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Fermented foods and food safety

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An evaluation is presented of risk factors associated with fermented foods, in comparison with fresh or alternatively processed foods. Cases of microbial food-borne infection have been reported in association with fresh cheese, sausages, fermented fish and fermented cereals. Another risk involves microbial food intoxications due to mycotoxin contaminated raw materials, production of bacterial toxins or possible mycotoxin production by fungal innoculants. In addition, toxic by-products of fermentation may be produced including ethyl carbamate and biogenic amines. From a food processing point of view, major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization, and use of poorly controlled natural fermentations. Also sub-optimal fermentation starters and inadequate storage and maturation conditions as well as consumption without prior cooking may reduce the safety of fermented foods. In addition to ensuring adequate processing conditions, the development of non-toxigenic starters with ability to antogonize pathogenic microorganisms and to degrade toxic substances needs continued attention.

Keywords: food-borne infection, intoxication, mycotoxin, ethyl carbamate, biogenic amines, starter.

INTRODUCTION

Food safety concerns us all as consumers, sometimes as politicians, and here particularly as food technologists. However, consumers and food professionals do not necessarily share the same view concerning the major food hazards (Table 1).

This paper deals with the following questions:

(1) Are fermented foods safer than fresh or alternatively processed foods?
(2) What risk factors can be identified in fermented foods?
(3) Can fermentation principles be used to increase their safety?

Like all other processed foods, fermented foods result from a manufacturing process involving the selection of raw materials, preparatory treatments, the fermentation operation proper, preservation, packaging and storage. Last but not least, treatment of the food by the consumer influences its condition. Thus, an integral approach should be taken when assessing the implications of individual risk factors for the safety of the consumer.

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FOOD INFECTION

Bacterial food-borne infections and intoxications constitute approximately 80% of all food-related illnesses (Waites & Arbuthnott, 1990). Food infections can occur if the following prerequisites are fulfilled: contamination followed by survival or growth by a pathogenic microorganism must take place, sufficient frequency and quantity of food must be consumed depending on the minimum infective dose of the pathogen, and the consumer must be susceptible to the pathogen. Particularly the young, old, pregnant and immuno-suppressed are more at risk than the average consumer.

Contamination

Contamination can take place during primary production of raw materials of plant and animal origin. In addition, it may occur during and after processing as a result of inadequate hygiene or packaging.

Table 1. Food hazards: perception versus epidemiology

Source	Consumers[a] (%)	Relative importance[b] (%)
Microbial contamination	22	49·9
Nutritional imbalance	—	49·9
Environmental contaminants	48	0·05
Natural toxicants	10	0·05
Food additives	12	0·0005
Others	8	—

[a] Survey held in The Netherlands, 1990.
[b] Ashwell (1990).

Survival

Most pathogenic microorganisms capable of infection are killed by pasteurisation and by exposure to acid conditions at pH ≤ 4·0. Other adverse environmental conditions such as reduced water activity, NaCl concentrations exceeding 10%, or chilled storage are usually inadequate to prevent pathogen survival. For instance, *Listeria monocytogenes* was shown to survive for 4 weeks at 5°C in a food system of pH 4·18 with 13% NaCl (Cole *et al.*, 1990).

Cases

Fermented milk products are of great economic importance. Numerous types of cheese are produced, from pasteurised or from raw milk. The latter procedure allows the survival of pathogens of animal origin, e.g. *Listeria* (McLauchlin *et al.*, 1990) and *Salmonella* spp. (Ratnam & March, 1986). The high buffering capacity of cheese curd prevents a significant pH decrease during ripening, even in the presence of active cheese starter lactic acid bacteria. Outbreaks of salmonellosis and listeriosis from raw milk cheddar and Mexican-style cheese have been reported. In hard cheeses, contaminating pathogens do not survive the maturation which involves several months of storage. If the cheese milk has been pasteurised, recontamination of the final product may occur. In particular, the manufacture of mould surface-ripened soft cheeses ('camembert', 'brie', etc.) requires much handling and is prone to re-contamination. In addition, the favourable pH caused by lactate degradation by the functional fungi, e.g. *Penicillium camemberti*, enables survival and growth. It has been estimated that 5–15% of mould ripened soft cheeses may contain *Listeria monocytogenes* due to re-contamination (Roberts, 1990). In recent years, outbreaks of listeriosis and *Escherichia coli* gastroenteritis have caused much concern.

