

Microorganisms as Food Additives

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ABSTRACT

Microorganisms, both bacteria and fungi, are used as additives in meats, milk, cereals, vegetables and fruits to produce fermented products. The fermented foods differ from the starting material in texture, flavor and keeping quality. Fermentation causes changes in the nutritional content of foods; vitamin and amino acid levels may increase, decrease or remain static, depending on the type of microorganism used and the product fermented. Microorganisms also impart desirable flavors, improve texture and enhance digestibility of foods. Fermentation destroys food spoilage organisms and permits preservation of food. Lactobacilli in cultured milks are used to supplement the normal intestinal flora in individuals suffering from digestive ailments or enteric diseases. Cultured milks are tolerated by lactose-intolerant individuals because of lactose utilization in the gastrointestinal tract by ingested lactobacilli. If sufficient acid is formed, foods which have undergone a lactic acid fermentation, such as fermented sausages or cheese, do not support growth of food poisoning microorganisms. Products which undergo controlled commercial fungal fermentations have been shown not to contain mycotoxins. Histamines and other biogenic amines are present in cheese and other fermented products. Fermentation offers a means of producing safe, nutritious foods with desirable organoleptic qualities and extended storage stability.

Many foods owe their production, flavor, texture, nutritional qualities and/or other characteristics to the activities of microorganisms. Although these changes in the food occurred historically by the accidental introduction of microflora from the environment, in recent years pure culture of specific microorganisms (starter cultures) have been added deliberately to food products under controlled conditions to ensure and to enhance production of the desired products.

Food additives are defined as those substances that may be incorporated during processing of foods to improve or maintain nutritional value, enhance con-

sumer acceptance, improve keeping quality and/or facilitate preparation (154). This definition should also include the deliberate addition of microorganisms to foods to give a desired product.

MEAT, POULTRY AND FISH PRODUCTS

Microbial additives used in meat, poultry and fish products (Tables 1 and 2)

Lactic acid starter cultures are added to meat for reliable and consistent acid production in preparation of fermented sausages. Acids produced by *Lactobacillus plantarum* and/or *Pediococcus cerevisiae* lower the pH and contribute to preservation of the sausage product; the lactic acid gives the tangy flavor as well as denatures the protein, resulting in the texture associated with fermented sausages. Since meats are naturally low in sugar content, a fermentable carbohydrate must be added to the sausage formulation. Employment of starter cultures for sausage production was suggested by Kurk (119) and Jensen and Paddock (104) but the meat industry has been slow to adopt their use. Only about half of the United States processors of fermented sausages use pure bacterial cultures in formulation of their fermented sausage products (10). The *Micrococcus* species added to European dry sausages reduce nitrate to nitrite to produce cured meat color, reduce spoilage, prevent possible flavor and color defects induced by lactic acid bacteria and decrease processing time (139,143). With the probable elimination of nitrate from meat processing formulae, use of the micrococcal starter culture for meat may no longer be necessary. More recently however, *Micrococcus varians* has been recommended as a starter culture in combination with *P. cerevisiae* in production of dry and semi-dry fermented sausages (85). *P. cerevisiae* produces lactic acid; *M. varians* is a weak producer of acid but its use does improve sausage flavor and color. Micrococcal catalase production removes the peroxide produced in meat by various oxidative mechanisms and eliminates color defects produced by peroxidative reactions. Uses of

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TABLE 1. *Microorganisms used as food additives in meat products.*

Product	Microorganisms added	Reference
Semi-dry fermented sausages		
Lebanon bologna	mixture of <i>Pediococcus cerevisiae</i> and <i>Lactobacillus plantarum</i>	186
Summer sausage	<i>P. cerevisiae</i>	3,47
Summer sausage	<i>P. cerevisiae</i> and <i>L. plantarum</i> mixture	37,109
Cervelat	<i>P. cerevisiae</i>	116
Cervelat	mixture of <i>P. cerevisiae</i> and <i>L. plantarum</i>	106
Thuringer	<i>P. cerevisiae</i>	116
Teewurst	<i>Lactobacillus</i> species	125
Pork roll	<i>P. cerevisiae</i>	131
Dry fermented sausages		
Pepperoni	mixture of <i>P. cerevisiae</i> and <i>L. plantarum</i>	187
Dry sausage	<i>P. cerevisiae</i>	127
European dry sausage	<i>Micrococcus</i> species	140,184
European dry sausage	mixture of <i>Micrococcus</i> species and <i>Lactobacillus</i>	142,184
Salami	mixture of <i>Micrococcus</i> species and <i>Lactobacillus</i> species	185
Salami	<i>L. plantarum</i>	106
Hard salami; genoa	<i>Micrococcus</i> species; mixture of <i>Micrococcus</i> species and <i>P. cerevisiae</i> ; mixture of <i>Micrococcus</i> species and <i>L. plantarum</i>	84,85
Mold ripened salami	<i>Penicillium</i> species; <i>P. janthinellum</i> ; <i>P. simplicissimum</i> ; <i>P. cyclopium</i> ; <i>P. viridicatum</i>	38
Fermented snack sausage		
Hot bar sausage	<i>P. cerevisiae</i>	116
Processed meat products		
Bacon	lactic acid bacteria	8,60,78
Bacon	<i>L. plantarum</i>	201
Canned ham	radiation-killed <i>P. cerevisiae</i>	124
Country style ham	<i>P. cerevisiae</i>	20
Frankfurters	<i>Micrococcus</i> species; <i>Vibrio</i> species	153
Fresh meat		
Ground beef	mixture of <i>Streptococcus lactis</i> and <i>Leuconostoc citrovorum</i>	163,164
Ground beef	<i>S. diacetilactis</i>	45
Ground beef	<i>Lactobacillus bulgaricus</i> ; <i>L. lactis</i> ; <i>P. cerevisiae</i>	72
Ground beef	<i>L. brevis</i>	61
Beef longissimus dorsi steak	mixture of <i>S. lactis</i> and <i>L. citrovorum</i>	163
Whole animal carcass	<i>Thamnidium elegans</i>	11

TABLE 2. *Microorganisms used as additives in poultry products.*

Product	Microorganisms added	Reference
Fermented sausages		
Semi-dry turkey sausage	<i>Pediococcus cerevisiae</i>	108
Dry turkey sausage	<i>P. cerevisiae</i>	18
Dry turkey sausage	mixture of <i>P. cerevisiae</i> and <i>Lactobacillus plantarum</i>	2
Processed poultry products		
Smoked vacuum packed turkey	radiation-killed <i>P. cerevisiae</i>	124
Cooked mechanically deboned poultry meat	<i>P. cerevisiae</i> and/or <i>L. plantarum</i>	158
Fresh poultry meat		
Ground chicken	<i>P. cerevisiae</i> and/or <i>L. plantarum</i>	161
Mechanically deboned poultry meat	<i>P. cerevisiae</i> and/or <i>L. plantarum</i>	160,161
Eggs		
Pasteurized liquid whole egg	<i>P. cerevisiae</i> and/or <i>L. plantarum</i>	159

bacterial starter cultures in fermented sausage manufacture (particularly in Europe) have been reviewed by Coretti (43).

Mold-fermented salamis are available widely in Europe; there are two types: Hungarian (smoked) and Italian (non-smoked). *Penicillium* mold spores are sprayed onto the casing surface and the mycelia are allowed to cover over the salami completely. Although the mold growth is confined to the surface, metabolic by-products from its growth influence the appearance, taste, odor and keeping quality of the sausage. Ciegler et al. (38) used pure cultures of *Penicillium* species to produce salami of good quality.

Lactic acid starter cultures have been suggested as food additives for nonfermented meats for various reasons. Addition of lactic acid bacteria to bacon produces acid from the cure sugar additive, thus reducing residual nitrite levels and nitrosamine formation during bacon frying (8,78). Microbial additives in bacon (201), canned ham and vacuum-packed smoked turkey (124) prevent food pathogen growth and toxin formation if the products are temperature-abused. When the product is kept refrigerated, the added lactic acid bacteria have limited metabolic activity; upon temperature-abuse (inadvertent warming) of the food, the organisms ferment the added sugar to acid preventing the growth of food pathogens. Bartholomew and Blumer (20) have suggested the use of *P. cerevisiae* for production of country cured hams to give a more consistent product and to shorten the aging period. Spraying of *Thamnidium elegans* mold onto beef carcasses inhibits growth of surface spoilage organisms; consequently the surrounding temperature and humidity can be increased to accelerate the aging process. Not only is there acceleration of aging (2-3 days vs. 14 days), but also the mold imparts a nutty flavor associated with aged beef (11).

