starters, through their diverse enzymatic systems, can be also heavily involved in the ripening process; their contributions are indispensable for the development of the typical flavor of many cheeses, e.g., Roquefort, Emmental, Camembert, and Limburger (54, 87).

Group 2 includes the nonstarter lactic bacteria. This group has been shown to contribute to flavor development in some varieties of cheeses and could therefore be considered a desirable contaminant of either the milk supply or the subsequent cheese. *Lactobacillus* strains are the most common and can be found in relatively high numbers; *L. casei*, *L. paracasei*, *L. plantarum*, and *L. curvatus* are the predominant species. *Pediococci* and enterococci are also members of the group but are usually present in smaller numbers (42).

Micrococci, which are not LAB, can also play a significant role in flavor formation in certain types of cheese (8).

### OCCURRENCE OF NSLAB IN CHEESE

Typically, factory-made Cheddar cheese contains $10^2$ to $10^4$ NSLAB/g after pressing. They reach $10^2$ to $10^6$ CFU/g within about 3 mo (42). However, if high quality raw milk is pasteurized and precautions are taken to avoid contamination from the environment, Cheddar cheese may remain free of detectable lactobacilli for up to 100 d (43).

Different factors may affect the growth of NSLAB in cheese. Lane et al. (65) demonstrated that within the ranges normally found in Cheddar cheese the water and salt content and the pH have little effect on the growth and final number of NSLAB. Ripening temperature was reported to influence the rate of NSLAB development but does not affect their final numbers in the cheese (40). It seems that lactose, AA, and sugars from nucleic acids are not used by NSLAB as energy sources; however, some species can use carbohydrates associated with the milk fat globule membrane for that purpose (42).

### THE ROLE OF NSLAB DURING CHEESE RIPENING

In the late 1960’s, Reiter et al. (88) added groups of NSLAB isolated from raw milk and cheese to experimental cheese or cheese milk made under controlled microbiological conditions. Although NSLAB accelerated Cheddar flavor development, some NSLAB groups developed off flavors.

More recently, Lane and Fox (64) manufactured cheese by chemical acidification (gluco-δ-lactone and lactic acid) as well as biological acidification (*Lactococcus lactis* ssp. cremoris). A mixed culture of six lactobacilli was added to biologically and chemically acidified cheese milk. Cheeses without lactobacilli addition were also made. A significant increase in proteolysis could be measured when the lactobacilli were added to the starter-free cheese. This increase was not as obvious in the cheese made with starter because of the lack of available substrate for the enzymes produced by both types of bacterial cultures. The role of lactobacilli was also studied by Lynch et al. (73, 74) who demonstrated that cheese with added lactobacilli developed a superior flavor when compared to control cheese. The presence of the lactobacilli led to increase levels of small peptides and AA in cheese.

### RATIONALE FOR USING ADJUNCT CULTURES

In recent years, procedures have been developed at the farm and plant levels to produce milk and obtain cheese with the lowest number of microorganisms possible. The introduction of a low-temperature pasteurization process coupled to microfiltration led to the production of almost sterile milk (66, 83). Although these methods provide the consumers with dairy products exhibiting a high degree of microbiological quality and safety, they also lead to the disappearance of or at least to a dramatic reduction in the number of desirable non-starter bacteria.

For the cheese industry to offer to the consumers safe and consistent cheeses with high organoleptic properties in a reasonable ripening time, they began to look for new technologies such as “adjunct cultures.” Adjunct cultures can be defined as selected strains of cheese related microorganisms that are added to the cheese milk to improve development of cheese sensory quality. They were also developed to accelerate cheese ripening, which may allow substantial cost savings to the cheese industry. In contrast to naturally occurring NSLAB, adjuncts are specifically selected and intentionally added to supplement the microflora of cheese milk to improve overall quality of finished cheese.

### THE USE OF ADJUNCT CULTURES

Research in the area of adjunct cultures has followed two main approaches: 1) nonviable (attenuated) adjuncts, and 2) viable (nonattenuated) adjuncts. Both approaches appear to improve the quality of Cheddar cheese and will be discussed in detail.

