

Effects of Fermentation on the Nutritional Properties of Food¹

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Fermented foods, such as breads, cheeses, various soybean products, cassava, vegetables, and sausages, have made important contributions to human diets for thousands of years and continue to do so. Certainly the most significant role of fermentation in human nutrition has been to help make the nutrients naturally present in the starting food materials more palatable and more widely available than would be possible without fermentation. Thus, even if fermentations had no direct effect upon the nutrient content and quality of foods, these processes would be very important to the food supply. However, it is clear that fermentation processes can have significant direct effects on the nutritive qualities of foods. It is the purpose of this chapter to review these direct nutritional consequences of fermentation and to consider some of the uncertainties and limitations of current data.

CHARACTERIZATION OF NUTRIENT CHANGES IN FERMENTED FOODS

Food fermentations are very complex processes because they normally involve the interaction of plant or animal tissues with a group of microorganisms. This means that changes depend upon the available nutrients and nutrient precursors in the starting materials, the metabolic capabilities of the starting materials, the metabolic abilities of

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the fermentative microorganisms, and possible interactions among all of these elements. To complicate matters further, many fermentations occur in solid or semisolid states so that particle sizes, diffusion rates of oxygen and nutrients, and distribution of fermentative organisms may be important factors in both the organoleptic quality and nutrient content of the product. Considering these complexities and the many types of food fermentations that are employed around the world, it is not surprising that our understanding of nutrient changes in most fermented foods is very incomplete. Particularly notable is the fact that examples are lacking in which the biochemical mechanisms underlying nutrient changes in fermented foods are understood well enough to allow control of final nutrient concentrations.

Since many groups of microorganisms participate in food fermentations, there should be opportunities to use advances in genetic modification and biochemical engineering techniques to increase critical nutrients in fermented foods. One example might be the increased production of a limiting amino acid, such as lysine, by fermentative organisms (Sands and Hankin 1974, 1976; Haidaris and Bhattacharjee 1978). However, the mechanisms by which nutrients are formed or degraded during food fermentations must be better defined before we can expect to produce consistent, useful changes in fermented foods.

Table 16.1 is a list of important questions that can be asked about almost any nutrient change in any food fermentation. With limited and scattered research efforts to characterize nutrient changes in fermented foods, undoubtedly it will be a number of years before all of the questions can be answered for any particular fermentation. Considering the variety and complexity of fermentation processes, it is not reasonable to expect research workers to address all of these questions, except in cases that are judged to be of special importance.

EFFECT OF FERMENTATION ON THE ENERGY CONTENT OF FOOD

Data have not been published on changes in the caloric content of food as a result of fermentation processes. Generally only small changes would be expected. In processes such as tempeh production, which are aerobic, the fermentation period is too short to allow large decreases in the total lipids, carbohydrate, or protein components of the food. During alcoholic or lactic acid fermentations, a large proportion of the sugars are metabolized. However, the energy produced by fermentation of sugars to either ethanol or lactic acid is only 2 mol ATP/mol hexose. This compares with the potential production of 38 mol ATP/mol of hexose when the sugar is completely oxidized. Therefore, approximately 95% of the energy available in the sugars remains after the fermentation.

Table 16.1. Questions for Characterization of Nutrient Changes in Fermented Foods

1. What are the most important nutrients in the fermented food?
2. What is the initial concentration and variability of a nutrient in the starting materials used in a fermentation?
3. What is the final concentration of a nutrient at the end of a defined process that results in a fermented product with acceptable chemical and organoleptic characteristics?
4. What is the final concentration of a nutrient after storage of a fermented product under a defined set of conditions of time, temperature, pH, humidity, microbiological flora, etc.?
5. If an increase in a nutrient occurs during a fermentation, is the increase a result of:
 - (a) moisture loss or other physical concentration effects during processing?
 - (b) synthesis of the nutrient by a fermentative microorganism?
 - (c) synthesis of the nutrient by the material which is undergoing fermentation?
 - (d) release of the nutrient from some bound, unavailable condition?
6. Which microorganism is responsible for synthesis of a nutrient?
7. What are the precursors used for the synthesis of a nutrient during fermentation?
8. What is the pathway used for synthesis of a nutrient during fermentation?
9. If a nutrient declines during fermentation, is the decrease a result of:
 - (a) removal of a nutrient due to washing, draining, or addition of water to the fermentation?
 - (b) degradation due to enzymes in the material being fermented?
 - (c) exposure of the nutrient to oxygen or light?
 - (d) change in pH, which results in decreased stability of the nutrient?
 - (e) metabolism of the nutrient by a microorganism in the fermentation?
 - (f) binding of the nutrient into a nonavailable form?
 - (g) uptake of the nutrient by microbial cells, which are removed after fermentation?
10. Which microorganism is responsible for degradation of a nutrient?
11. What is the pathway of nutrient degradation?
12. If a nutrient does not change during fermentation, is this a result of:
 - (a) stabilization of the nutrient due to a favorable pH change, exclusion of oxygen, exclusion of light, or another environmental factor?
 - (b) a balance between synthesis and degradation of the nutrient?
 - (c) a lack of any enzymatic or nonenzymatic mechanisms to cause a change?

Zimmer (1980) has estimated that the unavoidable energy loss due to microbial fermentation of silage is only 2–4%.

Fermentation processes will not be discussed in detail in this chapter. Descriptions for most of the fermentations considered in this review can be found in the literature references and in several recent books (Pederson 1979; Rose 1982; Steinkraus 1983). Unless otherwise indicated in the text, changes in nutrient content are stated relative to the nonfermented ingredients which were used in the experiments.

LACTIC ACID ISOMERS IN FOOD FERMENTATIONS

Lactic acid is the major product formed from sugars in vegetable, dairy and meat fermentations. Lactic acid bacteria produce two stereoisomers of lactic acid (Stetter and Kandler 1973), which have been designated D(-) and L(+). Since animals, including human beings, normally produce only the L(+) isomer of lactic acid when muscles are in oxygen deficit, the question of the fate of the D(-) isomer, when it is consumed, has been investigated.

Cori and Cori (1929) were the first to observe that the D(-) form of lactic acid is metabolized more slowly than the L(+) isomer. This basic observation has been confirmed in rabbits (Drury and Wick 1965), ducks (Brin 1964), cattle (Dunlop *et al.* 1964; Giesecke and Stangassinger 1980), and sheep (Giesecke and Stangassinger 1980). Even though the D(-) isomer is not produced in muscle tissue, it has been found that animals, whether ruminant or monogastric, normally absorb this isomer because it is formed by bacteria in the rumen or intestinal tract (Giesecke *et al.* 1980; Giesecke and Stangassinger 1980). Recent research has emphasized that mammals have normal mechanisms to metabolize the D(-) isomer. Giesecke *et al.* (1981) found that both the rabbit and the rat excreted only about 6% of an injected sample of the D(-) isomer, even though these animals metabolize the isomer differently. Thus, present data indicate that the energy yield from lactic acid will be similar regardless of the isomer consumed.

