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Lactic Fermentations of Dairy Foods and Their Biological Significance¹

ARUN KILARA and KHEM M. SHAHANI
Department of Food Science and Technology
University of Nebraska
Lincoln 68583

ABSTRACT

Fermentation is one of the methods of food preservation. Over years this method has evolved into a sophisticated art. Milk can be fermented by bacteria, yeasts, and molds to produce a variety of products such as yogurt, cheeses, sour cream, and buttermilk. Modification of milk by microorganisms affects both the physicochemical properties and the economic value of milk. The physicochemical changes are manifested in such properties as flavor, texture, and nutritive value. The economic value of milk is enhanced by the increased storage life of the products. A majority of these modifications are by the enzymes originating from microorganisms and acting on the protein, lipids, and carbohydrates in milk. The products resulting from such changes introduce variety into human diets.

Changes induced in milk constituents by microorganisms are discussed with regard to flavor, texture, and nutritive value. The significance of such modifications also is considered in terms of microbial synthesis of vitamins, natural antibiotics, natural anticarcinogenic substances, and enzymes. Although the mechanisms of these modifications are not yet understood, studies under *in vitro* conditions to delineate the physiological implications and nutritional and therapeutic significance of such modifications are described.

INTRODUCTION

From the time man appeared on earth, food production and manufacturing began, and to this day we are trying to transform the art of food production and manufacture to a science. Early nomadic man soon realized the relationship of climate to food as he experienced cyclic feasts and famines; and since the idea of facing famine did not appeal to him, food preservation evolved. Perhaps drying and smoking foods originated first, and when some dried foods were unwholesome, probably because of botulism or staphylococcal food intoxication, the addition of salt improved wholesomeness. The addition of salt led to the selection of microorganisms and produced a nutritious and tasty food. This natural selection process was presumably the inception of fermented foods, and this process was refined later to an art. Only during this century have serious scientific studies been conducted to understand fully the nature of the biochemical changes induced by microorganisms. Microorganisms are involved in the fermentation of various foods such as vegetables, meat, plant proteins, cereals, and dairy products.

In the dairy industry, fermentation organisms include bacterial lactic cultures which are used in the manufacture of cheese, cultured buttermilk, sour cream, butter, acidophilus milk, and bulgaricus buttermilk (11). Some varieties of cheese and products like yogurt require more than one type of bacteria. For example, the cultures used in Swiss cheese production consist of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Propionibacterium freudeureichii* var. *shermanii*. Some other varieties of cheeses require bacteria and mold as in blue or Roquefort cheese, or bacteria and yeast as with surface ripening cheeses such as brick (4). Therefore, microorganisms considered to be important in the dairy industry include lactic acid bacteria but also yeasts

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and molds.

The modifications that are brought about by these organisms can be classified as either physicochemical or economic. The physicochemical changes are reflected in such attributes as flavor, texture, appearance, and nutritive value. The economic alterations are reflected in the enhanced shelf life of milk resulting from the conversion of fluid milk to products which are more stable. These alterations in physicochemical quality and economic value of milk are brought about by changes in specific constituents of milk, namely milk fat, proteins, lactose, enzymes, minerals, and water. Certainly, the majority of these modifications involve fats, proteins, and lactose and are carried out by the enzymes inherent in the microorganisms. The ability of these organisms to modify milk lipids, proteins, and carbohydrates to a large number of new compounds produces unique products and introduces variety into our diet. This paper will review briefly the significance of dairy cultures with respect to the modifications of milk and dairy foods.

MODIFICATION OF MILK CONSTITUENTS

The three major constituents of milk — proteins, lactose, and lipids or milk fat — are modified to various degrees depending on the product, and the results are manifested in altered flavor, texture, appearance, and nutritive value.