In fermented meats, pathogens, e.g. *Salmonella typhimurium*, may survive in raw meat cured sausages if only marginal acidification takes place and is combined with high moisture content. For instance, a minor outbreak of salmonellosis was caused by fermented pork sausage. The product had a pH of 5·7 and a_w of 0·99 and was found to contain 10^6 cfu/g *Salmonella typhimurium*, among others (Van Netten *et al.*, 1986). Good quality sausage should have a pH 4·5–5·0 and a_w 0·92–0·99.

The addition or in-situ production of microbial inhibitory metabolites is considered to enhance safety. At present, the application of bacteriocins of lactic acid bacteria (Table 2) in food preservation is limited to nisin. Nisin is applied as an additive, or it is formed by *Lactococcus lactis* starter cultures in the product. The genetic information coding for nisin production has also been

Table 2. Some broad-spectrum bacteriocins of lactic acid bacteria

Bacteriocin	Produced by	Activity against	Heat stability	Approved	Applied	Reference
Nisin-A	*Lactococcus lactis*	Gram +	10 min, 100°C	GRAS	Yes	Delves-Broughton (1990)
Bulgarican	*Lactobacillus delbrücki* ssp. *bulgaricus*	Gram + and –				Abdel-Bar & Harris (1984)
Pediocin A	*Pediococcus pentosaceus*	Gram + and –	1 h, 100°C			Daeschel & Klaenhammer (1985)
Pediocin AcH	*P. acidilactici* (H)	Gram + and –				Bhunia *et al.* (1988)
Reuterin (non peptide)	*Lb. reuteri*	Gram + and –, fungi		FDA		Daeschel (1989)

cloned and expressed in different cheese starter bacteria (Coghlan, 1990), but these are not as yet applied commercially. Nisin is produced by *L. lactis* and has a rather broad-spectrum antimicrobial action including many Gram-positive bacterial species. It is able to inhibit spore germination of *Clostridium botulinum* in canned foods and kill *Listeria* spp. in raw (fermented) food. In order to be attractive as food preservatives, bacteriocins must have a broad-spectrum of antimicrobial activity, preferably including fungi. In addition, they must be stable to heat and other adverse conditions. Obviously, only non-toxic and non-allergenic products of GRAS-organisms may be considered for application.

In raw fish and fermented raw fish, *Vibrio parahaemolyticus* will survive quite well. In Japan, in particular, *V. parahaemolyticus* infections are common and can be associated directly with the custom of consuming raw (fermented) fish. Lack of heating or smoking is supposed to be the reason why fermented fish (salmon, halibut, herring) was much more frequently (25% of $n = 89$ samples) contaminated with *Listeria monocytogenes* than hot-smoked (9% of $n = 496$), or cold-smoked (14% of $n = 324$) fish (Jemmi, 1990).

In many tropical countries, raw cereals and pulses are allowed to undergo uncontrolled natural fermentations in order to enhance their flavour and digestibility. No salt is present in these high-moisture products. As a result of the activity of Enterobacteriaceae and lactic acid bacteria, a moderate extent of acidification occurs with pH values ranging from pH 4·5–5·5. These environmental conditions enable the survival of pathogenic bacteria. Normally this type of product requires cooking prior to consumption. Thus, if consumed immediately after cooking, one would not expect any risk of food infection. In practice, however, the food utilization habits of the consumer appear to play a crucial role. In particular, these naturally fermented cereal- or cereal/legume-based porridges are used as weaning foods in the tropics. Due to time constraints there is a tendency to prepare in advance and feed left-overs and there is a wealth of literature demonstrating the poor hygienic conditions of such traditional weaning foods. It would appear obvious that the daily intake of heavily contaminated food causes a significant incidence of food-borne infection symptoms including diarrhoea. However, there is very little evidence for a direct correlation between incidence of infectious diarrhoea and faecal contamination