The differences between the rates of metabolism of lactic acid isomers and indications that infants have difficulty metabolizing DL-lactic acid (Droese and Stolley 1962, 1965) resulted in a recommendation by the Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives that infants not be given foods containing D(-) lactic acid and that adults limit their intake of the D(-) isomer to not more than 100 mg/kg/day (World Health Organization 1966). Subsequently, the recommendation regarding limits on adult intake was dropped (World Health Organization 1974).

There are only limited data on the distribution of lactic acid isomers in foods. Kunath and Kandler (1980) found a mixture of isomers in both commercial and laboratory prepared yogurts. The proportions of the isomers varied with the fermentation and storage temperatures and the lactic acid bacteria present. Though an excess of L(+)-lactic acid was common, it usually did not exceed 70% of the total lactic acid. Alm (1982B) found 42% D(-)-lactic acid in yogurt which contained 1.2% total lactic acid. The D(-) isomer was not found in kefir, ropy milk fermented with *Streptococcus lactis* var. *longi* and *Leuconostoc cremoris*, low-fat acidophilus milk, and bifidus milk. Lactic acid isomers have not been analyzed in fermented cucumbers or sauerkraut. However, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, and

Lactobacillus brevis are known to produce DL-lactic acid and *Leuconostoc mesenteroides* D(-)-lactic acid (Garvie 1967; Stetter and Kandler 1973), so it would be expected that fermented vegetables would contain both isomers.

EFFECTS OF FERMENTATION ON PROTEIN CONTENT, QUALITY, AND AVAILABILITY

Many fermentations are done on foods such as cereals, legumes, dairy products, and meats, which are important protein sources. Changes in the nutritive value of proteins as a result of fermentation are particularly important for cereals and legumes. These sources of protein often are of lower nutritional quality than animal products, and they tend to be major dietary sources of protein for people with marginal or sub-marginal protein intake. Therefore, fermentation processes that consistently improve protein quality or availability of cereals or legumes could have a positive impact on the diets of many people. Conversely, any fermentation that resulted in unnecessary loss of protein content or quality could have a particularly negative impact.

Protein Content

The primary objectives for most fermentations of high-protein foods are to modify the flavor or texture characteristics of the starting-food ingredients. These changes generally are produced by fermentations that are limited both in the time and extent to which microorganisms are allowed to grow. Therefore, large changes in total protein content would not normally be expected. Available data tend to support this expectation. Fermentations have not been found to significantly affect the protein content of idli (Reddy *et al.* 1981; van Veen *et al.* 1967; Rajalakshmi and Vanaja 1967) or khaman (Rajalakshmi and Vanaja 1967). Small increases in protein were found after fermentation in the production of tempeh (Murata *et al.* 1967; Wang *et al.* 1968). Wang *et al.* (1968) attributed the increase in protein to the loss of other components during fermentation. Alm (1982C) observed an increase in protein content during the fermentation of several types of fermented milk, while Rao *et al.* (1982) found small, but statistically significant decreases. In both instances, the changes were attributed to the loss of volatile components from the samples. One exception to the general result that fermentations will not cause large changes in the amount of protein is the growth of *Candida tropicalis* on cassava flour to produce yeast biomass (Azoulay *et al.* 1980). The protein content of the flour increased from 3.1 to 18% as a result of the fermentation.

Table 16.2. Changes in PER of Proteins as a Result of Food Fermentations

Product	PER			Reference
	Before	After	Δ PER	
Chickpea tempeh	1.95	2.11	0.16	Kao and Robinson (1978)
Horsebean tempeh	0.89	1.51	0.62	Kao and Robinson (1978)
Horsebean tempeh + met + try	2.22	2.60	0.32	Kao and Robinson (1978)
Soybean tempeh	1.77	2.03	0.26	Kao and Robinson (1978)
Oncom	1.51	1.41	-0.10	Fardiaz and Markakis (1981B)
Idli				
4:1 black gram/rice	2.28	2.55	0.27	Rao (1961)
1:1 black gram/rice	1.99	1.84	-0.15	van Veen <i>et al.</i> (1967)
1:2 black gram/rice	1.50	2.00	0.50	Rajalakshmi and Vanaja (1967)
Wheat tempeh	1.28	1.71	0.43	Wang <i>et al.</i> (1968)
Soybean tempeh	2.17	2.27	0.10	Wang <i>et al.</i> (1968)
1:1 Wheat/soybean tempeh	2.49	2.79	0.30	Wang <i>et al.</i> (1968)
Wheat tempeh				
0 hr	1.25	—	—	Wang <i>et al.</i> (1968)
12 hr	—	1.28	0.03	Wang <i>et al.</i> (1968)
24 hr	—	1.78	0.50	Wang <i>et al.</i> (1968)
48 hr	—	1.84	0.56	Wang <i>et al.</i> (1968)
72 hr	—	1.73	0.45	Wang <i>et al.</i> (1968)
Soybean tempeh				
0 hr	2.63	—	—	Hackler <i>et al.</i> (1964)
12 hr	2.47	—	-0.16	Hackler <i>et al.</i> (1964)
24 hr	2.56	—	-0.07	Hackler <i>et al.</i> (1964)
36 hr	2.49	—	-0.14	Hackler <i>et al.</i> (1964)
72 hr	2.44	—	-0.19	Hackler <i>et al.</i> (1964)
Ontjom	2.17	2.17	0.00	van Veen and Steinkraus, (1970)
Ecuadorian rice	1.90	1.63	-0.27	van Veen and Steinkraus, (1970)
Fish paste	3.12	2.96	-0.16	van Veen and Steinkraus, (1970)
Dry sausage	3.24	3.92	0.68	Eskeland and Nordal (1980)

Nutritional Quality of Proteins in Fermented Foods

Even though changes in the quantity of protein as a result of food fermentation appear to be small or nonexistent, considerable effort has been made to investigate changes in the nutritional quality of the protein. Table 16.2 is a compilation of reported changes in the protein efficiency ratio (PER) of various foods as a result of fermentation. The results of these studies suggest that protein quality can be improved by fermentation in some instances. A number of studies showed no significant change in protein quality. None of the PER evaluations showed a significant decline in protein quality as a result of fermentation. The

data in Table 16.2 may be somewhat complicated by the fact that during fermentation other nutrients may change so that variations in growth response of rats may not be exclusively a result of changes in amino acids or proteins (Kao and Robinson 1978).