As early as 1914, it was hypothesized that lactic streptococci caused proteolysis in cheese (8). This hypothesis was verified later by inoculating aseptic curd with single strains of *Streptococcus lactis*, *Streptococcus cremoris*, and *Streptococcus lactis* var. *diacetylactis*, and observing the resultant increase in nonprotein nitrogen or amino nitrogen (19). It further was observed that some genera of bacteria were more proteolytic than others. For example, lactobacilli were more proteolytic than streptococci. The variation in proteolytic activity has been observed even between different strains of a given species, and this variation in proteolytic activity within strains can be greater than the degree of variation between species (21). The proteases of starter bacteria can be extracellular, bound to the cell wall membrane, or intracellular. The intracellular enzymes would

be released only when the bacterial cells autolyze. This process is illustrated in Figure 1.

Starter cultures are necessary for appropriate flavor development in cheese. Using aseptic vat techniques, Fryer et al. (5) produced three different types of cheeses: (i) In one case, a single strain starter comprising of *Streptococcus cremoris* or *S. lactis* was used as the inoculum along with a predetermined mixed flora. This predetermined mixed flora, called reference flora by Reiter et al. (18) and Fryer et al. (5), consisted of adventitious organisms introduced during cheesemaking as contaminants. The cheese so made developed intense Cheddar flavor, but the flavor quality was variable and often off-flavors predominated. (ii) In another vat, cheese was made in the absence of the reference flora, and the starter consisted of a single strain inoculum of *S. lactis* or *S. cremoris*. Such cheese had consistent flavor attributes, and the off-flavors resulting from the starter were reproducible, indicating these flavor defects were characteristic of the strain of culture used in making the cheese. Overall, the time required for the development of optimal flavor was more than in the first experiment. (iii) In the third vat, cheese was made by a direct acidification process, using glucono-delta-lactone instead of the starter. Under aseptic conditions of manufacture, such a cheese did not develop cheese flavor even after a prolonged ripening period. These experi-

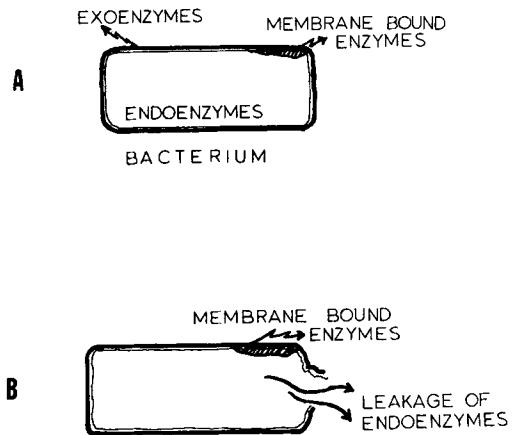


FIG. 1. Sources of bacterial enzymes. A. Enzymes in an intact bacterium; B. Release of enzymes upon autolysis of a bacterium.

ments indicated that the starter cultures were essential for flavor development (12).

The number of microorganisms in cheese declines rapidly in the initial stages of maturation or ripening. Flavor development follows. This phenomenon suggests that flavor development is related to the enzymes of starter bacteria that are released after the death of the cell rather than to the metabolic activity of the viable cells. Schormüller (22) has demonstrated that proteases, peptidases, transaminases, decarboxylases, and phosphatases are involved in the flavor and texture development of both soft and hard types of cheeses. The origin of these enzymes was not clear until the experiments with aseptic vats, for these enzymes could have been derived from milk, rennet, or the bacteria. They are now attributable to the starter culture, but the precise mechanism by which these enzymes produce flavor impact compounds has not been delineated. Although the relationship of starter organisms to flavor compounds and proteolysis is important, there are other modifications of equal significance that occur concomitantly. The degradation of proteins contributes to a softening of the cheese, thereby altering texture. Fat in cheese undergoes lipolysis and contributes to flavor. As in Figure 2, proteins are degraded by microbial proteases to yield peptides and amino acids. These amino acids can undergo a variety of changes, such as