Residual nitrite levels were lower in frankfurters produced with a micrococcal starter culture that reduced nitrate to nitrite (153). The author indicated that the flavor was improved in the frankfurters. Use of the additive nitrate in frankfurters has been discontinued, however, obviating the need for such starters. Various lactic acid bacteria have been used to increase the shelf life of ground beef (45,61,72,164), steak (163), ground chicken (161) and mechanically deboned poultry meat (160,161). The mechanism of shelf life extension of meats is unclear. Some workers found a decrease in the product pH (163,164), while other workers noted no change of pH (72,158,161). The combination of *L. plantarum* and *P. cerevisiae* which was effective in repressing growth of spoilage pseudomonads in pasteurized liquid whole egg (159) did not appear to act by changes in pH.

Raccach and Baker (157) indicated that production of H_2O_2 by *L. plantarum* and *P. cerevisiae* was insufficient to explain the increase in shelf life of fresh or cooked mechanically deboned poultry meat or pasteurized liquid whole egg. Inhibition of low-temperature

spoilage microorganisms by lactic acid bacteria may derive from production of acids, H_2O_2 , antibiotics, bacteriocins or a combination of these factors (158).

Burkholder et al. (28) have shown that cultures of *Geotrichum* species or *Candida lipolytica* can be used to convert menhaden (presently used only for fish meal and oil) into an acceptable human food. The microorganisms reduced the fat content by 30-50%, increased the protein content of the fish product and eliminated the fishy odor, resulting in a product with pleasant aroma and flavor. Use of microorganisms may be a method to make "trash" fish acceptable for human consumption.

Effect of microbial additives on nutritive quality of meat, poultry and fish products

The effect of microbial fermentation on the nutritive quality of meat has not been studied. Since lactic acid bacteria (either naturally occurring or added as starter cultures) can alter the nutritive quality including relative nutritive value (RNV) or protein efficiency ratio (PER) as well as the vitamin and amino acid content of foods such as cowpeas (224) and yogurt (1,91,111,162), it is probable that lactic acid bacteria also can alter the nutritive quality of meat during fermentation. However, experimental data are lacking and research is needed in this vital area.

Effect of microbial additives on chemical and microbiological safety in meat, poultry and fish products

Nitrosamines. The nitrosamine, N-nitrosopyrrolidine, has been isolated from fried bacon (80). The level of residual nitrite and the subsequent nitrosamine formation can be reduced by acidification of bacon before it is fried. If, during processing, the bacon is pumped with curing pickle containing lactic acid bacteria and a fermentable carbohydrate, the lactic acid bacteria ferment the sugar to lactic acid leading to reduction of the level of residual nitrite and reduced levels of N-nitrosopyrrolidine when the bacon is fried (78). Nitrosamine formation was not detected in lebanon bologna (148), thuringer (48) and European dry sausages (117).

Biogenic amines. Biogenic amines arise in foods from the metabolic activity of microorganisms, more specifically due to the action of amino acid decarboxylases. Presence of biogenic amines such as histamine or tyramine in foods has been reviewed (169).

Rice and Koehler (168) found that the tyrosine and histidine decarboxylase activity of the commercial meat lactic acid starter cultures, *P. cerevisiae* and *L. plantarum*, was quite low. Higher levels of amino acid decarboxylase activity were present in sausages undergoing natural flora fermentation in comparison with those fermented by *P. cerevisiae* (49). Use of *P. cerevisiae* in production of fermented sausages decreased the possibility of microflora, naturally present in the meat environment, that might have proteolytic and amino acid decarboxylase activity. Elimination of the undesirable microflora prevented formation of dangerous levels of biogenic amines. Commercial meat starter cultures used

in the United States are probably low in histidine and tyrosine decarboxylase (49,168); however, lactic acid bacteria proposed for use in meat (or any other food product) should be screened for amino acid decarboxylase activity.

Food-borne pathogens and/or toxins in fermented meat products. Because microorganisms cannot be excluded from fresh meats and the meat used for fermented sausage processing cannot be heated to eliminate spoilage organisms or pathogens, meat products may spoil or become hazardous if the lactic acid bacteria do not initiate a rapid pH drop.

Goepfert and Chung (74), studying the growth and survival of *Salmonella typhimurium* in simulated thuringer sausage (beaker sausage), found that low pH in conjunction with NaCl was the most important factor in destruction of salmonellae. Either *P. cerevisiae* or a *Lactobacillus* species, if used as starter culture, was effective in reducing the pH. Although the combination of low pH and salt was effective in reducing numbers of salmonellae when the initial level was 10^3 to 10^5 /g, it did not always produce a salmonella-free product. The authors recommended heating of fermented sausages to give a salmonella-free product because of the failure of the starter cultures to eliminate *Salmonella* species.

Lebanon bologna was usually free of *Salmonella dublin* or *S. typhimurium* (at initial levels of 10^4 /g) following a 4-day fermentation period when either a natural flora or the starter culture, a mixture of *P. cerevisiae* and *L. plantarum*, was used. The pH of these sausages was 4.6 or lower (186).

In pepperoni with an initial level of *S. typhimurium* of 10^4 /g, there was a slight reduction in the number of salmonellae at the end of the fermentation period when starter culture was used (a mixture of *P. cerevisiae* and *L. plantarum*); at the end of the drying period, no viable salmonellae were found (187). With natural-flora fermented or nonfermented pepperoni, *S. typhimurium* survived even the drying period. The final pH of the starter culture-fermented pepperoni was 4.6; natural-flora fermented, 5.0; and non-fermented pepperoni, 5.7. Smith et al. (187) found that *S. dublin* survived the pepperoni processing conditions regardless of the fermentation procedure. Cooking of the pepperoni after fermentation and before drying to an internal temperature of 60 C was recommended to ensure a product free of salmonellae, since the starter culture did not eliminate *S. dublin*.

Using *P. cerevisiae* as the fermenting organism in dry fermented turkey sausage, Baran and Stevenson (17) found that at initial inoculum levels of 10^6 /g, *Salmonella pullorum* or *Salmonella senftenberg* were not eliminated either during sausage fermentation or by subsequent processing of the sausage. Sirviö et al. (184), utilizing European dry sausage, found that *S. senftenberg*, initially present at 10^4 /g, was not detected at 7 days (sausage pH of 4.8) when a mixed starter culture consisting of *Lactobacillus* and *Micrococcus* species was

present. The pathogen was still present at 32 days of ripening when starter was not used (sausage pH of 5.4).

Production of acid by an active starter culture with the concomitant reduction in pH appears to be the principal factor in fermented sausages responsible for destruction of *Salmonella* species. However, as most investigators found that low numbers of salmonellae were often detected at the end of the processing period, the lactic acid bacteria alone cannot always produce a salmonellae-free fermented sausage.

The growth and concomitant toxin production by *Staphylococcus aureus* in dry, fermented sausages (Genoa, Italian dry sausage) has been responsible for a number of food poisoning outbreaks (12,32,33,34,35,36). In preparation of fermented sausages, meat and processing equipment are handled extensively by plant personnel. Most *S. aureus* contamination comes from human contacts; the nose, skin, hands, hair and feces are all common sources of staphylococcal contamination (26) in the fermented sausage processing line.

P. cerevisiae inhibited growth of *S. aureus* in thuringer anaerobically (core of the sausage) but not aerobically (19). Labots (120) found that the anaerobic growth of staphylococci was inhibited by nitrite in Dutch dry sausage; however, nitrite was not effective in inhibiting *S. aureus* growth aerobically. Barber and Deibel's inhibition of the anaerobic growth of *S. aureus* by *P. cerevisiae* reflects, partially, the effect of nitrite. *S. aureus* inoculated into dry fermented turkey sausages was not inactivated by fermentation with *P. cerevisiae* followed by heating (46 C for 5 h) and drying (17). The staphylococci actually showed increases during the processing of the turkey sausages, but enterotoxin B was not found.

Use of *Lactobacillus* and *Micrococcus* species as the mixed starter culture in production of European dry sausages prevented formation of staphylococcal enterotoxin A; the final pH of the sausages was 5.0 to 5.1 (142). In the absence of starter culture, enterotoxin A was formed by *S. aureus* (pH of sausages 5.6). Enterotoxin B was not produced in either the presence or absence of starter culture. Enterotoxin C was present during the early stages of ripening (with or without starter culture), but was not found in the final product (142).

In sausages fermented with a mixed culture of *P. cerevisiae* and *L. plantarum*, destruction of *S. aureus* was related to the concentration of glucose added to the sausage formulation (188). To reduce the population of *S. aureus* from 10^8 to <0.3 /g in the presence of 2% glucose, about 58 h (at 35 C) of fermentation were required; 1% glucose required approximately 100 h and 0.5% glucose required about 130 h of fermentation. In the absence of glucose and/or starter culture, no destruction of *S. aureus* occurred.