Evaluations of protein quality changes also have been made by techniques other than PER measurements. Hargrove and Alford (1978) found that the growth rate and feed efficiency of yogurt prepared with *Lactobacillus bulgaricus* and *Saccharomyces thermophilus* were improved, compared to nonfermented milk, when fed to rats. There was no improvement when other fermented milks were tested, including cultured buttermilk, acidophilus milk, kefir, and Bulgarian buttermilk. The effect of natural fermentations on the protein quality of corn, chickpea, and cowpea flours has been investigated by Fields and co-workers (Hamad and Fields 1979; Zamora and Fields 1979), using the growth response of *Tetrahymena pyriformis* relative to casein. They found significant increases in nutritive value as a result of fermenting each type of flour.

The results of protein quality studies suggest that fermentations can improve protein quality, but also that there is no improvement in many instances. Therefore, if fermentation is to be used for this purpose, the ingredients, conditions, and fermentative organisms that can give improvement need to be defined for each case.

Amount and Availability of Limiting Amino Acids

The nutritive value of proteins will depend primarily upon the amount and availability of the limiting essential amino acid in a food. There is the possibility that during fermentation the total amount of any particular amino acid may increase or decrease or that the availability may change significantly. For most foods, including those that are fermented, the limiting amino acids are lysine or the sulfur amino acids.

In an investigation of several types of fermented milks, Rao *et al.* (1982) found that both lysine and the sulfur amino acids tended to decline as a result of fermentation. Lysine decreased by nearly 40% when skim milk was fermented by *Lactobacillus acidophilus*. Buttermilk was the only product to show an increase in lysine during fermentation. The largest methionine loss, 30%, occurred when whole milk was fermented by *L. acidophilus*. Several studies of tempeh fermentations generally showed little change in either lysine or methionine (Wang *et al.* 1968; Stillings and Hackler 1965; Kao and Robinson 1978; Murata *et al.* 1967). Lactic acid fermentation of dry sausages also showed no change in these amino acids.

An instance in which fermentation increased the level of the most limiting amino acid was a 60% increase in methionine during the preparation of idli from black gram (Padhye and Salunkhe 1978).

Table 16.3. Modified Essential Amino Acid (MEAA) Indexes and Percentage Digestible Crude Protein of Single-Cell Protein (SCP) from Certain Lactobacilli

SCP Source	MEAA index	Digestible crude protein (%)
Casein	91	98.5 ± 0.2
<i>L. acidophilus</i> 3532	73	79.3 ± 0.5
<i>L. acidophilus</i> 3205	86	83.7 ± 0.5
<i>L. bulgaricus</i> 2217	76	89.2 ± 0.1
<i>L. bulgaricus</i> 3533	69	81.3 ± 0.4
<i>L. casei</i> 14435	80	82.3 ± 0.2
<i>L. delbrueckii</i> B-443	80	82.5 ± 0.2
<i>L. fermenti</i> 3954	85	86.5 ± 0.5
<i>L. fermenti</i> 3957	69	81.6 ± 0.5
<i>L. plantarum</i> 14431	59	79.9 ± 0.8
<i>L. plantarum</i> 8014	62	80.6 ± 0.3
<i>L. thermophilus</i> 3863	69	88.0 ± 0.3

Source: Erdman *et al.* (1977).

However, van Veen *et al.* (1967) did not find any change in methionine when idli was fermented to give optimum product quality. Padhye and Salunkhe (1978) attributed the differences between the results of the two studies to variations in preparation techniques and different microflora in a natural fermentation. This points up the need to control the organisms and conditions of fermentation if a positive nutritional effect is to be consistently attained.

There are limited data on the amino acid profiles and content of the organisms that carry out fermentations. Stillings and Hackler (1965) reported that a strain of *Rhizopus oligosporus* was low in most essential amino acids. *Saccharomyces cerevisiae* has an excellent amino acid profile with the exception of a low methionine content (Kihlberg 1972). Erdman *et al.* (1977) found that lactobacilli have good amino acid profiles and good digestibilities (Table 16.3). They are relatively high in lysine and low in methionine, though the methionine content is generally higher than *S. cerevisiae* cells, soy protein, or wheat flour. There were quite large differences in amino acids, both among species and among strains within a single species, indicating a potential for selecting favorable organisms from the standpoint of amino acid profile. Unfortunately, the strains of *L. plantarum* evaluated had both the lowest essential amino acid index and lowest digestibility. This species dominates the later stages of most natural lactic acid fermentations (Pederson 1979).

Information is needed on the amount of protein that is provided by microbial cells in food fermentations and the quality of protein in other organisms to determine whether there may be opportunities to improve overall protein quality of fermented foods with the proteins from the cells of the fermentation microorganisms.

In addition to the amino acid content, the nutritional quality of a food may be improved if, in the process of fermentation, the amino acids become more available. This can occur as a result of proteolytic activity by the fermentation microorganisms. Molds used in food fermentations have active proteolytic enzymes (Ko 1982). Most lactic acid bacteria used in dairy fermentations have limited proteolytic activity, though the species that participate in other lactic acid fermentations appear to have almost no proteolytic activity (Law and Kolstad 1983).

Increases in soluble amino acids during fermentation have been observed in milk products (Alm 1982A; Rao *et al.* 1982), peanuts (Cherry and Beuchat 1976), tempeh (Murata *et al.* 1967; Robinson and Kao 1977), and natural fermentations of corn (Tongnual *et al.* 1982), chickpeas, and cowpeas (Zamora and Fields 1979). Whether increases in free amino acids contribute to the improvement of protein quality remains to be clarified.

EFFECTS OF FERMENTATION ON CHANGES IN VITAMINS

As indicated from the questions in Table 16.1, fermentations may result in changes in vitamin content by several mechanisms, including (1) synthesis of vitamins by the fermentation organisms, (2) loss of vitamins by metabolism of the fermentation organism or the food which undergoes fermentation, (3) loss of vitamins by chemical reactions not directly related to fermentation, (4) increase or decrease in the stability of vitamins as a result of pH changes, and (5) soaking or cooking losses associated with preparation of a product before or after fermentation. We will review the limited information available for the vitamins that have been investigated in fermented foods. Shahani and Chandan (1979) have previously reviewed vitamin changes in cultured dairy products. Smith and Palumbo (1981) have reviewed vitamin changes in a variety of fermented foods.