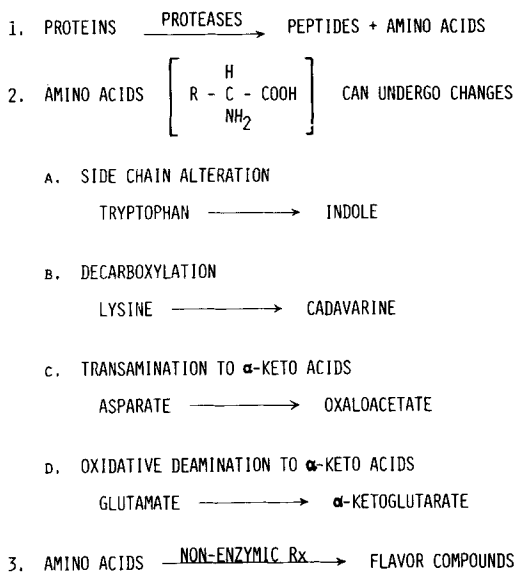


FIG. 2. Mechanisms for the degradation of proteins.

side chain alterations, decarboxylation, transamination and oxidative deamination to alpha-keto acids. Additionally, the amino acids can enter into nonenzymatic browning reactions with sugars to yield flavor compounds. Some of the precursor amino acids and their degradation products are in Table 1. Some of these compounds, such as cadaverine, contribute to

TABLE 1. Precursor amino acids and their metabolites.

Precursor	Mechanism	Metabolite
	Decarboxylation	
Lysine		Cadaverine
Glutamate		Aminobutyric acid
Tyrosine		Tyramine
Tryptophan		Tryptamine
	Deamination	
Alanine		Pyruvate
Tryptophan		Indole
Glutamate		α-Ketoglutarate
Serine		Pyruvate
Threonine		α-Ketobutyrate
	Strickland reaction	
Alanine		Acetate
Leucine		Isovalerate
Proline		γ-Amino valerate
Hydroxyproline		γ-Amino-α-hydroxyvalerate

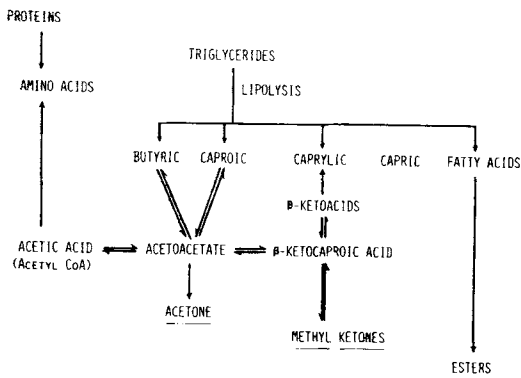


FIG. 3. Mechanisms for the degradation of lipids.

flavor. In mold ripened cheeses, such as blue and Roquefort, lipolysis plays a key role in producing the flavor impact of certain ketones and aldehydes. As described by Harper and Kristoffersen (7), fats are converted after lipolysis to β -keto acids and acetoacetate. The β -keto acids further are metabolized to methyl ketones whereas the acetoacetate can be converted to acetyl CoA and amino acids or to acetone. Some fatty acids also are esterified (Figure 3).

In a product like cheese, a portion of the lactose of the milk is metabolized by the microorganisms to produce acidity, but the major part of the lactose is lost in the whey. In other cultured products such as yogurt, sour cream, and buttermilk, degradation of lactose gives rise to a variety of flavor compounds such as diacetyl and acetaldehyde. A significant amount of lactose does remain intact in products. Figure 4 shows the degradative pathways for citrate and lactose through the common intermediate, pyruvate. The pyruvate can be converted to a variety of flavor compounds.

Based upon the utilization of lactose, lactic microorganisms have been classified as being either homofermentative or heterofermentative. Homofermentative organisms produce 1.8 moles of lactic acid per mole of glucose; i.e., most of the glucose utilized is converted to lactic acid. Heterofermenters produce by-products like ethanol, acetate, glycerol, mannitol, and carbon dioxide. Acetaldehyde, the flavor compound of importance in yogurt, normally results from the breakdown of pyruvate by bacteria (3). Additionally, Collins (2) observed that acetaldehyde can be produced by strepto-