Smith and Palumbo (188) observed also that the acid produced by starter culture during the fermentation was injurious to *S. aureus*. More injured cells were noted in sausages fermented with 0.5% glucose than in those

containing 1 or 2% glucose. At high levels of glucose (2%), killing of *S. aureus* by the mixed starter culture was achieved because acid production was at a maximum; injury was minimal. Cells were not injured in the absence of glucose and/or starter culture. Other workers (42,69) reported that injured *S. aureus* can repair injury, grow and produce toxin if removed from the stressed environment and placed in suitable nutrients.

Strict sanitation is necessary for control of growth and subsequent toxin formation of *S. aureus* in fermented sausages by limiting the numbers of staphylococci introduced into the processing environment and raw materials. Use of starter cultures of proven acid-producing ability, with addition of the maximum amount of fermentable carbohydrate consistent with the sausage formulation can also assist in control.

Christiansen et al. (37) inoculated a summer sausage mix with *Clostridium botulinum* spores and studied the influence of starter culture (a mixture of *P. cerevisiae* and *L. plantarum*), glucose (2%), and nitrite level on production of botulinum toxin in the sausages. Results of their study are summarized in Table 3. When starter culture, glucose and 50 ppm of nitrite were present, sausages did not contain toxin. Sausages lacking starter culture but containing glucose and nitrite were also toxin-free; the natural lactic microflora of the meat fermented glucose to acid and contributed to inhibition of botulinum toxin production. Omission of nitrite or glucose led to toxic sausages. Thus lactic acid bacteria were effective in preventing growth and toxin formation by *C. botulinum* only if glucose and nitrite were present.

TABLE 3. Effect of additives on botulinum toxin formation in summer sausage held at 28 C.

Nitrite level (ppm)	Starter culture ^a	Glucose	Number of toxic sausages at 112 days
0	+ ^b	+	2/25
50	+	+	0/25
150	+	+	0/25
0	-	+	8/25
50	-	+	0/25
150	-	+	0/25
0	+	-	22/25
50	+	-	22/25
150	+	-	15/25
0	-	-	20/25
50	-	-	21/25
150	-	-	14/25

Table modified from Christiansen et al. (37).

^aMixture of *P. cerevisiae* and *L. plantarum*.

^b+ indicates that starter culture and/or glucose was added.

- indicates that starter culture and/or glucose not added.

At initial levels of 10^2 to 10^6 /g, *Clostridium perfringens* could not be eliminated completely during processing of fermented dry turkey sausages in which *P. cerevisiae* was the starter organism (17). The extent of

persistence or destruction of *C. perfringens* during fermented sausage manufacture is not known.

The processing conditions employed in the manufacture of fermented sausages appear not to be destructive to viruses. Fermentation by lactic acid starter cultures, nitrite level, drying or heating had little or no effect on destruction of Coxsackie virus, echovirus or poliovirus present in the meat used in sausage manufacture (95,106). Controls are needed to prevent contact of raw materials or equipment used in sausage fermentation with viruses.

In commercial practice, pure cultures of mold are not used to produce mold-ripened salami (38). Various species of *Penicillium* are found in both Hungarian and Italian mold-ripened salamis. Ciegler et al. (38) studied the potential for the production of penicillic acid in mold-ripened salamis by inoculating the sausages with cultures of *Penicillium* known to produce penicillic acid in broth. Even after 70 days of ripening, no trace of penicillic acid was detected in the sausages. While *Penicillium* species predominate in mold-ripened salami, occasionally other fungi take over the ripening process. Bullerman et al. (29) inoculated the surface of Hungarian or Italian salamis with a toxigenic strain of *Aspergillus flavus*. Where aflatoxins were found, levels were approximately 3 µg/g. On a rice substrate, however, toxin production was 330 to 480 µg/g, indicating that meat does not appear to be a good substrate for aflatoxin production.

Limited amount of work has been done on mycotoxin production in mold-ripened salami. The work of Ciegler et al. (38) and Bullerman et al. (29) suggests that mycotoxin production may not be a problem. Use of pure cultures of the desired *Penicillium* species (which have been screened to eliminate mycotoxin producers) would prevent the chance contamination by undesirable molds which might be toxigenic, and, in addition, would give a uniform consistent product.

Prevention of the growth and/or toxin formation by food-borne pathogens in processed or fresh meat products and poultry products

Lactic acid bacteria have been used as additives in meat and poultry products to prevent growth and/or toxin formation by foodborne pathogens under temperature abuse conditions. Daly et al. (45) showed that the addition of *Streptococcus lactis* subsp. *diacetylactis* to ham sandwich spread was effective in inhibiting growth of *S. aureus* when the spread was incubated at 25 C. Ham and vacuum-packed, smoked turkey inoculated with *C. botulinum* spores did not develop toxin when meat products were formulated to contain radiation-killed *P. cerevisiae* and 1% glucose (124). Under conditions of temperature-abuse (30 C), acid was produced by the radiation-killed pediococci which then prevented growth of *C. botulinum* in the food. Radiation-killed *P. cerevisiae* do not grow but they retain their fermentative capacities. Similarly, ham and turkey inoculated with *S. aureus* and subjected to temperature-abuse showed

destruction of staphylococci as well as lack of enterotoxin A production when radiation-killed *P. cerevisiae* and glucose were present. Products formulated without pediococci and glucose and subjected to temperature-abuse contained botulinal toxin and staphylococcal enterotoxin (124).

Bartholomew and Blumer (20) pumped hams with *P. cerevisiae* and glucose and found that growth of the pediococci (with acid production) prevented proliferation of coagulase-negative *S. aureus*, the predominant flora in cured and aged hams. Presumably *P. cerevisiae* would also prevent multiplication of coagulase-positive *S. aureus*. Bartholomew and Blumer, however, did not study the effect of their process on the growth of foodborne pathogens.

Raccach and Baker (158) showed that addition of a mixed culture of *P. cerevisiae* and *L. plantarum* to cooked, mechanically deboned poultry meat was effective in preventing growth of *S. typhimurium*. In control poultry meat incubated at 11 C, *S. typhimurium* increased from 10^3 /g to 10^8 /g in 7 days; there was no increase in numbers when the mixed starter culture was present. *S. aureus* inoculated into cooked, mechanically deboned poultry meat increased from 10^3 /g to 10^7 /g in 7 days; in the presence of a mixed culture of *P. cerevisiae* and *L. plantarum*, there was no increase in staphylococci numbers (158). The work of Raccach and Baker is interesting because glucose was not added to the poultry product and there was little change in pH. Therefore, the lactic acid bacteria inhibited *S. typhimurium* and *S. aureus* by a mechanism other than acid formation. The inhibitory effect on the foodborne pathogens was not from H_2O_2 production, since *L. plantarum* and *P. cerevisiae* do not accumulate H_2O_2 (157).

P. cerevisiae or *L. plantarum* or a mixture of the two cultures did not repress growth of *S. typhimurium* in pasteurized liquid whole egg (159) at pH 7.4, the normal pH of the liquid egg. When the pH was reduced to 6.8, salmonellae were repressed by lactic acid bacteria. In 7 days at 11 C, control pasteurized liquid whole egg (pH 6.8) inoculated with *S. typhimurium* showed an increase of salmonellae from 10^4 /g to 10^8 /g; egg product containing *P. cerevisiae* showed a salmonellae increase to only 10^6 /g in the 7-day period. *L. plantarum* was not effective in repressing growth of salmonellae (159). On further incubation, *S. typhimurium* growth in the presence of *P. cerevisiae* would probably reach a level comparable to that of the control. Use of lactic acid bacteria to protect pasteurized liquid whole egg appears to have limited usefulness.

Tanaka et al. (201) have shown that in bacon containing 10^3 *C. botulinum* spores/g, inoculation with *L. plantarum* and 0.9% sucrose gave a product that did not develop botulinal toxin upon temperature-abuse at 27 C for 8 weeks. Nitrite at 120 ppm was not always effective in preventing toxin formation when a fermentable carbohydrate was absent. Tanaka and his coworkers stated that the natural flora of bacon did not always

produce sufficient acid from the added sucrose and recommended addition of *L. plantarum* to ensure *C. botulinum* control. In addition to protecting the bacon from developing toxin during temperature-abuse, acid produced by *L. plantarum* from sucrose during bacon processing reduced the residual level of nitrite and prevented formation of nitrosamine during the frying of bacon (78).

Daly et al. (45) found that *S. lactis* subsp. *diacetylactis* inhibited growth of *S. aureus* added to ground beef held at 25 C. Ground beef containing *C. botulinum* spores or *S. aureus* and incubated at 30 C was free of toxin produced by either organism when the ground beef was formulated with radiation-killed *P. cerevisiae* and glucose. Growth of both pathogens was prevented and the numbers of *S. aureus* decreased to undetectable levels (124).

When *S. lactis* subsp. *diacetylactis* was present, growth of *S. aureus* was prevented in certain temperature abused food products, e.g., vanilla cream filling, chicken gravy, and soy milk (45).