Riboflavin

Riboflavin changes have been investigated primarily in cereal and legume fermentations. No increase was found in a natural fermentation of chickpeas (Zamora and Fields 1979), in the fermentation of coconut press cake to produce oncom (Reddy *et al.* 1982), and in one study of idli fermentation (van Veen *et al.* 1967). Riboflavin concentration was also unchanged after fermentation of milk with several lactic acid bacteria (Alm 1982B). However, increases in riboflavin have been the most often observed result of fermentation. Products in which increases have been observed are (1) tempeh prepared from soybean (Roelofsen and Talens 1964; Murata *et al.* 1967; van Veen and Steinkraus 1970;

Robinson and Kao 1977), chickpea, and horsebean (Robinson and Kao 1977), (2) miso prepared from soybeans, chickpeas, or horsebeans (Robinson and Kao 1977), (3) idli (Rajalakshmi and Vanja 1967; Ramakrishnan *et al.* 1976), (4) khaman (Rajalakshmi and Vanaja 1967), (5) ogi (Akinrele 1970), (6) dhokla (Aliya and Geervani 1981), and (7) ambali (Aliya and Geervani 1981). Aliya and Geervani (1981) observed decreases in riboflavin when products were steamed after fermentation.

There have been few data which indicate the specific organisms or reactions leading to increases in riboflavin concentration. Akinrele (1970) sterilized ogi batter and inoculated it with either *Aerobacter cloacae* or *L. plantarum* and compared the vitamin content with a natural fermentation and a nonfermented control. *Aerobacter cloacae* caused a doubling of riboflavin compared to the controls, while the vitamin concentration decreased in the *L. plantarum*-inoculated sample. This result suggested that *A. cloacae* was the microorganism responsible for the fact that, after a natural fermentation, the riboflavin content was slightly higher than the nonfermented batter.

Extensive studies have been carried out to investigate the characteristics of idli prepared with soybeans replacing the traditional blackgram (Ramakrishnan *et al.* 1976). Changes in thiamin, niacin, and riboflavin were measured after pure culture fermentations of idli batter with microorganisms isolated from natural fermentations, including lactobacilli, *Streptococcus faecalis*, and *Aerobacter aerogenes*. A 2.5-fold increase in riboflavin occurred during natural fermentation. Fermentation with *Lactobacillus delbrueckii* resulted in a riboflavin concentration equal to the natural fermentation. Fermentations with other lactobacilli resulted in riboflavin levels intermediate between the sterilized batter and the idli made with *L. delbrueckii*.

Niacin

Niacin, like riboflavin, generally has been found to increase as a result of fermentation, increases up to fivefold have been observed in soy tempeh (Roelofsen and Talens 1964; van Veen and Steinkraus 1970; Robinson and Kao 1977). A time course study by Murata *et al.* (1967) indicated that nicotinic acid concentration continued to increase throughout a 72-hr fermentation (Fig. 16.1). Organoleptically, a 24-hr fermentation tends to give the best quality product.

Increases in niacin have also been observed in natural idli and khaman fermentations (Rajalakshmi and Vanaja 1967; Ramakrishnan *et al.* 1976). Ramakrishnan *et al.* (1976) measured niacin changes in batters fermented with several lactobacilli, *A. aerogenes*, and *S. faecalis*. The niacin content increased significantly above the sterilized control in every case. An unidentified lactobacillus and a *Lactobacillus fermenti*

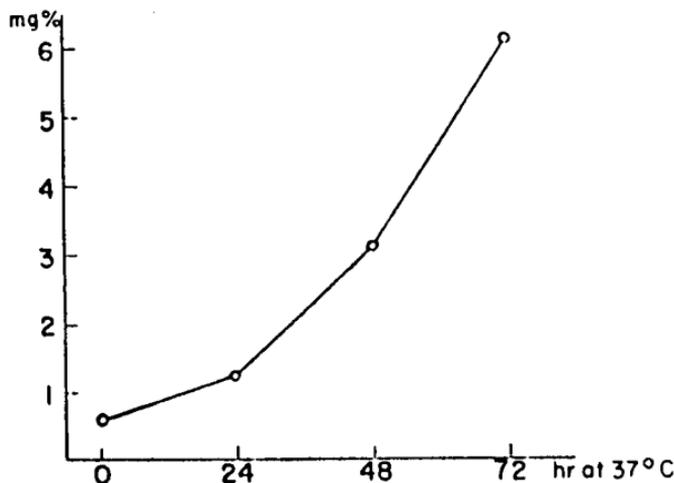


Fig. 16.1. Changes in nicotinic acid content of tempeh during fermentation. Source: Murata *et al.* (1967).

strain caused increases which were similar to the 40% increase found in a natural fermentation.

In ogi fermentations, Akinrele (1970) obtained a 25% increase in niacin concentration with a traditional fermentation. Inoculation of a sterile batter with *L. plantarum* isolated from ogi caused no change, but inoculation with *A. cloacae*, which is also found in the natural fermentation, resulted in an 84% increase in niacin.

Shahani and co-workers have studied changes in several B vitamins during the manufacture of cottage cheese (Reif *et al.* 1976) and Cheddar cheese (Nilson *et al.* 1965). In both studies, the vitamin retention in the cheese relative to the starting milk was evaluated. The effect of draining and washing cheese curd on the retention of niacin in the curd was evaluated. Only 22% of the total niacin in the milk was retained in Cheddar curd. However, the niacin concentration in the curd was doubled relative to the concentration in the milk due to the loss of whey. In cottage cheese, 63% of the niacin from the skim milk was retained in the curd, and the concentration of the niacin in the curd was 3.6-fold greater than in the milk.

During fermentation and aging of Cheddar cheese (Nilson *et al.* 1965), niacin increased about 25% in the first month, remained nearly constant from 1 to 6 months, and then increased slowly from 6 to 12 months if the storage temperature was 10°C or higher (Fig. 16.2). More than a doubling of niacin in the cheese could be induced if lactose was added early in the ripening period (Fig. 16.3). Vitamin B₆ also increased after lactose addition. This was attributed to increased microbial activity as a result of extra substrate availability. Only a slight increase in niacin content was observed as a result of the activity of the cottage cheese starter culture. However, almost a doubling of the niacin content of the curd was obtained by the addition of rennet. No explanation of this effect was given.