**The Use of Viable Adjuncts**

Viable adjunct cultures have been used in commercial cheese making for several years. However, the need to enhance flavor development in low-fat cheese has spurred additional interest in their utilization. Some
work was published in the 1980’s and early 1990’s on the impact of such cultures on cheese ripening (28, 41, 67). It is only in the last 5 yr that research groups joined efforts to select and characterize cultures. Workers in this area have noted the importance of the activities of adjunct cultures during ripening for the development of the characteristic flavor of the cheese.

Puchades et al. (86) compared the impact of adding different lactobacilli such as L. casei, L. paracasei, L. plantarum, and L. brevis during Cheddar cheese making at a concentration of 10^5 cfu/ml. A desirable flavor was obtained when homofermentative cultures were used. The addition of L. brevis led to off flavors and gas production. Further, Lee et al. (70) demonstrated that although the use of some homofermentative mesophilic lactobacilli led to more intense desirable cheese flavor, others caused high acidity, bitterness, off flavors and open and crumbly textures, which clearly indicates the importance of culture selection.

Drake et al. (18) have reported on the addition of 10^2 or 10^3 cfu/ml of L. helveticus WSU 19 during Cheddar cheese making. Cheeses containing the higher concentration of adjunct obtained higher organoleptic scores by dairy judges. Cheese with added lactobacilli exhibited higher rates of proteolysis when compared to the control cheese. In fact, a 11-kDa band could be detected on the sodium dodecyl polyacrylamide gel electrophoresis gels of the cheese made with L. helveticus WSU 19 after 3 and 6 mo of ripening, which was absent in the control cheese.

The same culture (L. helveticus WSU 19) was added to 33% reduced-fat Cheddar cheese at concentrations of 10^2 or 10^3 cfu/ml (19). The resultant cheese was significantly less bitter and exhibited a more intense oaky/ nutty flavor. Higher levels of protein degradation were also noticed in the L. helveticus treated cheese.

Studies involving the joint efforts of the Western Center for Dairy Research and the Wisconsin Center for Dairy Research compared different strains of L. casei, L. helveticus, and Brevibacterium linens in low-fat cheese (12). The authors demonstrated that low levels of lactobacilli had a positive impact on cheese quality, while high levels of the same culture led to flavor defects. This work showed the importance of Br. linens to produce methanethiol (a compound believed to be an important contributor to the development of characteristic Cheddar cheese flavors). However, the role of this organism in AA production and whether it has debitterase action is unclear. The same group (99) also reported that a consumer panel found that 60% reduced-fat Cheddar cheese made with brevibacteria was preferred to cheese made with L. casei and L. helveticus. The addition of adjunct cultures for the enhancement of low- and reduced-fat cheese was also considered at the Southeast Center for Dairy Research USA (20). The authors indicated that the use of a combination of two adjuncts from cultures of L. casei, L. helveticus, Lac. lactis ssp. lactis biovar. diacetylactis and Br. linens produced best flavored Cheddar cheese according to trained and consumer panelists. The authors also reported that L. helveticus alone as an adjunct significantly improved the sensory quality of 33% reduced-fat Cheddar cheese. An integrated culture system composed of Lac. lactis ssp. lactis biovar. diacetylactis JVI and Lac. lactis ssp. cremoris SKII and L. casei led to the production of a non-bitter reduced-fat cheese with a clean acid buttery flavor (51).

The work carried out by the previously mentioned centers, which focused on live cells, indicated the importance of the metabolic activities of live cells during cheese ripening especially for the production of methanethiol by Br. linens. However, the role of the metabolic activities of L. helveticus was not clearly explained regarding the undesirably high concentrations of this adjunct during ripening. The role of the intracellular metabolic activities of the LAB, which is now well established (28, 44, 58, 71), appears to be indispensable to changes in cheese quality during the ripening process.

The Use of Nonviable (Attenuated) Adjuncts

This approach can be described as a means of providing packages of intracellular microbial enzymes, which can be added to the cheese milk and subsequently retained in the coagulum without interfering with the cheese-making process. These enzymes are expected to be well distributed and quickly released in the cheese through the autolytic process. Because the microorganisms in the adjunct cultures were originally isolated from ripened Cheddar cheese, they are expected to contribute to the formation of the typical Cheddar cheese sensory qualities.

Early approaches to increase the number of desirable bacteria in cheese involved increasing the amounts of starter in the cheese milk by using a higher starter level (92) or by adding growth stimulants (52, 53, 80). This approach led to a cheese with lower pH, higher moisture content, and undesirable flavors. Attempts were therefore made to add cultures that have been attenuated so that they could play a role during ripening without producing excess lactic acid.