cocci from the substrate, thymidine. The conversion of thymidine to acetaldehyde is via a sequential reaction involving the enzymes thymidine phosphorylase, deoxyriboaldolase, and deoxyribomutase. Diacetyl production, important in flavor of buttermilk, sour cream, and butter, is thought to be a by-product of pyruvate utilization. Excess pyruvate is toxic to bacteria, so the organisms convert excess pyruvate to nontoxic compounds like diacetyl and acetoin. This detoxification process occurs via one of the two proposed methods. In the first method, organisms convert α -acetylacetate to valine and pantothenic acid. Pantothenic acid is converted to coenzyme A which participates in the formation of diacetyl. In this group of organisms, both valine and pantothenate are synthesized. In another group of organisms, exogenous pantothenate is consumed to produce acetyl coenzyme A (2). To recapitulate, lactose is used to produce pyruvate and lactate, both of which can be used to produce acetaldehyde and acetoin. In our laboratory, we have observed that lactose is utilized through both the hexose monophosphate (HMP) shunt and the Embden Myerhoff Parnas (EMP) pathways. The production of lactic acid not only contributes to flavor but also causes the milk to coagulate and changes its texture; i.e., the conversion of fluid milk to a solid milk curd and liquid whey.

Lipolysis is important in some dairy foods. Extensive lipolysis of milk leads to off-flavors and is undesirable; however, by partial lipolysis of butteroil under carefully controlled conditions, a butter flavor concentrate can be pro-

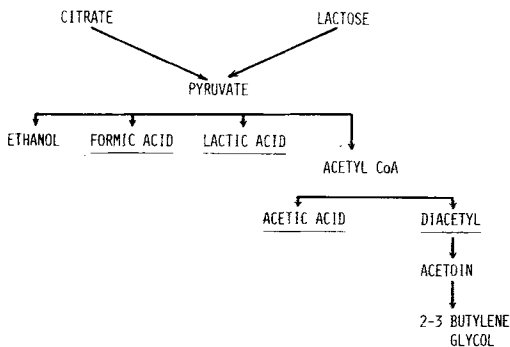


FIG. 4. Utilization of citrate and lactose by lactic streptococci to produce flavor impact compounds.

duced which will enhance greatly the buttery flavor of the foods (1). Also, *Penicillium roqueforti* grown in milk as a submerged culture can produce blue cheese flavor readily and quickly. Such a product can be used to flavor salad dressings (15).

Thus, proteins, lactose, and fats can be modified by microbial enzymes to induce changes in flavor, texture, and appearance of dairy products. If lactic microorganisms were not used in such products, texture and appearance could perhaps be modified by other means, but the flavor of the product would be inadequate. For example, studies with direct acidified yogurt and sour cream in our laboratory show that while body and texture were essentially similar to those of the cultured products, the flavor scores for directly acidified products were lower than the corresponding scores of the cultured products (9, 17).

Some of the cultured products being introduced commercially include frozen yogurt, yogurt mayonnaise, and sweet acidophilus milk. Sweet acidophilus milk is prepared by adding *Lactobacillus acidophilus* cell concentrates to milk in an effort to provide these beneficial microorganisms without the attendant acidity

and sharpness of conventional acidophilus milk. Although numerous benefits have been postulated, there is an imminent need for experimental verification and such research work is in progress. More recently, lysine-excreting mutants or isolates of *Lactobacillus acidophilus* and *L. bulgaricus* have been described. These types of organisms may play a significant role in improving the nutritional properties of cultured foods (20).

SIGNIFICANCE OF MODIFICATIONS

Lactic microorganisms modify significantly the proteins, lipids, and lactose in dairy products and produce desirable qualities. However, other modifications are less obvious and their significance is not understood fully because we lack studies correlating observations in vitro to those in vivo. These modifications pertain to the production of antibiotics, vitamins, and enzymes by lactic microflora. Numerous lactic microorganisms produce natural antibiotics. For example, *S. lactis* produces nisin (13), *L. bulgaricus* produces bulgarican (23), *L. acidophilus* produces acidophilin (24), acidolin (14), etc. In vitro, these antibiotics have a broad

TABLE 2. In vitro antibacterial activity of acidophilin.