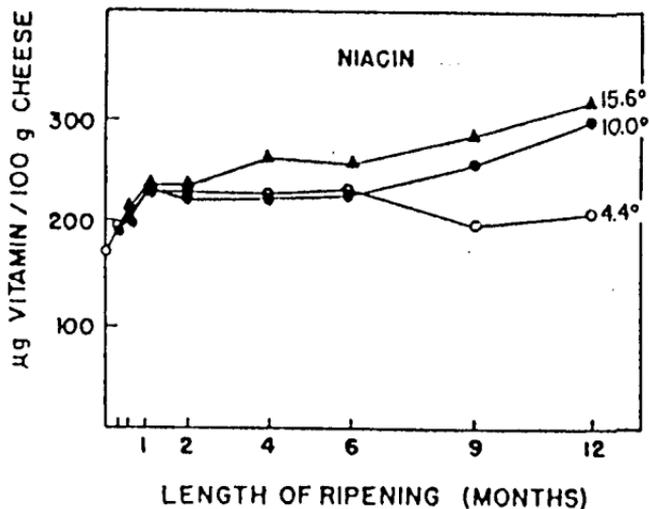


Fig. 16.2. Effect of temperature and length of ripening upon niacin content of Cheddar cheese. Source: Nilson *et al.* (1965).

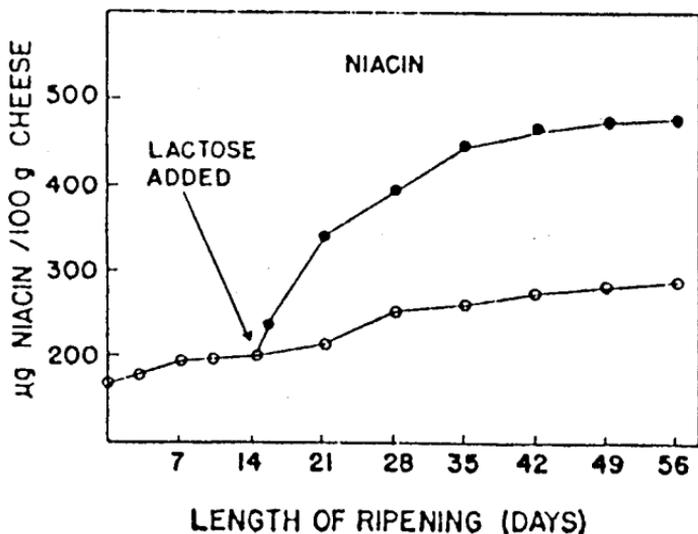


Fig. 16.3. Relationship between lactose metabolism and the biosynthesis of niacin in Cheddar cheese. Source: Nilson *et al.* (1965).

Alm (1982A) found almost no change in the niacin content of fermented milks prepared with the usual cultures of lactic acid bacteria. Costilow and Fabian (1953) found no substantial changes in the niacin content of cucumber brine after pure culture fermentations with *L. plantarum* and four yeasts, which had been isolated from cucumber fermentations. Zamora and Fields (1979) observed an unusual case in which a significant decrease of niacin occurred during a natural fermentation of cowpeas and chickpeas.

Folic Acid

Studies of folic acid changes have been limited, probably due to the difficulties in the assay of the different forms of this vitamin. Rao (1961) reported a 59% increase of folate in fermented steamed idli compared to the nonfermented starting material. Akinrele (1970) observed no change in folic acid in ogi fermentations.

In their studies of cheese fermentations, Shahani and co-workers found an increase in folic acid from 1 to 14 $\mu\text{g}/100\text{ g}$ during a 16-hr fermentation of cottage cheese (Reif *et al.* 1976; Fig. 16.4). As a result of this synthesis, there was over 10 times more folic acid in the cottage cheese than in the skim milk used in the manufacture of the cheese. Folic acid concentration also tripled during the first week of Cheddar cheese ripening (Nilson *et al.* 1965; Fig. 16.5). However, after the initial increase, it decreased until after 2 months of aging, the folate level was the same as at the beginning of aging. Alm (1982A) found large increases in folic acid in all of the fermented milks she prepared,

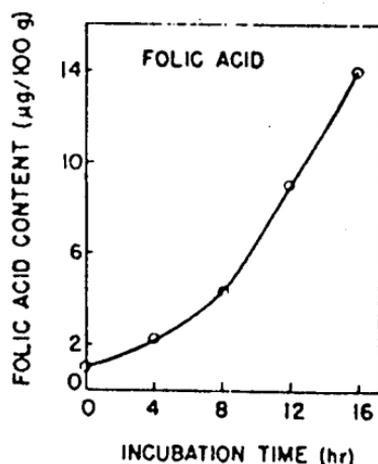


Fig. 16.4. Biosynthesis of folic acid by cottage cheese starter culture. Source: Reif *et al.* (1976).

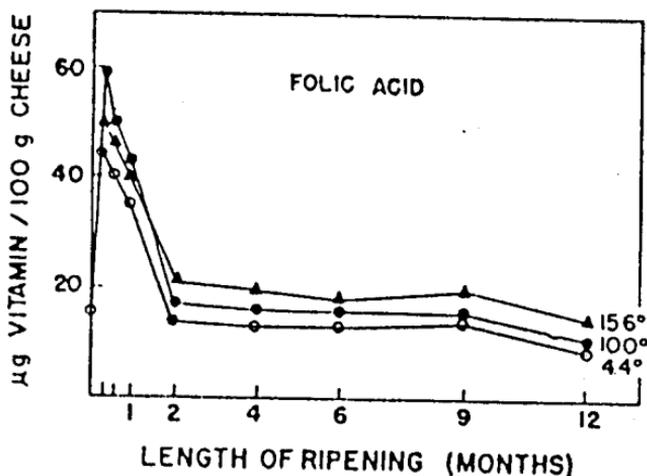


Fig. 16.5. Effect of temperature and length of ripening upon folic acid of Cheddar cheese. Source: Nilson *et al.* (1965).

except acidophilus milk, which showed over a 30% decline. It appears that folic acid can be synthesized by a number of the organisms used in the fermentation of dairy products.

Thiamin

Increases of thiamin from 30 to 150% were observed in dhokla and ambali fermentations (Aliya and Geervani 1981). Thiamin decreased when the fermented batter was steamed, but fermentation after steaming resulted in the thiamin content returning to levels equal to or greater than before steaming. Rajalakshmi and Vanja (1967) found a 176% increase of thiamin in the fermentation of idli and a 49% increase in khaman. Thiamin content also increased in soy idli with either a natural fermentation or fermentation of sterilized batters inoculated with lactobacilli or *S. faecalis*. *Lactobacillus delbrueckii* caused the largest increase, just as it did for riboflavin. *Aerobacter aerogenes* caused a decrease of thiamin. Akinrele (1970) found a doubling of thiamin in a natural ogi fermentation. However, he was unable to show an increase in inoculated fermentations. Thiamin decreased when sterilized batters were inoculated with *L. plantarum*, *A. cloacae*, or a combination of these organisms.