Five methods for cell attenuation have been described (22, 27, 44), but most research has focused on physical treatments: heat shocking, freeze shocking, and spray drying. The methods described in the literature can be summarized as follows: 1) Methods involving a physical treatment including heat shocking, freeze shocking, spray drying, and freeze-drying. 2) Methods involving
the use of enzymes; lysozyme was the only enzyme proposed for this purpose. Treatment with lysozyme does not seem to be effective for cell attenuation because of the difference in LAB sensitivity to lysozyme. In addition, the relatively high price of lysozyme represents another obstacle (69). 3) Methods involving chemical treatments (i.e., use of organic solvents). The addition of organic solvents such as butanol (93) represents a major drawback to the methods involving a chemical treatment due to the concerns over residual solvent in the cheese. 4) Methods involving genetic modifications of the cells. In these methods, lactose negative (Lac−), proteinase negative (Prt−), or Lac− and Prt− cells were used (1, 37, 44, 47, 57).

We will expand on what we believe can be the most applicable methods. The physical methods are those most commonly used for culture cell attenuation. These techniques also result in varying levels of cell viability, modification of ability to produce acid, and cellular proteinase and esterase activities. The autolytic properties of the cells are also altered as a result of the physical treatments. In previous reviews the impact of the various physical treatments on the different intracellular enzymatic activities were documented (27, 28, 41, 44) and are summarized in Table 1. The focus here will be on the impact of these treatments on the autolytic properties of the cells. This process is of major importance during cheese ripening. Most of the bacterial enzymes important for cheese ripening are intracellular; therefore, these enzymes cannot be released until autolysis occurs. This release can then allow intimate contact with desired substrates and improve flavor and texture development during ripening (14, 15, 33, 72).

Studies on the influence of heat shocking on the rate of cell autolysis are contradictory. Bie and Sjostrom (9) reported an increase in autolysis when _L. helveticus_ cells were subjected to a heat treatment of 54, 59, or 64°C for 20 s. On the other hand, El Soda et al. (30) showed that cells of various cheese related microorganisms from the following genera _Lactobacillus_, _Leuconostoc_, _Propionibacterium_, _Lactococcus_, and _Bifidobacterium_ exhibited a considerable delay in cell autolysis when subjected to a heat shocking treatment of 65°C for 16 s. Other authors later confirmed these findings. Thiboutot et al. (94) showed that very little autolysis could be measured over a period of 120 h in cells of _L. casei_ ssp. _casei_ if they were subjected to a heat treatment of 65°C for 16 s. Most recently, El Soda et al. (32) determined the rate of autolysis of one _L. helveticus_ strain and two of _L. casei_ strains after the following treatments: 1) heat shocking at 60, 60, and 70°C for 15 s; 2) freeze shocking at −20°C for 48 h followed by thawing at 38°C; and 3) spray drying with an outlet temperature of 82°C.

The results indicate that freeze-shocked cells showed the highest rates of autolysis, followed by untreated cells (Figure 1). The heat shock treatments significantly affected autolysis. The rate of autolysis decreased when the heat shock temperature was increased (Figure 2). The decrease in autolysis after the heat shock treatments may be partially attributed to a thermal denaturation of the autolytic system of the cultures studied. Spray drying using an outlet temperature of 82°C resulted in autolysis rates that were lower than other treatments. Contrary to heat shocking, the freeze shocking process had a positive effect on autolysis. Bie and Sjostrom (9) demonstrated a 100% increase in the amount of DNA released from cells subjected to three cycles of freezing at −20°C. When cells of _L. acidophilus_ were stored at −20°C overnight and then thawed, the rate of autolysis was 45% higher than cells stored at 3°C for the same time period (82). In a study involving several species of lactobacilli, El Kholy et al. (24) indicated that, as a general rule, cells subjected to freezing and thawing cycles resulted in autolysis at a faster rate when compared to untreated cells. The differences in the rate of autolysis between untreated cells and cells subjected to freezing and thawing varied according to the strain tested. Information concerning the impact of spray drying on cell autolysis is rather limited. Johnson and Etzel (55) and To and Etzel (95) studied the effect of freeze drying, freezing, and spray drying on autolysis and concluded that the slowest rate of autolysis was in spray-dried cells.