No.	Test organism	Strain	IC ₅₀ ^a (μg/ml)
1	<i>Bacillus subtilis</i>	ATCC 6633	30
2	<i>Bacillus cereus</i>	Difco 902072	29
3	<i>Bacillus stearothermophilus</i>	ATCC 7954	43
4	<i>Streptococcus faecalis</i>	ATCC 8043	45
5	<i>Streptococcus faecalis</i> var. <i>liquefaciens</i>	ATCC 4532	42
6	<i>Streptococcus lactis</i>	NU C ₁₀	30
7	<i>Lactobacillus lactis</i>	LY-3 France	40
8	<i>Lactobacillus casei</i>	ATCC 7469	42
9	<i>Lactobacillus plantarum</i>	ATCC 8014	60
10	<i>Lactobacillus leichmannii</i>	ATCC 7830	59
11	<i>Sarcina lutea</i>	ATCC 9341	30
12	<i>Serratia marcescens</i>	NU	29
13	<i>Proteus vulgaris</i>	NU	32
14	<i>Escherchia coli</i>	NU	32
15	<i>Salmonella typhosa</i>	ATCC 167	30
16	<i>Salmonella schottmulleri</i>	ATCC 417	30
17	<i>Shigella dysenteriae</i>	ATCC 934	30
18	<i>Staphylococcus aureus</i>	NU (coagulase + ve)	50
19	<i>Staphylococcus aureus</i>	Phage 80/81	60
20	<i>Klebsiella pneumoniae</i>	ATCC 9997	60
21	<i>Vibrio comma</i>	ATCC 9459	30

^aIC₅₀ = concentration inhibiting 50% of growth.

TABLE 3. Effect of feeding yogurt upon proliferation of Ehrlich Ascites tumor cells in mice.

Series	Total tumor cells (millions/mouse)		Inhibition* (%)	DNA content ($\mu\text{g/ml}$ suspension)		Inhibition* (%)
	Control	Experimental		Control	Experimental	
1	26.61 \pm 3.9	18.72 \pm 4.1	29.6	351 \pm 82	250 \pm 75	28.8
2	20.95 \pm 2.6	15.25 \pm 3.7	27.3	347 \pm 40	260 \pm 32	25.1

*Significant ($P < .05$).

spectrum of activity, are generally low molecular weight compounds, have a low pK, and are elaborated only when the organisms are grown in milk (23, 24). Such antibiotics are inhibitory to both gram positive and gram negative, pathogenic and nonpathogenic bacteria (Table 2). Even though their elaboration in cultured milk has been demonstrated, the beneficial effects to humans of ingesting such cultured milk products have not been demonstrated conclusively.

Our laboratory trials have been with Ehrlich ascites tumor cells carried in the peritoneal cavity of male Swiss mice (Table 3). Feeding

cultured milk products such as yogurt, acidophilus, and bulgarian buttermilk inhibits both the total tumor cell numbers as well as the DNA content of the ascitic fluid (Fig. 5). It is unclear whether this antitumor activity is related to the natural antibiotics produced by the organisms. In other experiments, we found that many of the lactic microorganisms are capable of synthesizing some B vitamins (Fig. 6). In comparative studies where cottage cheese, yogurt, and sour cream (25) were prepared by conventional culture techniques and by direct acidification, folic acid or folacin, biotin, and pantothenic acid were definitely

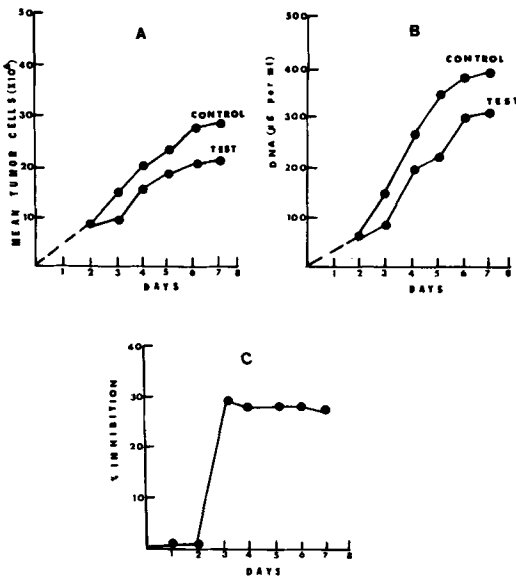


FIG. 5. Progression of Ehrlich ascites tumor in vivo as determined by (A) daily cell counts; (B) DNA determinations; (C) percentage inhibition of tumor cell proliferation. Test animals were fed yogurt ad libitum.