Consistent decreases of thiamin have been found as a result of fermentation of soybeans to tempeh (Roelofsen and Talens 1964; Murata *et al.* 1967; van Veen and Steinkraus 1970; Robinson and Kao 1977). No change in thiamin concentration was found as a result of fermentation of chickpeas and horsebeans to tempeh (Robinson and Kao 1977). Zamora and Fields (1979) saw no change in thiamin during a natural fermentation of cowpeas and a 25% decrease during chickpea fermentation. Little or no change in the thiamin occurred as a result of fermentation of milk with different lactobacilli (Alm 1982A).

Vitamin B₁₂

Vitamin B₁₂ is absent or present in extremely low concentrations in foods from plant sources. For people on a vegetarian diet, formation of B₁₂ in a fermented food can be very important. Robinson and Kao (1977) found small increases in B₁₂ in tempeh prepared from soybeans, chickpeas, and horsebeans. Van Veen and Steinkraus (1970) reported an increase of over 30-fold from 0.15 to 5 µg/kg in the B₁₂ concentration of tempeh compared to the starting soybeans. Liem *et al.* (1977) found that tempeh prepared with a pure culture of *R. oligosporus* had very low levels of B₁₂ compared to tempeh prepared by a traditional method. They concluded that the B₁₂ was formed by contaminating bacteria normally present in the traditional procedure. Subsequently, Ro *et al.* (1979) increased the B₁₂ content of kimchi by addition of *Propionibacterium freudenreichii* subsp. *shermanii* to the natural fermentation (Fig. 16.6).

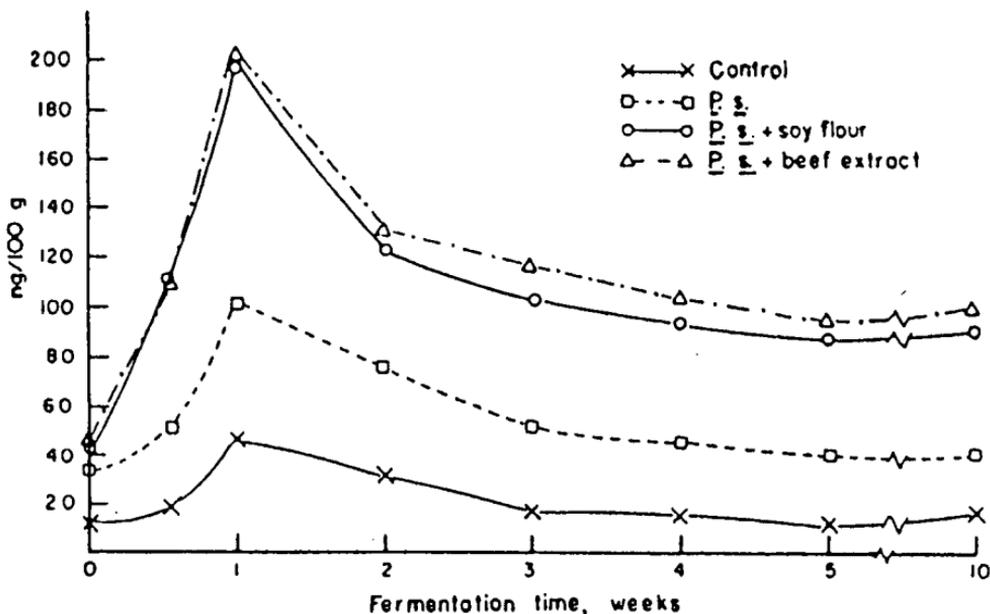


Fig. 16.6. Content of vitamin B₁₂ in control and *Propionibacterium freudenreichii* subsp. *shermanii*-inoculated (*P. s.*) kimchi, with or without soy flour or beef extract, fermented at 4°C. Source: Ro *et al.* (1979).

Milk contains substantial amounts of B₁₂. Alm (1982A) found decreases of up to 50% in yogurt and other fermented milk products inoculated with lactic acid bacteria. In Cheddar cheese, there was little change in the B₁₂ content during 9 months of aging (Nilson *et al.* 1965). However, from 9 to 12 months the vitamin concentration increased. Vitamin B₁₂ increased approximately fourfold during the production of cottage cheese with starter culture.

Vitamin B₆

Murata *et al.* (1967) observed increases in vitamin B₆ concentrations of 4.4- and 14-fold in two batches of soybean tempeh. Figure 16.9 shows the time course of vitamin B₆ changes. Increases of pyridoxine were also found in tempeh and miso prepared from chickpeas, horsepeas, and soybeans (Robinson and Kao 1977).

In dairy fermentations, Alm (1982A) found only slight changes in pyridoxine in milks fermented with lactic acid bacteria. Almost no change occurred in the preparation of cottage cheese (Reif *et al.* 1976). In Cheddar cheese ripening (Fig. 16.7), B₆ concentration increased initially and then declined until, after 2 months, the vitamin level was the same as in the initial curd (Nilson *et al.* 1965). Over the next 10 months, the B₆ concentration gradually increased until the final concentration was two to three times the initial level. When lactose was

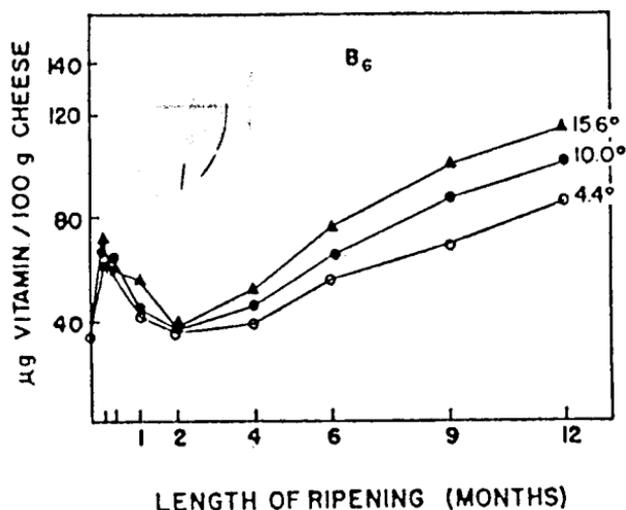


Fig. 16.7. Effect of temperature and length of ripening upon vitamin B₆ of Cheddar cheese. Source: Nilson *et al.* (1965).

added to the cheese during ripening, B₆ increased rapidly and then declined until it was similar to the concentration in the nonsupplemented cheese.