Comparable studies on the impact of physical attenuation of bacterial cells on cheese ripening is limited and to some extent contradictory. Although Ezzat and El-Shafei (35) suggested that heat shocked cells are better candidates for the enhancement of Ras cheese flavor; Aly (3) found no significant differences between the two treatments on low-fat cheese quality. El-Abboudi et al. (22) suggested that the development of typical Cheddar cheese flavor was accelerated by addition of homogenized, thermally treated cells of _L. casei_ ssp. _casei_. This treatment did not increase gross proteolysis in cheese as measured by soluble nitrogen but accelerated the hydrolysis of peptides, thus increasing the amount of amino nitrogen and reducing bitterness. Johnson et al. (56) compared the performance of freeze-shocked, freeze-dried and spray-dried adjunct cells of _L. helveticus_ to enhance flavor and body development in reduced fat Cheddar cheese. Cheese flavor intensity was enhanced in all adjunct cheeses, but cheeses made with an adjunct spray dried at high outlet air temperature had the lowest off-flavor intensity. Other sensory measures were not significantly different among the cheeses in spite of differences in the cellular properties of the
Table 1. Summary of conditions used for attenuation of dairy-related microorganisms.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Methods of physical attenuation</th>
<th>Reduction in enzymatic activity</th>
<th>Impact on cheese ripening</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shocking</td>
<td></td>
<td></td>
<td>Increase in amount of tyrosine. Marked increase in proteolysis</td>
<td>84</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>69°C/17 s</td>
<td>10 to 30%</td>
<td>Increase in proteolysis, a moderate increase in flavor intensity, a less bitter taste, a larger number of eyes and increase in AN level</td>
<td>34</td>
</tr>
<tr>
<td>Mixed strain lactococci</td>
<td>56°C/17 s</td>
<td>15%</td>
<td>Increase proteolysis. Enhance cheese flavor, free amino acids</td>
<td>4</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>67°C/15 s</td>
<td>AP (74%), EP (35%)</td>
<td>Develop in flavor, high scores for body texture, proteolysis proceeded rapidly</td>
<td>96</td>
</tr>
<tr>
<td>L. bulgaricus</td>
<td>63°C/20 s</td>
<td>N.D.</td>
<td>Increase proteolysis and development of flavor of cheese</td>
<td></td>
</tr>
<tr>
<td>L. helveticus</td>
<td>64°C/18 s</td>
<td>10%</td>
<td>Increase is soluble N, enhance cheese flavor with decrease in bitterness</td>
<td>13</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>70°C/18 s</td>
<td>N.D.</td>
<td>Increase in % of total volatile acidity</td>
<td>35</td>
</tr>
<tr>
<td>L. casei</td>
<td>67°C/22 s</td>
<td>DAP (37%)</td>
<td>Increase proteolysis, no bitter flavor</td>
<td>23</td>
</tr>
<tr>
<td>L. plantarum, L. casei</td>
<td>50°C/15 s</td>
<td></td>
<td>Decrease in acidification rate, increase in AN, increase in aminopeptidase activity, higher degradation of αS-casein than of β-casein</td>
<td>6</td>
</tr>
<tr>
<td>L. helveticus, L. casei</td>
<td>60, 65 &amp; 70°C/15 s</td>
<td>30%, 62 to 93% AP</td>
<td>Moderate levels of proteolysis</td>
<td>32</td>
</tr>
<tr>
<td>Freeze shocking</td>
<td></td>
<td></td>
<td>Substantial increase in water soluble nitrogen. Debittering effect</td>
<td>7</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>−24°C/24 h</td>
<td></td>
<td>50% reduction of Ras cheese ripening with L. helveticus</td>
<td>2, 3</td>
</tr>
<tr>
<td>L. helveticus, L. casei</td>
<td>−20°C/20 h</td>
<td></td>
<td>Mutant starter gave moderate increase in proteolysis (1–2%)</td>
<td>49</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>−36°C/1 wk</td>
<td>24 to 35% EP, 13% ES</td>
<td>Enhanced proteolysis of Cheddar cheese. Acid flavor defect with both microorganisms and calcium lactate crystals with pediococci</td>
<td>29</td>
</tr>
<tr>
<td>L. casei, Pediococcus sp.</td>
<td>−20°C/20 h</td>
<td></td>
<td>Enhancement of proteolysis and lipolysis in Ras cheese</td>
<td>26</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>−20°C/24 h</td>
<td>75–95%</td>
<td>Enhancement of proteolysis. Debittering effect</td>
<td>62</td>
</tr>
<tr>
<td>L. casei</td>
<td>−24°C/24 h</td>
<td>AP (15%)</td>
<td>Decrease bitterness in Ras cheese</td>
<td>25</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>−20°C/2 wk</td>
<td></td>
<td>High extent of enzyme release. Higher levels of water soluble nitrogen and FAA, enhancement in cheese flavor and prevented bitterness</td>
<td>75, 77</td>
</tr>
<tr>
<td>L. casei</td>
<td>−20°C/24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. helveticus</td>
<td>−82 &amp; 120°C</td>
<td>AP (85%)</td>
<td>Greater proteolysis and enhancement in cheese flavor</td>
<td>55, 56</td>
</tr>
</tbody>
</table>