DAIRY PRODUCTS

Microbial additives used in dairy products (Table 4)

The prime role of the streptococci and lactobacilli in cheese-making and manufacture of fermented milks is to provide lactic acid. In fermented milks, lactic acid prevents growth of undesirable microorganisms, curdles the milk and provides an acid flavor. In cottage cheese, cream dressing for cottage cheese, cream cheese, buttermilk, sour cream and butter, the acid conditions induced by lactic acid bacteria are necessary for development of maximum amounts of flavor compounds by *Leuconostoc* species. *Leuconostoc* species or *S. lactis* subsp. *diacetylactis* produce the flavor constituents, diacetyl and acetic acid, from the citric acid normally present in milk (64,180).

The role of lactic acid bacteria in cheese-making is to produce lactic acid to suppress growth of undesired microorganisms that cause flavor defects in the finished cheese. Additionally, the acid condition speeds up the action of rennet and aids in syneresis (expulsion of whey from the curd). This decrease in water content improves the keeping quality of cheese. The lactic acid bacteria also have proteolytic activity which contributes to flavor and texture of the finished cheese (180).

Other microorganisms as well as lactic acid bacteria are used as additives to dairy products. Propionibacteria are important in the manufacture of Swiss cheese; propionic and acetic acids and CO_2 are formed from lactic acid. Propionic and acetic acids contribute to the desired "nutty" flavor and the carbon dioxide gas forms the holes or "eyes" (180). In blue-veined blue or Roquefort cheeses, the mold *Penicillium roqueforti*, in addition to lactic acid bacteria, is added to milk before manufacture. After the solid cheese is hooped it is

TABLE 4. *Microorganisms used as additives in dairy products.*

Product	Microorganisms added	Reference
Cheese		
Parmesan, Romano	mixture of <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>	165
Cheddar, Colby	<i>S. lactis</i> ; <i>S. cremoris</i>	39,88,222
Swiss, Emmenthaler	mixture of <i>L. bulgaricus</i> (or <i>L. lactis</i> or <i>L. helveticus</i>) and <i>S. thermophilus</i> and <i>Propionibacterium shermanii</i>	88,152,166
Provolone	mixture of heat-resistant <i>Lactobacillus</i> species and <i>S. thermophilus</i>	165
Blue, Gorgonzola, Roquefort, Stilton	<i>S. lactis</i> plus <i>Penicillium roqueforti</i>	64,88
Camembert	<i>lactic streptococci</i> plus <i>Penicillium camemberti</i>	64,88
Brick, Limburger	mixture of <i>S. thermophilus</i> and <i>S. cremoris</i> ; mixture of <i>S. thermophilus</i> and <i>L. bulgaricus</i>	145
Brick, Limburger	mixture of <i>S. lactis</i> and <i>S. thermophilus</i>	64,88
Muenster	mixture of <i>S. thermophilus</i> and <i>Lactobacillus</i> species	145
Gouda, Edam	<i>S. lactis</i>	64,79
Mozzarella	mixture of heat resistant <i>Lactobacillus</i> species and <i>S. thermophilus</i>	165
Cottage cheese, cream cheese	<i>S. lactis</i> or <i>S. cremoris</i> ; mixture of <i>S. lactis</i> or <i>S. cremoris</i> and <i>S. diacetylactis</i> (or <i>Leuconostoc</i> species)	52,152
Fermented milks		
Bulgarian buttermilk	<i>L. bulgaricus</i>	180
Acidophilus milk	<i>L. acidophilus</i>	52
Buttermilk, sour cream	<i>S. lactis</i> ; mixture of <i>S. cremoris</i> and <i>Leuconostoc citrovorum</i> (or <i>L. dextranicum</i>)	64
Yogurt	mixture of <i>L. bulgaricus</i> and <i>S. thermophilus</i>	52
Milk		
Fresh milk	<i>S. diacetylactis</i>	45
Fresh milk	mixture of <i>S. diacetylactis</i> and <i>Leuconostoc cremoris</i>	220
Fresh milk	<i>L. acidophilus</i>	73,192
10% non-fat milk solids	<i>L. bulgaricus</i>	72
25 and 40% non-fat milk solids	<i>L. acidophilus</i>	54
Miscellaneous		
Butter	mixture of <i>S. diacetylactis</i> and <i>S. lactis</i>	64
Cream dressing for cottage cheese	<i>S. lactis</i> ; <i>S. cremoris</i> ; <i>S. diacetylactis</i>	51

pierced with needles to allow growth of the aerobic mold throughout the cheese. Mold growth uses some of the lactic acid in the cheese, raising the pH toward neutrality. Flavor development induced by *P. roqueforti* involves lipolysis, proteolysis and production of various methyl ketones (64). A white mutant of *P. roqueforti* produces cheese identical except for color to that produced by the blue mold (115). A related *Penicillium* mold is used for Camembert cheese production. This mold, *Penicillium camemberti*, grows only on the cheese surface, producing extracellular proteolytic enzymes that migrate toward the interior of the cheese and produce softening. Use by the mold of these proteolytic products and lactic acid raise the pH and produce various ammoniacal flavor components. A secondary fermentation involving yeast and bacteria also occurs (64).

Cheese such as brick and Limburger are naturally surface ripened by the action of *Brevibacterium linens* that is not added to the milk or cheese (145). Tuckey et

al. (206), however, did add cultures of an oxidative yeast and *B. linens* to the lactic acid bacteria during manufacture of Limburger.

In addition to providing flavor, starter organisms in cream dressing for cottage cheese increased the shelf life of the cottage cheese (51). This increased shelf life was derived from competitive growth inhibition of spoilage bacteria by the lactic acid organisms and was related directly to the quantity of *S. lactis* subsp. *diacetylactis* present in the finished product.

Acidophilus milk, used as a dietary adjunct, has an extremely sour taste that many people find objectionable. Gilliland et al. (73), Espina and Packard (54) and Speck (192) indicated that fresh acidophilus milk can be utilized in the diet instead of the fermented product. The purpose of ingestion of acidophilus milk is to supplant the intestinal flora present in some humans with the more desirable *Lactobacillus acidophilus* organisms. In the fresh acidophilus milk product, the milk acting as a

carrier medium is inoculated with high levels of *L. acidophilus* but is not allowed to ferment. The keeping qualities of refrigerated fresh acidophilus milk are similar to those of normal fresh milk.

Various investigators (45,72,220) reported that addition of lactic acid bacteria to milk increased shelf life, apparently unrelated to pH change. Gilliland and Speck (72) indicated that H₂O₂ produced by *Lactobacillus bulgaricus* inhibited growth of psychrotropic bacteria in milk, but inhibition was abolished by addition of catalase. Juffs and Babel (105) also demonstrated that commercial starter cultures prevented growth of low temperature spoilage organisms with the inhibitory effect eliminated by addition of catalase.

Effect of microbial additives on nutritive quality of dairy products

Effect of microbial fermentation on the nutritive quality of dairy products. It is generally assumed that the nutritional quality of cultured milk products is approximately similar to that of the milk from which they are made. Reddy et al. (162) and Kilara and Shahani (111) showed that there is a large increase in the folic acid content of yogurt and sour cream as compared to the unfermented starting materials. However, the levels of niacin, pantothenic acid, biotin, B₆, and B₁₂ either did not change or were reduced slightly. Acott and Labuza (1), comparing yogurt to whole milk, found substantial decreases in vitamin A, thiamin, riboflavin, biotin, B₁₂, choline and ascorbic acid, but an increase in nicotinic acid. The decreases in many of the B-vitamins were not unexpected since the lactic acid bacteria are known to have stringent growth requirements for B-vitamins.

By using a simulated gastric digestion system (pepsin), Breslaw and Kleyn (25) found that the protein of yogurt was more easily digested than that of the raw mix. Protein from finished yogurt was digested in about one-half the time required for protein from the raw starting materials.

Hargrove and Alford (91) studied the growth response of rats to a variety of cultured milks. Yogurt consistently gave greater weight gains than the control milk. The weight gain with Bulgarian buttermilk, lactic buttermilk, kefir, acidophilus milk and sweet acidophilus milk was the same as that with the control milk. Milk directly acidified with lactic acid resulted in reduced weight gains in rats. Thus feeding yogurt to healthy growing rats offered a distinct nutritional advantage. Various aspects of the nutritional and healthful benefits of cultured dairy products have been reviewed (181).

Cultured milk products as dietary adjuncts. The beneficial activities of certain lactobacilli in the intestinal tract led to their use as dietary adjuncts. Gilliland et al. (73) and Hargrove and Alford (91) showed that ingestion of acidophilus milk leads to colonization of *L. acidophilus* in the intestinal tract of humans and animals. Speck (190), Sandine (172) and Gilliland (71) discussed the relationship between lactobacilli, particularly *L. acidophilus*, and the microflora of the gut.