Biotin

Very limited data concerning changes in biotin in food fermentations are available. Only small changes were observed in fermented milks (Alm 1982A). Generally, the biotin concentration declined by less than 20%. During Cheddar cheese ripening (Fig. 16.8), the biotin level increased during the first 2 months by 60%, but then declined such that after 6 months the concentration was less than the initial concentration (Nilson *et al.* 1965).

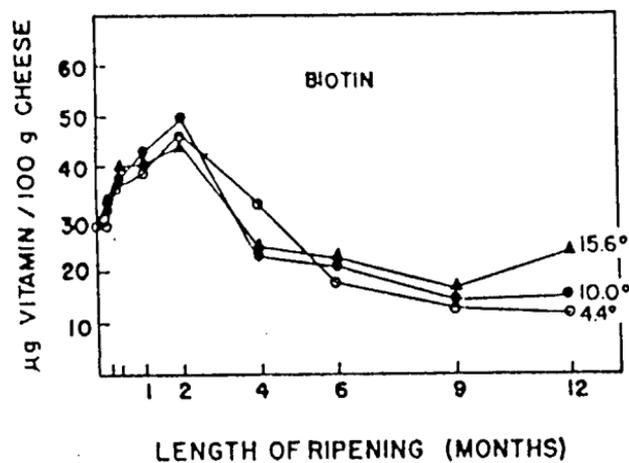


Fig. 16.8. Effect of temperature and length of ripening upon biotin content of Cheddar cheese. Source: Nilson *et al.* (1965).

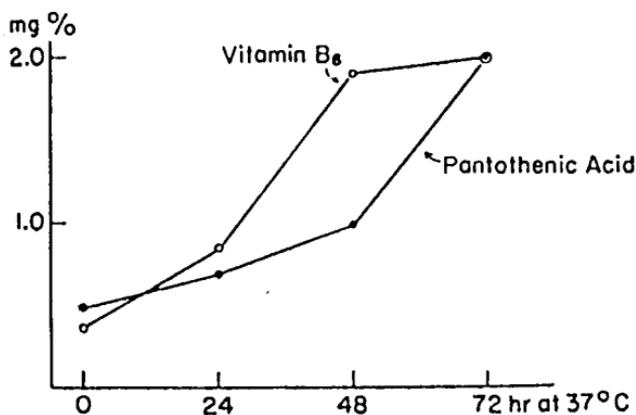


Fig. 16.9. Changes in pantothenic acid and vitamin B₆ content of tempeh during fermentation. Source: Murata *et al.* (1967).

Fermentation of cucumber brine with *L. plantarum* resulted in a 25% decline in biotin, while fermentation with four salt-tolerant yeasts caused decreases of 10–25% (Costilow and Fabian 1953).

Pantothenic Acid

Pantothenic acid did not change in fermented milks, except for a 20–30% decline during the fermentation of yogurt (Alm 1982A). During Cheddar cheese aging, there was a decline for the first 2 months, then a gradual increase. Storage at 15.6°C resulted in almost no net change in the pantothenate content, but there was an overall decline at lower temperatures (Nilson *et al.* 1965).

Van Veen and Steinkraus (1970) found a 28% decrease in pantothenic acid during tempeh fermentation, but Murata *et al.* (1967; Fig. 16.9) and Robinson and Kao (1977) observed substantial increases in tempeh compared to the starting materials. Increases in pantothenate were also found for the production of miso from chickpeas, horsebeans, and soybeans (Robinson and Kao 1977). Pantothenate declined during ogi fermentation whether a traditional fermentation or fermentations with inoculated *A. cloacae* or *L. plantarum* were tried (Akinrele 1970).

Cucumber brine fermented with *L. plantarum* showed a 26% decline in pantothenic acid concentration, while yeast fermentations resulted in 3–17% increases (Costilow and Fabian 1953).

Ascorbic Acid

Ascorbic acid is stabilized by acid conditions and the exclusion of oxygen (Kahn and Martell 1967; Kurata and Sakurai 1967; Huelin *et al.* 1971). Since it is desirable to ferment and store vegetables under these conditions, good retention of ascorbic acid might be expected. However, little is known about the ability of either fermenting vegetable tissue or lactic acid bacteria to metabolize ascorbic acid. Vegetable

materials are often exposed to oxygen during preparation for fermentation and during tank-emptying operations. Also, vegetables may be drained or desalted after fermentation (Jones and Etchells 1944), which may result in large losses of the water-soluble vitamins.

Jones (1975) reported nearly complete loss of ascorbic acid when salt-stock cucumbers were desalted from 8–16% NaCl to 2–4% NaCl for use in finished products. Fellers (1960) found an 86% loss of vitamin C in desalted cucumbers. A range of 1–35 mg ascorbic acid/100 g of sauerkraut was found in commercially canned sauerkraut in the 1950s (Pederson *et al.* 1956). This wide range of concentrations was attributed to variations in handling and processing procedures and to variations in the fresh product. Kimchi, which consists of a fermented mixture of Chinese cabbage, radishes, onions, spices, and sometimes shrimp, decreased 50% in ascorbic acid concentration during the first 5 weeks of fermentation, then remained constant for the next 5 weeks (Ro *et al.* 1979; Fig. 16.10). Lee *et al.* (1960) observed a transient increase of