1AP = Aminopeptidase; EP = endopeptidase; N.D. = not determined; DAP = dipeptidyl aminopeptidase; ES = esterase; AN = amino nitrogen.
adjunct cultures and chemical measures of cheese ripening.

Bartels et al. (7) found that Gouda cheese made with freeze-shocked adjunct cultures of *L. helveticus* had better flavor quality than cheese with untreated cultures. Our work (77) on the impact of attenuated adjunct cultures on cheese quality indicated that the attenuated adjunct cultures with enhanced autolytic properties provide a more controlled and consistent ripening resulting in flavor and texture improvement. The stimulating effect of attenuation, in particular, freeze shocking on release of enzymes from lactobacilli can be of a great importance in assuring the desired aminopeptidolysis early during cheese maturation (7, 24, 77).

### Selection Criteria for Adjunct Cultures

The selection of appropriate adjuncts is becoming widely used for successful cheese production. However, if they are not making a contribution through peptidase and esterase activities, some adjunct cultures may contribute little to cheese flavor development. In fact, in a study in which several commercial adjuncts were compared, El Soda et al. (31) demonstrated that some of the adjuncts exhibited little peptidase and esterase activity and autolyzed at a very slow rate. We believe that in addition to production of specific flavor compounds, the selection of adjuncts should be based on enzyme profiles (enzyme level and enzyme specificity) and autolytic properties.

**Selection Based on Enzyme Profile**

The literature indicates that there are wide differences in the concentrations of proteinases, peptidases, and esterases among genus, species, and strains of LAB (85). It was shown (21) that X-prolyldipeptidyl peptidase may not play a decisive role during Cheddar cheese ripening, while the debitterase role of pep N is now well established in LAB.

Amino acid catabolism during ripening is thought to play an important role in flavor development. Gao et al. (45) reported that dead unlysed cells of *Lac. lactis* 51 were able to degrade aromatic AA, and they suggested that cell lysis may not be important for AA catabolism by some lactic culture strains. However, Yvon et al. (100) reported that little alteration of the cytoplasmic membrane is needed to increase penetration of AA in the cells. These authors also suggested that after cell rupture and the progressive release of the intracellular contents of the cell in the cheese matrix, the aminotransferases from *Lac. lactis* could still be active since the co-factors appear to be tightly bound to the enzyme. The enzyme was still active at the pH and salt concentration of cheese. Further work is however needed for a better understanding of the positive and negative impact of AA catabolism on cheese flavor and to determine the role of autolysis during this event.

Laan et al. (63) studied the effect of pH and NaCl and CaCl₂ concentration on the aminopeptidase activity of lactococci and lactobacilli to assess the potential role of these enzymes on proteolysis during cheese ripening. The authors concluded that the aminopeptidase activity of the various cultures was partially inhibited at pH 5.2. The inhibition was restored by the addition of NaCl or CaCl₂ at concentrations found in the moisture phase.
of Cheddar cheese. Nonstarter lactic acid bacteria seem, therefore, to play a significant role during the ripening process, and their presence is essential for the development of a full-flavored cheese. Several reviews (27, 28, 44, 67) reported methods to improve the enzyme potential of LAB using genetic approaches. Recently Arnau et al. (5) expressed bovine plasmin in \textit{Lac. lactis}, and the plasmin produced in \textit{Lac. lactis} was biologically active. The authors did not apply the genetically modified culture in a cheese system.