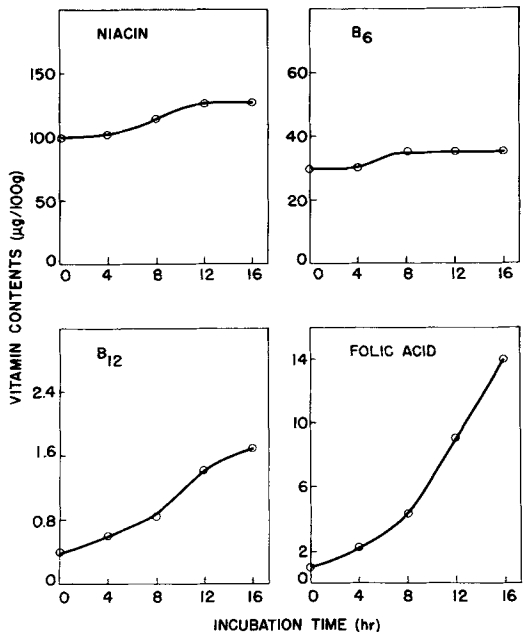


FIG. 6. Biosynthesis of certain B-vitamins by the cottage cheese starter culture.

TABLE 4. Comparison of B-vitamins in standardized mix, cultured sour cream, and acidified sour cream.

Vitamin	Standard- ized mix	Sour cream	
		Cul- tured	Acidi- fied
		(µg/100 g)	
Folic acid	1.7	10.8	3.1
Niacin	60	67	64
Pantothenic acid	370	360	330
Biotin	3.4	2.6	3.1
Vitamin B ₆	19	16	17
Vitamin B ₁₂	.3	.3	.3

higher in cultured products (Table 4).

Lactose-intolerant people are reportedly more tolerant to cultured milk products. This is presumably because a part of the lactose is metabolized to produce lactic acid, flavor compounds, and cell mass. Apart from the partial conversion of lactose to its metabolites, the microbial cells contain lactose or β -galactosidase, the enzyme responsible for hydrolyzing lactose. In the case of *S. lactis*, *S. thermophilus* and *L. bulgaricus*, this enzyme is bound to the cell wall (10). It has been postulated that this lactase can aid in hydrolyzing lactose in the small intestine. Experiments with rats indicated that this enzyme and the starter microorganisms may survive the low pH encountered in gastric digestion (6). Thus, cultured milk products may have beneficial effects, especially for the segment of the population that cannot tolerate lactose. Sweet acidophilus milk has been suggested to confer the benefits of improved lactose tolerance and alteration or balancing of the intestinal flora. Again, these need to be verified by in vivo experiments. For *L. acidophilus*, methods for enumeration and identification of organisms in feces may pose problems in evaluating the colonization process.

More research is required to relate observations of in vitro conditions to benefits in man and animal. The use of starter cultures as biological control agents against foodborne pathogens may be desirable, because this would eliminate the use of food additives in certain instances (26).

In summary, some of the important changes in milk and milk products by dairy cultures have been reviewed. These changes deal with preserving food, altering flavors through modifi-

cation of proteins, lipids, and carbohydrates, and producing vitamins, natural antibiotics, and anticarcinogenic compounds. Most of these changes cannot be achieved in the absence of lactic microorganisms. Although many metabolic pathways and many kinds of degradation products that are produced by lactic cultures in milk and milk products have been identified, some of the beneficial or nutritional aspects of such reactions have not been elucidated fully. Research in progress should help unravel these mysteries.

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