Hawley et al. (93) and Sandine et al. (173) reviewed the use of lactobacilli (with emphasis on *L. acidophilus*) in controlling enteric diseases in both animals and man. In recent research, Daniels et al. (46) showed that cultured colostrum was effective in preventing scouring in calves. Feeding colostrum plus *Lactobacillus lactis* prevented establishment of enteropathogenic *Escherichia coli* and subsequent scouring in piglets; use of the lactobacilli alone delayed scouring but did not prevent it (136).

Lactose-intolerant individuals are able to consume cultured milks even though consumption of unfermented milk leads to intestinal distress (70). Goodenough and Kleyn (77) found that the raw mix for yogurt preparation contained 8.5% lactose, which decreased to 5.6% in the finished product; milk contains approximately 5% lactose. Since yogurt did not produce distress in lactose-intolerant individuals, such distress may be from some factor other than lactose level. Lactase (β -galactosidase) activity of viable starter culture organisms or from lysed organisms may be responsible for the more complete hydrolysis of lactose in the gastrointestinal tract of lactose-intolerant individuals (76,110,191). Data obtained by Goodenough and Kleyn (76) utilizing rats fed yogurt indicated that there was significant survival of the lactic acid starter culture organisms in the upper gastrointestinal tract and significant β -galactosidase activity in rat intestinal extracts. Thus the potential β -galactosidase activity in the intact and lysed bacterial cells found in the gastrointestinal tract could reduce the lactose level of the ingested cultured milk product and might explain tolerance of cultured milks by lactose-intolerant individuals.

Feeding yogurt to mice injected with Ehrlich ascites tumor cells in their peritoneal cavities resulted in inhibition of tumor cell proliferation as well as a reduction in the DNA content of the ascitic fluid (111). Feeding milk, lactose or lactic acid had no tumor inhibitory effect. There was a linear relationship between the amount of yogurt consumed and tumor inhibition (181). The mechanism of the yogurt anti-tumor activity as well as its significance are unknown at the present time.

Effect of microbial additives on chemical and microbiological safety in dairy products

Nitrosamines in cheese. Nitrate is added to certain cheeses, such as Gouda and Edam, to prevent the texture defect caused by the gassy butyric fermentation carried out by *Clostridium tyrobutyricum*. However, the nitrosamine contents of such cheeses are quite low and do not constitute a health hazard (50,79,81).

Biogenic amines in cheese. Voight and Eitenmiller (208) showed that strains of *Streptococcus lactis* and *Leuconostoc cremoris* possess tyrosine decarboxylase; however, they did not find the enzyme in commercial dairy starter cultures. The biogenic amines are decomposed by monoamine and diamine oxidases; monoamine oxidase was present in *S. lactis* and *S. lactis* subsp. *diacetilactis* and diamine oxidase was present in

L. cremoris and *Streptococcus cremoris* (209). While the work of Voight and Eitenmiller (208) indicates that amino acid decarboxylases may be either lacking or low in commercial starter cultures, the cultures used in the dairy industry should be screened to eliminate those that produce tyrosine and histidine decarboxylases. Another aspect of control of biogenic amine formation in cheese would be selecting starter cultures containing mono- and diamine oxidases, since these enzymes would destroy any amine present (209).

Siderophores in cheese. Siderophores are iron-transport compounds produced by microorganisms when grown in a low-iron environment. The siderophores have large stability constants for ferric ion, which enables microorganisms to compete effectively for the environmental complexed iron needed for their metabolic activities (146).

Siderophores have been found in mold-ripened cheeses but not in other types of cheese (146). Since cheese is low in iron, growth of *P. camemberti* or *P. roqueforti* during ripening of cheese leads to the excretion of siderophores. Presence of siderophores in foods such as cheese or saké (or other low iron containing fermented foods) may or may not pose health problems. Ong and Neilands (146) pointed out that microbial siderophores in the intestinal tract may combine with iron and make it more available for absorption by the body. However, it is possible that the siderophores may complex the iron and render it nutritionally unavailable. A bacterial siderophore, pacifarin, inhibits growth of virulent salmonellae in mice (218). However, some siderophores produced by non-pathogenic microorganisms can be utilized by pathogens (219). Degradation or modification of microbial siderophores (which are either catechols or hydroxamic acids) by enzymatic activity in the gastrointestinal tract may lead to products that are toxic to humans (146). Since microorganisms are used extensively in the preparation of foods, the role of microbial siderophores in those foods should be examined further.

Foodborne pathogens and/or toxins in fermented dairy products. Marth (129) reviewed the incidence and control of *Salmonella* in dairy products and concluded that salmonellosis from ingestion of dairy products would not be a health hazard if incidence of contamination of raw milk were low, the milk were pasteurized and the pasteurized (and fermented) product were protected from recontamination.

Hargrove et al. (92) indicated that the rate and amount of acid produced during cheesemaking, the pH and the type and size of inoculum of the starter culture significantly suppressed growth and survival of salmonellae in Cheddar and Colby cheeses. Cheese made with *S. cremoris* was more inhibitory to salmonellae survival than were cheeses made with *S. lactis*. A starter culture inoculum of 3% was more effective than that of 0.5 to 2%. There was no loss of viability in salmonellae when the starter failed to produce acid (pH of such cheeses was around 5.7). At pH 5, cheese with a salmonellae count of

$10^5/g$ showed survival of the pathogen for at least 3 months. Goepfert et al. (75) previously showed a slow rate of decline in numbers of salmonellae in cheese at low pH. Acid conditions in the presence of salt do not always inactivate *Salmonella* nor ensure a product free of salmonellae.

When slow acid-producing strains of *S. lactis* were used to manufacture Cheddar cheese from milk inoculated with *S. typhimurium*, Park et al. (151) found that salmonellae grew rapidly during manufacture of the cheese; limited additional growth occurred during the early part of cheese ripening followed by a decline in salmonellae numbers. Salmonellae survived ripening of cheese for 7 months at 13 C and 10 months at 7 C. Inactive or slow starters lead to extended survival of salmonellae because of high pH and moisture content of the cheese.

Park and Marth (149) inoculated skim milk with *S. typhimurium* and then fermented the milk with a variety of lactic acid bacteria. *S. cremoris*, *S. lactis*, or a mixture of the two cultures repressed growth of salmonellae during fermentation of skim milk at 21 or 30 C but did not completely inactivate the pathogen in the 18-h fermentation period. Both *S. diacetylactis* and *L. cremoris* were less inhibitory to salmonellae than were *S. cremoris* or *S. lactis*. Mixtures of *Streptococcus thermophilus* and *L. bulgaricus* completely inactivated *S. typhimurium* in skim milk fermented at 42 C. *S. thermophilus* was more inhibitory than *L. bulgaricus*. Lactic acid was considered to be the most important factor for inactivation of salmonellae (149). Yogurt containing *S. typhimurium* and fermented for 18 h at 42 C utilizing *S. thermophilus* and *L. bulgaricus* became free of salmonellae (171). Lactic acid was responsible for almost all bactericidal activity against salmonellae present in yogurt, but the low pH and low oxidation-reduction potential augmented the inhibitory activity of lactic acid.

Park et al. (150) and Frank et al. (67) studied the fate of enteropathogenic *E. coli* (EEC) during manufacture and ripening of Camembert cheese utilizing commercial lactic starters. During making of the cheese, there was an increase in EEC numbers followed by a decline when the pH decreased to 4.5 to 4.6. As ripening of Camembert proceeds from the surface into the core of the cheese, lactic acid is degraded and the pH rises. The surface of the ripened cheese will support rapid growth of EEC. As ripening proceeds into the center, the rise in pH provides conditions favorable for EEC growth and survival. Contamination of the surface of ripening Camembert with EEC can lead to an unsafe product because of the favorable growth conditions for the pathogen which was induced by the action of the mold, *P. camemberti*. During the manufacture of brick cheese in which commercial lactic starter cultures were used, EEC showed a rapid increase in numbers, followed by a slow decline during ripening and refrigerated storage (68). In the finished brick cheese, viable EEC were present after 7

weeks of refrigerated storage. The survival of EEC during Camembert and brick cheese manufacture and storage indicates that strict sanitation must be observed in preparation of the cheese to ensure that EEC does not come in contact with ingredients or equipment.

When EEC-containing skim milk was fermented with commercial lactic starter cultures, a combination of low temperature of fermentation (21 C) and a large starter culture inoculum (2%) was effective in controlling EEC (66). Fermentation at 32 C permitted growth of EEC, whereas no growth took place at 21 C. At the low temperature and with a large inoculum, pHs of 4.5 to 4.6 were achieved in 12 to 15 h with complete destruction of EEC. At 32 C, some growth of EEC took place; the pH reached 4.5 to 4.6 in 9 to 12 h. Even though increases in EEC numbers occurred at 32 C, ultimately all pathogens were destroyed; however, growth of pathogens in a food should be discouraged.