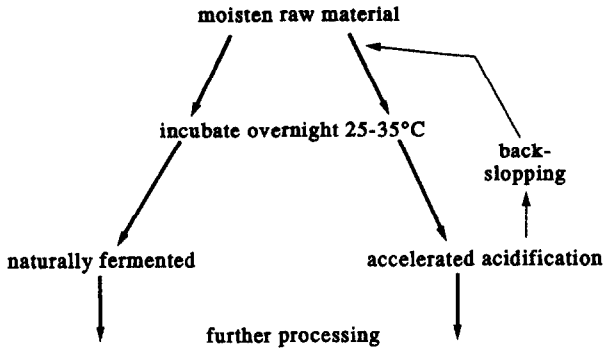


Fig. 1. Principle of accelerated acidification by 'back-slopping'.

of weaning food (Lloyd-Evans *et al.*, 1984). This can be due to several reasons. Firstly, viruses (Enterovirus, Rotavirus) also play an important role in the etiology of diarrhoea. Virus control requires proper cooking of food and water; no untreated water must be used to 'dilute' boiled porridges. Secondly, the acidity of the traditional products may be inadequate to kill the contaminating pathogens.

It has been shown that under simple processing conditions, the rate and extent of acidification of natural fermentations can be improved significantly by enrichment of inocula using the 'back-slopping' method (Fig. 1). This approach was successful with a variety of root crops, cereals and legumes and had significant bactericidal effect in challenge tests with a variety of Enterobacteriaceae (Nout *et al.*, 1989). In acidifying maize of pH 4·1–4·4 faecal coliforms died at a rate of approximately 1 log cycle per hour.

The fermentative preservation of animal feed by ensiling is of importance. Fresh grasses, fodder crops or industrial by-products, e.g. sugar beet pulp, are packed or heaped while creating anaerobic conditions to stimulate the activity of lactic acid bacteria and fermentative yeasts which will decrease the pH and compete with less desirable microorganisms. A regularly occurring problem is that of fungal spoilage of silage, notably with *Penicillium roqueforti* and *Aspergillus fumigatus*. It was demonstrated that the increased pH in silage spoiled by *P. roqueforti* enabled the survival of pathogens including *Listeria* spp. In turn, this could contribute to the maintenance of contamination cycles of pathogenic microorganisms.

FOOD INTOXICATION

Food intoxications, either acute or chronic, may occur depending on the quantity and nature of

the ingested toxin. Consumer sensitivity towards toxins may vary considerably with general state of nutrition and health, and with the dietary pattern. In the context of this paper, three sources of toxins will be discussed: those already present in the raw material, microbial toxins produced during or after processing, and toxic by-products of fermentation.

Raw materials

A number of raw materials naturally contain toxic substances, for instance cyanogenic glycosides (Reddy & Pierson). In addition, environmental contaminants such as pesticides, herbicides and hormones may be present. There is little evidence that food fermentation has a diminishing effect on such residues. In the field and during storage, plant foods, in particular, may become contaminated with mycotoxins. The fate of aflatoxins during food fermentation has been studied by several investigators.

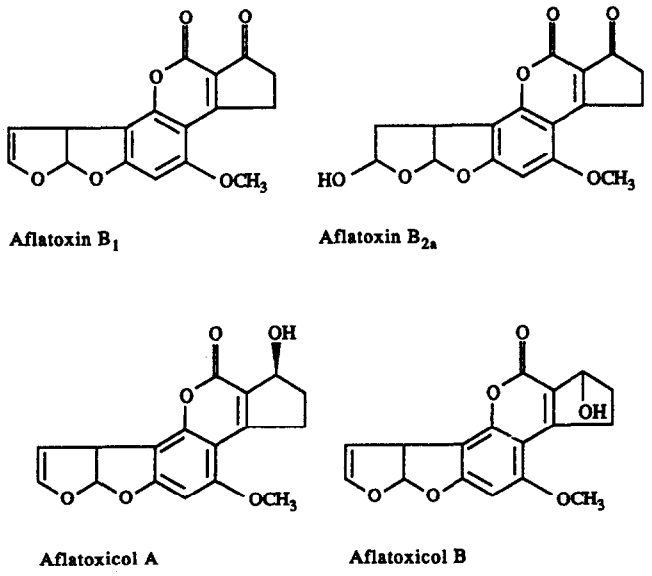


Fig. 2. Detoxification of aflatoxin B_1.