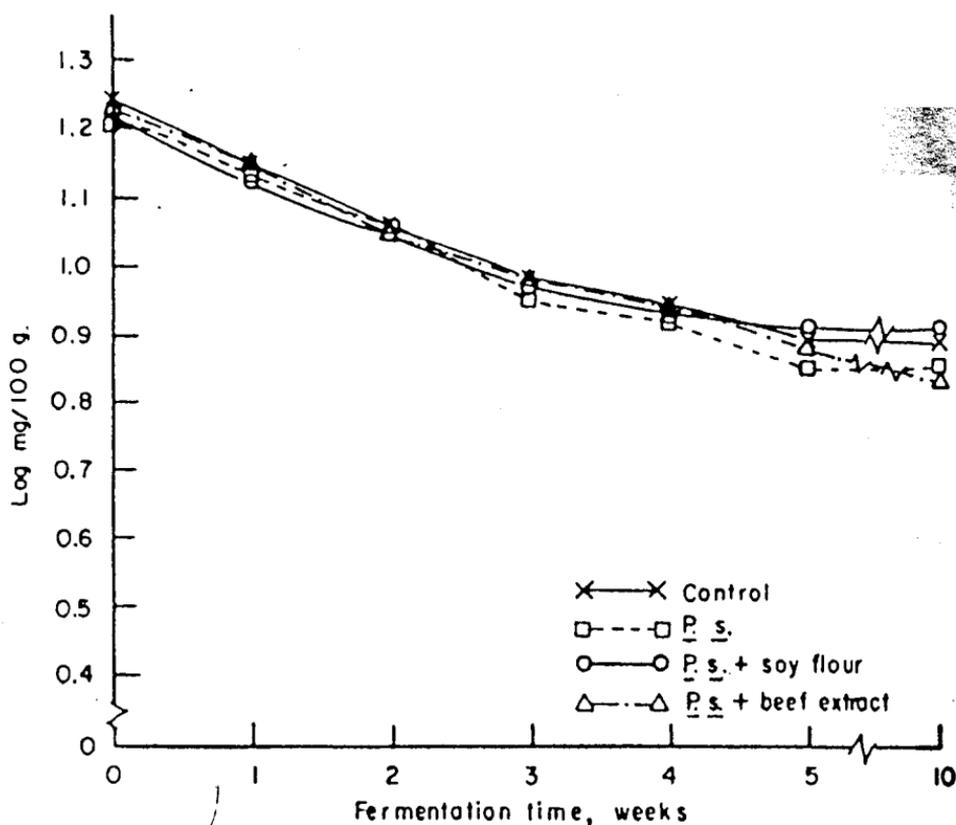


Fig. 16.10. Ascorbic acid content of control and *Propionibacterium freudenreichii* subsp. *shermanii*-inoculated (*P. s.*) kimchi, with or without soy flour or beef extract, and fermented at 4°C. Source: Ro *et al.* (1979).

ascorbic acid in kimchi during the second week of fermentation. Kimchi held at 0°C increased in vitamin C over a 60-day period by about 150% and then gradually declined (Rhie and Chun 1982). Lee and Lee (1981) studied vitamin C changes in radish kimchi fermented at 22°–23°C in a nitrogen atmosphere. Vitamin C initially decreased, then increased and reached a maximum when the kimchi was most acceptable organoleptically, and declined. The increase in vitamin C was attributed to synthesis by the radish enzymes. Addition of galacturonic acid to either kimchi or radish juice fermentation increased the amount of vitamin C formed. Large percentage increases of ascorbic acid were observed in both tempeh and miso prepared from chickpeas, horsebeans, and soybeans (Robinson and Kao 1977).

REMOVAL OF PHYTIC ACID BY FERMENTATION

The presence of high concentrations of phytic acid in cereals and legumes is of nutritional concern because of its apparent ability to reduce the bioavailability of minerals, particularly divalent cations including calcium, zinc, iron, and magnesium (Reddy *et al.* 1982). It has been found in a number of cases that phytates are significantly reduced

Table 16.4. Losses of Phytic Acid Phosphorus during Fermentation and Cooking of Foods

Nature of flour used	Nature of product	Phytic acid phosphorus present originally in flour (mg/100 g)	Amount of phytic acid phosphorus hydrolyzed (mg/100 g)	Phytic acid phosphorus hydrolyzed (%)
White flour (70% extraction)	Bread made with yeast	51	43.4	85.0
National wheat meal (85% extraction)	Bread made with yeast	127	87.6	69.0
Wheat meal (92% extraction)	Bread made with yeast	214	66.3	31.0
	Baking powder bread	214	10.7	5.0
	Steamed pudding	214	34.2	16.0
	Pastry	214	0.0	0.0
White flour with added sodium phytate	Baking powder bread	214	32.1	15.0
	Steamed pudding	214	128.4	60.0
	Pastry	214	32.1	15.0

Source: Calculated from the data of Widdowson (1941). From Reddy *et al.* (1982).

during fermentation processes, as a result of the presence of phytase in the grain itself or production of the enzyme by the fermentation organisms. Thus, in wheat bread, phytic acid was hydrolyzed to a greater extent when yeast rather than baking powder was used to raise the dough (Table 16.4; Widdowson 1941, as calculated by Reddy *et al.* 1982). Ranhotra *et al.* (1974) found complete hydrolysis of phytic acid in wheat bread and over 75% removal in bread prepared from soy-fortified wheat flour. Addition of yeast to Iranian whole wheat meal (Reinhold 1975) or to traditionally unleavened Indian chapaties prepared from whole wheat meal (Swaranjeet *et al.* 1982) also resulted in larger decreases in phytic acid concentration than occurred without yeast addition.

Fermentation of rice-black gram blends to make idli has been found to reduce the phytate content by 30% (Rajalakshmi and Vanaja 1967) and 41% (Reddy and Salunkhe 1980). A 45% decrease in phytate was observed by Ramakrishnan *et al.* (1976) in soy idli in which black gram dal was replaced by soy dal. They fermented sterilized idli batter with a number of bacteria found in natural idli fermentations. Only those bacteria which produced measurable levels of phytase activity caused significant reductions in phytate. *Lactobacillus buchneri*, an unidentified *Bacillus*, and *Microbacterium flavum* reduced the phytate by 16, 39, and 57%, respectively, during a 14-hr fermentation. *Aerobacter aerogenes* and several lactic acid bacteria produced no measurable phytase.

Markakis and co-workers have analyzed phytic acid changes during fermentation of tempeh (Sudarmadji and Markakis 1977) and oncom (Fardiaz and Markakis 1981A). In both studies reduction of phytic acid was attributed to phytase production by the inoculated mold, since the soybeans and peanut press cake were boiled prior to fermentation. A 33% reduction of phytic acid occurred in the tempeh fermentation. *Rhizopus oligosporus* caused almost complete destruction of phytic acid, while fermentation with *Neurospora sitophila* resulted in about a 50% decline.

OUTLOOK

Given the many examples cited in which substantial increases in nutrients have been observed, it seems that there must exist many opportunities to improve the nutritional consequences of food fermentations. The development of genetic transfer and modification technologies for microorganisms would appear to expand these opportunities. Newman *et al.* (1984) have recently described efforts to introduce mutant cultures of *L. plantarum*, which produce high levels of lysine in traditional cereal fermentations, in areas where protein availability is limited.

Morishita *et al.* (1981) have shown that it is possible to mutate lactobacilli to recover their ability to synthesize amino acids. These pathways were apparently inactivated, but not completely lost, during the course of evolution of this group of organisms.

Before the potential for consistent nutritional improvements can be translated into practical manufacturing technologies, much more must be done to establish mechanisms of nutrient synthesis and degradation during fermentations. At its most basic, this must include identification of the microorganisms and substrates responsible for nutrient improvements in important food fermentations.

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NUTRITIONAL EVALUATION OF FOOD PROCESSING

Third Edition

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