Several intracellular enzymes from nonstarter adjunct cultures have been investigated and demonstrated the powerful ability to enhance cheese flavor development and quality due to their specific aminopeptidolytic action on casein during cheese maturation (22, 50). Adjunct culture of lactobacilli were reported to have 5 to 100 times higher intracellular enzyme activity than lactococci with high potential to degrade hydrophobic AA and reducing bitter off-flavor (50, 63). Recently, our group has investigated (77) proteolysis and flavor development in Cheddar cheese as function of addition of some strains of lactobacilli. The study revealed that cheese made with adjunct strains selected for high peptidolytic potential especially those with low acidification ability (33) exhibited the highest levels of proteolysis and flavor appreciation without detrimental effect on cheese-making procedure or cheese quality. This indicated the important role of high intracellular enzyme activities of lactobacilli, in particular those appropriately selected for their high aminopeptidase specificity.

**Selection Based on the Rate of Cell Autolysis**

The work of Kawabata et al. (59) and Lepeuple et al. (71) confirmed that early lysis of \textit{Lactococcus} cultures allowed the release of intracellular peptidases, which are then active in cheese. These publications also demonstrated that the extent of starter lysis could be related to the level of proteolysis (59). Furthermore, it was shown that starter strain lysis resulted in higher free AA levels and a decrease in bitterness (74).

Studies on the autolytic properties of both starter and nonstarter LAB have shown that the autolytic properties of a cell are strain dependent (Figure 3) (14, 15, 30, 58, 72, 89).

The environmental conditions and the physiological conditions affected the rate of autolysis. Sodium chloride at a concentration of 0.2 M was reported to enhance the autolysis of lactococci (58). El Soda et al. (30) reported that concentration of 0.5 M and up to 1 M of NaCl was required to obtain higher rates of autolysis from \textit{Lactobacillus} cultures. Several studies indicated that cells harvested during log phase autolyzed faster when compared to late stationary phase cells (10, 11, 14, 58, 72). The previously described studies were carried out in a buffer system, which may not reflect what happens in a cheese system. More recently, Boutrou et al. (15) followed the autolysis and proteolytic activities of lactococci in a pseudo-curd. The authors classified their cultures into three groups: low, moderate, and high lytic capacity. Boutrou et al. (10) also demonstrated that cell lysis positively influenced the ripening of cheese. Proteolysis was more advanced in cheese made with the highly autolytic cultures.

El Soda et al. (31) compared the autolytic properties, peptidase and esterase activities of commercially available adjunct cultures. The cultures were classified into four groups according to their levels of autolysis and aminopeptidase and esterase activities. Some cultures exhibited high rates of autolysis combined with high levels of aminopeptidase and esterase activities, whereas others possessed high autolytic activity with relatively low enzymatic activity. The third group was low in autolysis and high in enzymatic activity. The fourth group was composed of cultures low in autolysis and enzyme activity. The corresponding cheese slurry experiments carried out by the same group (75) confirmed the data obtained by Boutrou et al. (10, 11) for the lactococci. The slurries with added lactobacilli exhibited considerably higher aminopeptidase and esterase activity when compared to the control. The slurries made with cultures selected for their high enzymatic potentials and high autolytic properties exhibited the highest concentrations of released peptidases and esterases. On the other hand, slurries to which the poorly autolytic strain was added displayed very little enzyme.

![Figure 3. Rate of autolysis of different strains of \textit{Lactobacillus helveticus} (H, K, J, U, I, L, M, G) and \textit{Lactobacillus casei} (A, B, C, D, E, R, T). ●: A, H; ■: B, K; ▲: C, J; ○: D, U; □: E, I; ●: R, L; ○: T, M; ★: G. From El Soda et al. (29).](image-url)
activity. Higher concentrations of water soluble nitrogen and free fatty acids could also be measured in the slurries made with the cultures showing high levels of enzymatic activities combined with high autolysis (75).

The autolysis of starter and adjunct during the ripening process is assumed to result in the release of intracellular enzymes into the curd matrix leading to enhancement of flavor development. A selection could therefore be made based on the autolytic properties of the cells to ensure a sufficient release of intracellular enzymes.