Cheddar cheese made from milk contaminated with *Bacillus cereus* and use of commercial starter culture contained high numbers of spores after 52 weeks of ripening; no vegetative cells were found (130). Similarly, fermented milks containing *B. cereus* showed no vegetative cells, but spores were not destroyed by the acid conditions.

Cheese has been implicated in staphylococcal enterotoxin food poisoning outbreaks (133,203,206). Several kinds of cheese have been investigated to determine the growth and survival potential of staphylococci.

Tuckey et al. (206), using commercial lactic starter cultures, prepared Cheddar, Colby, Swiss, and Limburger cheeses from milk containing *S. aureus*. Initially during manufacture, all cheeses supported growth of *S. aureus*; during ripening, staphylococcal numbers decreased, but all the cheeses contained high numbers ($10^5/g$) at the end of the ripening period. More *S. aureus* survived when Cheddar and Colby were ripened at 7 C than at 10 to 12 C.

Reiter et al. (167) showed that staphylococci multiplied rapidly during the early steps of Cheddar cheese manufacture when the starter culture was destroyed by bacteriophage and little destruction of the pathogen occurred during aging. In contrast, normal Cheddar cheese showed rapid declines of *S. aureus* numbers. Staphylococci subjected to sublethal heating (60 to 65 C for 17 sec) in milk were unable to survive Cheddar cheese making operations with lactic starter cultures; the salt and acid prevented growth and survival of injured cells (167).

In Colby or Cheddar cheese prepared from *S. aureus*-containing milk, failure of the starter culture allowed enterotoxin formation (203). Staphylococcal enterotoxin A formation in cheese could be prevented by use of milk with less than 10^3 staphylococci/ml and by use of a starter culture capable of producing a titratable acidity of more than 0.5% in the whey at the milling stage. Zehren and Zehren (225) considered the vat of Cheddar cheese abnormal when the titratable acidity in

the whey was less than 0.4% at milling. When the acidity was above that level, staphylococcal enterotoxin was not found in the cheese.

Enterotoxin was found in brick and Swiss cheeses, depending on the level of *S. aureus* initially present in the milk and on the starter culture used but was not found in mozzarella cheese (when an active starter was used) or in blue cheese even under conditions of starter culture failure (204). The effect of the cheese molds on production and/or destruction of staphylococcal enterotoxin needs further study.

The most effective control of *S. aureus* in cheese is use of staphylococci-free milk, pasteurization and an active starter culture which provides adequate acid production to prevent staphylococcal growth.

Minor and Marth (132) added *S. aureus* to buttermilk, sour cream and yogurt and determined the length of survival of the pathogen during cold storage. Growth did not occur in any of the products. If the numbers of staphylococci added were low ($10^2/g$), the pathogen could not be detected at 24 h. A larger inoculum of staphylococci ($10^5/g$) ensured longer survival: 2-4 days in yogurt, 3-6 days in buttermilk, and 4-7 days in sour cream. Survival of staphylococci was longer in the fermented products stored at 7 C than in those stored at 23 C.

Botulism does not appear to be a problem in fermented milk products or cheese. In fermented milks, the pH is too low to permit growth of *C. botulinum*. In cheese, the combination of salt, low pH and low water activity does not support growth of *C. botulinum*. However, *C. botulinum* can grow and produce toxin in pasteurized cheese spreads (82,107).

Viruses have been transmitted to humans by raw and underpasteurized milk (41). Cliver (40) showed that influenza virus, vesicular stomatitis virus and poliovirus present in milk were not destroyed during manufacture and ripening of Cheddar cheese even when starter culture was used. Proper pasteurization (71.7 C for 15 sec) of the milk did lead to inactivation of these viruses.

P. camemberti strains isolated from commercial Camembert cheese produced cyclopiazonic acid, a mycotoxin toxic to rats, when grown in culture media (123,194). Camembert cheese ripened and stored at 14 to 16 C did not contain cyclopiazonic acid, but cheese ripened at 14 to 16 C and then stored at 25 C (not a normal storage temperature) did contain the mycotoxin (194). LeBars (123) found that *P. camemberti* produced cyclopiazonic acid in culture media over a temperature range of 4 to 25 C, suggesting that temperature-abuse may not be a condition necessary for production of the mycotoxin in Camembert cheese. Mycotoxin was found in 11 of 20 commercial Camembert cheeses and was limited to the outer crust of the cheese; no cyclopiazonic acid was found in the inner part of the cheese (123). While the toxicity of cyclopiazonic acid toward humans is unknown, its presence and significance as a natural contaminant in Camembert cheese (as well as its

production by *P. camemberti*) should be assessed. *P. roqueforti*, also used for cheese manufacture, produces toxic metabolites, including roquefortine, isofumigaclavine A and PR toxin (179). PR toxin, toxic to rats and mice, is unstable in blue cheese, probably because of reaction with free amino acids and amines present in the cheese (177). Lafont et al. (122) examined 100 samples of blue-molded cheeses and found 38 contained the mycotoxin, mycophenolic acid, at levels ranging from 0.01-15 ppm. Strains of *P. roqueforti* isolated from the toxic cheeses produced the mycotoxin in culture media. These workers suggested that *P. roqueforti* strains used in cheese manufacture be screened for their mycophenolic acid-producing capacity.

VEGETABLES AND FRUIT PRODUCTS

Microbial additives used in vegetable and fruit products (Table 5)

Traditional fruit and vegetable fermentations have occurred from microbial populations normally present on the food. More recently, pure culture fermentations have been used in several of these processes. Pickled cucumbers (57), olives (56) and sauerkraut (53) have been produced by addition of *L. plantarum* to the brine containing the vegetables. Lactic fermentation of vegetables represents an economical means of obtaining a product resistant to growth of undesirable microorganisms and prevents spoilage and microbial food poisoning. Wines (with their desirable sensory characteristics) offer a means of preserving fruit juices that can be used as safe and flavorful substitutes when water supplies are unsatisfactory.

Fermentation of cassava corrects a different problem. Cassava contains linamarin, a cyanogenic glucoside, that is toxic (103). Ngaba Lee (138) reported that the lactic acid produced by various species of lactic acid bacteria resulted in removal of the toxic hydrocyanic acid and made the food safe for consumption. However, Okafor (144) found that linamarase, an endogenous enzyme in cassava, is mainly responsible for breakdown of linamarin.

In current commercial practice, sauerkraut (a lactic fermentation of cabbage) is prepared by natural flora fermentation. Such uncontrolled fermentations can lead to inferior or unacceptable products. Engelland (53) demonstrated that satisfactory sauerkraut can be prepared by inoculation of shredded cabbage with *L. plantarum*, but his method has been limited to use in canned sauerkraut.

Certain wines contain increased quantities of malic and tartaric acids, making them acidic in flavor. The malo-lactic fermentation by certain lactic acid bacteria converts malic acid to lactic acid and CO₂ and reduces the perceptible acidity of wines to acceptable levels. Kunkee (118) and Lafon-Lafourcade (121) added selected strains of *Leuconostoc gracile* (*L. oenos*) or *Lactobacillus hilgardii* to wines to control excessive acidity. The yeast *Schizosaccharomyces pombe* has also been used to decrease acidity in wines (21); the yeast cells decompose malic acid to ethanol and CO₂.

The desired stage of ripeness in bananas persists only for a short time; therefore, a significant amount of the fruit is wasted because it cannot be sold or consumed. Fermentation of banana pulp by lactic acid bacteria increases the shelf life of the fruit and may permit greater

TABLE 5. *Microorganisms used as food additives in vegetable and fruit products.*

Product (vegetable or fruit)	Microorganisms	Reference
Pickles		
Carrots	mixture of <i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>Leuconostoc mesenteroides</i> , and <i>Pediococcus cerevisiae</i>	141
Cucumbers	mixture of <i>P. cerevisiae</i> , <i>L. plantarum</i> and <i>L. brevis</i> ; mixture of <i>P. cerevisiae</i> and <i>L. plantarum</i> ; <i>L. plantarum</i>	57,59
Cucumbers-sliced	<i>L. plantarum</i>	63
Cucumbers-diced	<i>L. plantarum</i>	83
Mixed vegetables; green tomatoes; hot cherry peppers	mixture of <i>P. cerevisiae</i> and <i>L. plantarum</i> ; <i>L. plantarum</i>	55
Various vegetables-diced	<i>L. plantarum</i>	83
Olives	<i>L. plantarum</i>	56,58
Sauerkraut (cabbage)	<i>L. plantarum</i>	53
Gari (cassava)	<i>L. plantarum</i> ; mixture of <i>L. plantarum</i> and <i>Streptococcus</i> species; mixture of <i>L. plantarum</i> and <i>L. acidophilus</i>	138
Banana pult	<i>L. bulgaricus</i> ; <i>S. thermophilus</i> ; <i>S. faecalis</i> ; <i>L. fermentum</i> ; <i>Leuconostoc mesenteroides</i>	4
Wines (various fruits; alcoholic fermentation)	<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i> ; <i>Saccharomyces</i> species	7,152
Wines (grape; deacidification)	<i>Leuconostoc gracile</i> (<i>L. oenos</i>); <i>Lactobacillus hilgardii</i> ; <i>Schizosaccharomyces pombe</i>	21,118,121

use of bananas as food (4).