Methods to Enhance Cell Autolysis

Among the methods used for cell attenuation, freeze shocking seems to be the most promising for high autolysis and high enzyme activity (24, 30, 35). Approaches have also been suggested to enhance and control lysis in live cells. Freitag and McKay (38, 39) isolated a Lactococcus strain harboring a thermoinducible prophage, which exhibited normal growth at 30 to 32°C and lysed when the cells were shifted to 38 to 40°C. Although this approach seems attractive because of the possibility of releasing the intracellular content of the cells at a given time during the cheese-making process neither cheesemaking trials nor further work with the cultures was reported.

Addition of bacteriocin-producing cultures was shown to promote autolysis in lactococci. Lactococcus lactis ssp. lactis DPC 3286, which produces bacteriocins A, B, and M, was shown to exhibit a lytic effect on other lactococcal strains. Cheese made with the bacteriocin-producing culture showed higher levels of AA and higher sensory evaluation scores than the control. The mechanism of cell lysis is, however, not understood and it is thought that a combination of the three bacteriocins may be necessary to initiate the lysis process (79). De Ruyter et al. (17) used the lytic genes of bacteriophage δ US3 (Lyt A and Lyt H) to accomplish controlled lysis of lactococcal cells. This expression system has the advantage of allowing the gene of interest to be present and silent in the host until the system is triggered by sublethal amounts of nisin. The authors suggest the use of an adjunct culture containing Lyt A and Lyt H genes under control of the nisin-incisive promoter. Other L. lactis present in the cheese will lyse after induction with nisin. The presence of nisin, which is essential to trigger the lytic system, can be obtained through the addition of a nisin producing culture. Mejier et al. (78) have reported the production of a nisin-immune transconjugant producing nisin, which could serve that purpose.

Bacteriophage genes were also cloned and expressed in lactococcal strains by Shearman et al. (90, 91), which led to the normal growth of the cells through exponential phase then lysed prematurely in the stationary phase. Studies were also carried out to construct systems in which change in temperature could trigger gene expression. Naut et al. (81), using comparative molecular modeling, isolated a derivative that became totally inactive at 42°C. Such a system can be useful in cheese making, where temperature shift constitutes an initial step in the process.

The bacteriophage-based methods may have potential for cheese-ripening enhancements but may be unacceptable to cheese manufacturers, and more particularly when the actual phage is needed to assist the lytic process (15, 59, 68). While the methods described above have improved our understanding of autolysis, their practical application has not been demonstrated. Thus, it appears that more traditional physical approaches to enhancing autolysis will be more widely used.

In addition to their role in cheese flavor enhancement, adjunct cultures were also used as probiotics. Gardiner et al. (46) manufactured Cheddar cheese with L. salivarius or L. paracasei, which were isolated from the human small intestine and have been characterized for their probiotic potential; L. paracasei was found to grow and sustain high viability in cheese during ripening, but the L. salivarius cultures declined over the ripening period. The authors suggest that Cheddar cheese can be an effective vehicle for delivery of some probiotics organisms to the consumer.

CONCLUSION

The literature survey on the use of adjunct cultures for cheese quality enhancement clearly indicates the need for more research on the difference between live and attenuated (nonviable) adjuncts for the improvement of the sensory quality of cheese. Although live cells favor the production of desirable metabolic products of adjuncts, the production of free fatty acids and AA will be delayed because these activities can only occur after cell autolysis. Live cells can also hardly be involved in the debittering process known to be important in cheese (22, 50). However, the productions of compounds like methanethiol are unlikely to be obtained if 100% attenuated (nonviable) cells are used as adjuncts.

A significant role of viable cells in improving the flavor and texture of mild Cheddar beyond methanethiol production is unlikely. Viable cells may have a larger impact on full-flavor Cheddar if the combined effects of their metabolic activity and associated enzyme activity from subsequent cell lysis are important. In young, mild cheeses, Cheddar flavor is less complex. Here, early high enzymatic activity may be desirable. Therefore nonviable, attenuated adjunct cells could rapidly re-
lease intracellular enzymes to accelerate flavor and texture development. However, there is little information to support or refute such an approach.

A study of the same adjuncts added in viable and nonviable attenuated forms could help to address these questions. Studies that clarify the role of lysed cells versus metabolically active cells and the impact of both types of adjuncts on the rate of flavor and texture changes are needed.

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