Effect of microbial additives on nutritive quality of vegetable and fruit products

Effect of microbial fermentation on the nutritive quality of vegetable products. The protein content of cassava flour increased from 3.1 to 18% following growth of *Candida tropicalis* on this product. Levels of lysine increased from 1.1 to 7.7%, methionine from 0.7 to 2.7% and cystine from 0.5 to 2.0% (15). The increased nutrient content of the cassava was due to the presence of synthesized yeast biomass. *C. tropicalis* possesses α -amylase which permits the yeast to use the cassava starch directly for its growth. Research is needed to determine if the nutritive value of other starchy vegetables such as potatoes, yams, sweet potatoes, pumpkin and winter squash might be upgraded by fermentation with *C. tropicalis* or by other yeasts.

Effect of microbial additives on chemical and microbiological safety in vegetable and fruit products

Nitrosamines. A number of vegetables which can be fermented or are part of a fermented product (including cabbage, celery and beets) contain high levels, >500 ppm, of nitrate in the fresh state (94,183). Lactic acid bacteria, including *L. plantarum*, which is normally used to ferment vegetables, reduce nitrate to nitrite (189). Therefore, the possibility exists that fermented vegetables can contain nitrosamines; however, Tate and Alexander (202) found none in sauerkraut.

Mycotoxins in wine. Patulin, a mycotoxin produced by *Aspergillus* and *Penicillium* species, has been found in grape juice prepared from mold-infested grapes but was not detected in wines produced from such juice (178). Patulin is either destroyed during the yeast fermentation of the juice or is transformed into another product. Fermentation-modified patulin (if indeed formed) may also be toxic.

Saccharomyces cerevisiae fermentation of apple juice containing ^{14}C -patulin indicated that approximately 90% of the radioactive isotope appeared as unidentified water soluble materials in the fermented juice (195). Very little ^{14}C -patulin was transformed into $^{14}\text{CO}_2$. The toxicity of the unidentified products from yeast fermentation was not determined. The potential toxicity resulting from use of moldy fruit in the production of wine should be investigated more thoroughly.

PLANT SEED PRODUCTS

Microbial additives used in plant seed products (Table 6)

Use of fermentation of grains to provide food and drink predates recorded history. Cereal flours, mixed with water and allowed to ferment before baking, produced breads which were more desirable in taste and texture than unleavened breads. Brewing of grains provided nutritious and satisfying beverages. Some plant seed products such as soybeans in the unfermented state cannot be used extensively as foods. Fermentation of soybeans removes beany flavor, reduces its flatulency and increases its nutritional quality.

Soy or peanut milks (made by extracting soybeans or peanuts with water) have been fermented by a variety of lactic acid bacteria to give products similar to buttermilk or yogurt (9,23,27,135,213). Mital and Steinkraus (134) reviewed the fermentation of soy milk by *Lactobacillus* species. The curd can be precipitated from soy milk to give a fresh curd cheese or tofu which can be further fermented to sufu or soy cheese (89,90,176,211,212). Precipitation of soybean protein with Ca^{++} and fermentation of curd with *Actinomucor elegans* followed by brining and aging is the traditional process for making sufu (212). Proteases extracted from the mold mycelium by the brining step penetrate the tofu cubes to hydrolyze the soy proteins.

Whole soybeans or the residue remaining after formation of soy milk can be fermented with *Rhizopus oligosporus* to give tempeh, a food more satisfying than raw or cooked soybeans (6,212,214).

In manufacture of soy sauce, a koji is prepared by inoculating a soybean-wheat mixture with *Aspergillus soyae* or *Aspergillus oryzae*. The mold growth produces enzymes which reduce starch to fermentable sugars and degrade proteins. After fermentation, the resulting koji is added to a salt solution to produce the moromi which undergoes a secondary fermentation carried out by a natural flora of yeast and bacteria (212). Some manufacturers add pure cultures of strains of *Saccharomyces rouxii*, *Torulopsis* species, and *Pediococcus soyae* to accelerate the fermentation and improve flavor. Yong and Wood (223) prepared an acceptable soy sauce by using *A. oryzae* under aseptic conditions to produce the koji and added a mixture of *S. rouxii* and *L. delbrueckii* at the moromi stage.

In miso manufacture, the koji is usually prepared from rice inoculated with *A. oryzae*. However, Ilany-Feigenbaum et al. (101) have used other plant seeds to produce the miso koji. The fermented koji is mixed with salt and soybeans and a further fermentation by yeast and bacteria takes place (152). Use of pure cultures of strains of *S. rouxii*, *Torulopsis* species, *Pediococcus halophilus*, and *Streptococcus faecalis* reduces the fermentation time of miso manufacture from 6-12 months to 2-3 months (212). Miso has been prepared entirely with starter cultures. The koji, prepared by inoculating rice with *A. oryzae*, was added to salt and soybean grits, inoculated with *S. rouxii*, and allowed to ferment (96,182). An acceptable product was obtained in approximately one-half the normal processing time.

Hamanatto or black beans is prepared by mixing steamed soybeans with parched wheat. The wheat-coated beans are inoculated with *A. oryzae*, and the molded beans are aged in salt brine for several months. A secondary fermentation is carried out by *Streptococcus* and *Pediococcus* species (212).

A koji of rice and *A. oryzae* is used in the fermentation of rice to saké. The koji is analogous to malt in beer production; starches are degraded by the amylases, and proteins undergo proteolysis. The sugars and nitro-

genous compounds produced by *A. oryzae* action are then utilized by the saké yeast in production of alcohol (152).

Arnaud and Farr (14) have found antioxidant materials present in the soy protein fermented by *R. oligosporus* that could be mixed with other foods to give antioxidant protection. Peanut flour and corn meal also have been fermented to improve their nutritive qualities (156,215).

Various aspects of the fungal fermentation of peanut press cake (residue remaining after oil extraction) have been reviewed by Beuchat (22). The natural fermentation carried out by *Neurospora sitophila* or *R. oligosporus* produces the food called ontjom. Wang (210) has used pure cultures of *Neurospora intermedia* to produce ontjom.

Pederson (152) indicated that, in addition to yeast, lactic acid bacteria may also be involved in manufacture of conventional bread. Since these breads are not sour, the action of lactic acid bacteria is unknown. In the manufacture of San Francisco sour dough bread, however, both *Lactobacillus san francisco* and the yeast *Saccharomyces exiguus* are necessary for the fermentation of wheat flour to produce the sour dough bread (113,199). The recommended recipe for San Francisco sour dough bread is given by Kline and Sugihara (114). The sour dough yeast, *S. exiguus*, frequently has been unstable as a starter culture organism, but modified bakers' yeast can often be substituted (196).

Lactic acid bacteria are involved in the soda cracker process and have been isolated and identified by Sugihara (197). Combination of bakers' yeast (*S. cerevisiae*) with *L. plantarum*, *Lactobacillus delbrueckii*, or *Lactobacillus leichmannii* or with mixtures of *L. plantarum* and *L. delbrueckii* or *L. plantarum* and *L. leichmannii* gave an acceptable soda cracker with decreased processing time (198).

Effect of microbial additives on nutritive quality of plant seed products

Effect of microbial fermentation on the nutritive quality of plant seed products. Fermentation does not always improve the nutritive value of foods. Tempeh (fermented soybeans), ontjom (fermented peanut press-cake), and idli (fermented mixture of black gram and rice) did not show increased PER (protein efficiency ratio) compared to similarly heated raw ingredients (207). However, the PER of tempeh prepared from 1:1 wheat-soybean mixture was 2.5, equal to that of casein (212). Hackler et al. (86) reported the PER of tempeh prepared by fermenting soybeans with *Rhizopus oligosporus* varied from 2.0 to 2.5. An increase in fermentation time of the soybeans resulted in decreased intake of tempeh by weanling rats with resultant decrease in weight gains. Increase in time of deep-fat frying of tempeh (normal preparative method) resulted in decrease in PER (2.02 at zero time decreased to 0.61 with 7 min of frying) as well as decreased feed intake and weight gain. Hackler and his coworkers (86) concluded

that fermentation by *R. oligosporus* did not improve the nutritional quality of soybeans.

The free amino acid content of *R. oligosporus*-fermented soybeans (tempeh) increased compared to that of unfermented soybeans (137). The increase for individual amino acids ranged from 1- to 85-fold. The riboflavin, niacin, and B₁₂ levels increased in tempeh as compared to those in unfermented soybeans; pantothenate and thiamin decreased (207). Since *R. oligosporus* does not produce B₁₂ (98), the increase in this vitamin in native tempeh analyzed by van Veen and Steinkraus probably came from the growth of bacterial contaminants. The content of niacin, riboflavin, pantothenate and B₆, but not thiamine, in tempeh increased over that of the unfermented beans (137). *R. oligosporus* lacks the synthetic capacity to produce thiamin. Fermenting wheat with *R. oligosporus* led to increases in niacin and riboflavin and a decrease in thiamin (212).

Utilizing corn meal, Wang and Fields (215) showed that the relative nutritive value (RNV) and lysine content were increased by fermentation with *S. cerevisiae* or *C. tropicalis*; methionine decreased. Azoulay et al. (15) fermented corn flour with *C. tropicalis* with an increase in protein from 8.8% to 20.6%. The lysine level doubled upon fermentation by the yeast, with little or no change in the methionine and cystine content (15). Hamad and Fields (87) fermented water-grain mixtures with a natural lactic microflora and found that the RNV increased in fermented wheat, barley, rice, millet and corn but not in fermented oats. Available lysine increased in all of the fermented grains. A natural fermentation of rice meal (dominated by lactic acid bacteria) led to significant increases in the isoleucine, lysine, riboflavin and the RNV content; niacin and thiamin levels of rice were decreased by fermentation (205). In the production of tapé ketan, fermentation of rice with a mixed culture of *Amylomyces rouxii* and *Endomyopsis burtonii* led to a 3-fold increase in thiamin (44).

Peanut flour fermented with *A. oryzae*, *Mucor hiemalis*, *N. sitophila*, *R. oligosporus*, or *Actinomucor elegans* showed increases in the thiamin and riboflavin levels when compared to those in unfermented peanut flour; the pantothenate level did not change. *M. hiemalis*, *N. sitophila*, and *R. oligosporus* increased the niacin content in the peanut flour, whereas *A. oryzae* decreased it (156).

A natural lactic fermentation of cowpeas led to an increase in RNV as well as increases in methionine, isoleucine, niacin and riboflavin, but no change in the thiamin level (224). Fermented chickpeas showed an increase in methionine, isoleucine and tryptophane; the RNV increased also. However, niacin and thiamine decreased in the naturally fermented chickpeas with no change in the riboflavin level (224). In both types of peas, fermentation reduced the level of trypsin inhibitor and the flatulence-causing sugars, raffinose and stachyose.

Sands and Hankins (174) suggested using amino

acid-producing mutants of fermenting microorganisms to upgrade fermented foods. They used a lysine-producing mutant of *L. bulgaricus* to ferment soy milk and found that the lysine content increased from 4% (wild-type *L. bulgaricus*) to 14% (mutant). Products which are fermented by lactic acid bacteria or other microorganisms could be upgraded in amino acid levels by the use of the proper mutant.

Effect of microbial additives on chemical and microbiological safety in plant seed products

Nitrosamines in beer. Certain beers contain high levels of nitrosamines (13,126). The presence of nitrosamines is probably from the manner in which the malt is dried and not from the microorganisms.

Siderophores in saké. The iron-free siderophore, deferrichrysin, has been found in saké (200). *A. oryzae* growing on steamed rice (koji) produced deferrichrysin, whereas the saké yeast (*S. cerevisiae*) did not produce siderophores when growing on steamed rice. Therefore, *A. oryzae* produced the siderophore on rice during the koji stage of saké fermentation (175). The significance of siderophores in saké is unknown at the present time.

Aflatoxins in fermented plant seed products. Hessel-tine et al. (100) examined approximately 50 strains of *A. oryzae* and found that none produced mycotoxin in media. Shoyu (soy sauce), miso, Chinese black beans, and tempeh were produced with these strains of *A. oryzae*; little or no aflatoxin was detected in the products. *R. oryzae*, which has been used to produce ontjom, inhibited aflatoxin-forming *A. flavus* when the fungi were grown together, and *R. oryzae* metabolized preformed aflatoxin (102). Maing et al. (128) prepared soy sauce with *A. oryzae* or *A. oryzae* plus a toxigenic strain of *A. parasiticus*. Fermentation of soybeans with *A. oryzae* did not lead to aflatoxin formation, but *A. parasiticus* plus *A. oryzae* did give aflatoxin in the fermented soybeans. The mycotoxin persisted during the brining stage of soy sauce manufacture. If fermented by the proper fungal starter cultures, mold fermented foods (tempeh, soysauce, miso, as well as others) will not contain mycotoxins (212).

MISCELLANEOUS FERMENTATIONS

Coffee bean fermentation

Coffee beans have an outer, mucilaginous envelope which must be removed before the beans can be dried and roasted. In the natural fermentation, the outer layer is removed by pectinolytic microorganisms (152). Agate and Bhat (5) employed the pectinolytic yeasts, *Saccharomyces marxianus*, *S. bayanus*, and *S. cerevisiae* var. *ellipsoideus* to free beans of this mucilage layer, and *Erwinia dissolvens* has also been used for the same purpose (65). The fermentation of coffee beans by microorganisms does not appear to contribute to flavor and aroma of the roasted product (103).

Cacao bean fermentation

Microbial fermentation of the cacao bean is an essential preliminary step for the chocolate flavor formed with roasting of the bean (170). Roasted unfermented beans do not have the characteristic chocolate flavor. The complicated microbial fermentation involves both yeast and bacteria (31,147), and additional studies are needed for development of starter cultures that will give consistent chocolate flavor development.

Maple sap and syrup fermentation

Normally, sugar maple produces a sap that gives a syrup of characteristic "maple" flavor (62). "Buddy" syrup produced from sap obtained during tree budding cannot be utilized as a table syrup because of unpleasant flavor and aroma and consequently represents an economic loss to the maple syrup producer. Inoculation of "buddy" sap or syrup with *Pseudomonas geniculata* destroys the "buddy" characteristics and produces syrup with satisfactory table qualities (216,217).

Maple syrup producers occasionally find that some syrups are low in the characteristic maple flavor and are less desirable to the consumer. Fermentation of such maple sap with strains of *P. geniculata* has been shown to intensify the maple flavor and color of the resultant syrup (221).

Other fermentations

Pederson (152) suggested that microbial fermentations probably occur in the production of the flavorings vanilla, ginger, citron and tabasco sauce. Further study of these microbial fermentations is warranted in order to develop starter cultures for their production.

SUMMARY AND CONCLUSIONS

Use of starter culture technology-microorganisms as deliberate food additives-offers many advantages to both the consumer and the food processor. Starter culture will give uniform quality to the products and avoid the inconsistencies from batch to batch arising from wild natural flora fermentation. The processor, by producing a consistently superior commodity, avoids economic loss due to uncontrolled and inconsistent flavor and texture development. Use of the proper fungal or bacterial starter culture eliminates the incidence of product failure caused by the growth of undesirable microorganisms.

Use of starter cultures reduces the processing time. More time is required for the natural flora to multiply to levels where fermentation can be initiated. There are savings in equipment, space, manpower, time and fuel; more food is made available in shorter time at cheaper prices when microorganisms are used as deliberate food additives.

Fermentative organisms can increase, decrease or not affect the vitamin and amino acid composition of foods. In addition to affecting the chemical composition of

foods, fermentation destroys undesirable factors in raw foods and increases the sensory qualities of foods. Without fermentation, cassava is toxic; microorganisms liberate HCN from the toxic glucoside and make cassava safe for human consumption (103). Fermentation of soybeans eliminates beany flavor and flatulence; consumption of tempeh did not cause flatulence in normal healthy individuals in contrast to ingestion of cooked soybeans (30). Certain materials, inedible and unpalatable to humans, are converted to edible and digestible foods by fermentation; ontjom is made from peanut press cake and bongkreng is prepared from coconut press cake (207). The ability of lactose intolerant individuals to ingest cultured milks without distress gives them access to a nutritious food (70).

Starter culture technology offers advantages to the consumer. Fermentation is an excellent preservation technique (where lactic acid is produced) that permits the extended use of a food long after harvest. Further, the consumer's exposure to toxic substances produced by microorganisms is reduced by the use of known starter cultures.

With the use of large inocula of the proper organisms, growth of food poisoning bacteria to levels where they can cause infections or intoxications is prevented. In natural fermentations when the numbers of desired organisms are low, any natural pathogen present could outgrow the fermentative microorganisms. Additionally, with lactic acid starter cultures, the acid produced from carbohydrate fermentation aids in controlling the growth of pathogens. Use of the proper mold starter culture in mold fermented foods decreases the chance of toxigenic fungi replacing fermentation. By producing a safe product, the processor also benefits because he suffers less economic loss due to recall by regulatory agencies.

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