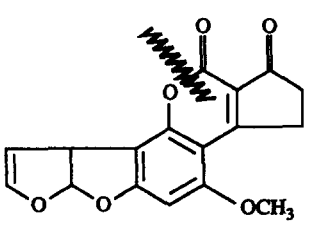


Fig. 3. Detoxification of aflatoxin B_1 by opening of the lactone ring.

Groundnut presscake or maize used as a raw material for the production of fermented products, e.g. Indonesian oncom and Ghanaian kenkey, may be contaminated with aflatoxins. Also, animal feed ingredients may have considerable mycotoxin levels. The fate of aflatoxin B_1 during food fermentation has been investigated in a variety of products. Fungi involved in food fermentations, for instance *Rhizopus oryzae* (= R. arrhizus) and *R. oligosporus* are able to reduce the cyclopentanon moiety which results in aflatoxicol A (Fig. 2). This appears to be a reversible reaction. Under suitable growth medium conditions (e.g. presence of organic acids), aflatoxicol A is irreversibly converted into its stereo-isomer aflatoxicol B (Nakazato *et al.*, 1990). Aflatoxicol is approximately 18 times less toxic than aflatoxin B_1.

In lactic fermentations at pH $\leq$ 4·0, aflatoxin B_1 is readily converted into aflatoxin B_{2a} (Fig. 2) which is also less toxic. Both biotransformations thus reduce the toxicity but there is no complete detoxification unless the lactone ring of the aflatoxin molecule is broken (Fig. 3). This would correspond to loss of fluorescence at 366 nm. It was found that such loss of fluorescence correlates with reduced mutagenicity. Screening fungi for the ability to reduce fluorescence in aflatoxin B_1 medium revealed that certain *Rhizopus* spp. were able to degrade 87% of aflatoxin B_1 into non-fluorescent substances, of as yet unknown nature and toxicity (Bol & Smith, 1989). This might provide opportunities for detoxification of food and feed in solid substrate fungal fermentations.

Microbial toxins

Microbial toxins may be produced by contaminating microorganisms. In some cases, the functional flora has been found to be toxigenic.

Contaminants

In large pieces of meat, e.g. country cured ham or in insufficiently heated or cured sausages, there is a realistic chance that *Clostridium botulinum* or *Clostridium perfringens* could grow and produce toxins, if brining and drying are inadequate. It is therefore essential to ensure an adequate combination of inhibitory factors (NaCl, nitrite, water activity, pH) or to apply heat treatments to avoid clostridium poisoning.

In cheese made from raw milk, *Staphylococcus aureus* may grow and produce enterotoxins. As

S. aureus is inhibited in the presence of competing microflora, the presence of actively growing starter cultures strongly reduces the chance of enterotoxin formation.

Tempe technology plays an important role in providing high quality protein from plant origin, especially in south-east Asia. Most commercial tempe makers use soybeans as a raw material. It has been shown that the acidification taking place during the preparatory soaking of the soybeans, plays a role in the development of the tempe microflora. In particular, poorly acidified beans allowed the survival and growth of pathogenic and toxinogenic bacteria including *Bacillus cereus*, *Yersinia enterocolitica* and *S. aureus*. It has been shown that growth of *Salmonella* spp., Enterobacteriaceae and *S. aureus* during the stage of fungal fermentation is inhibited if competing lactobacilli are present; if *S. aureus* does grow it is unable to produce significant amounts of enterotoxin (Nout & Rombouts, 1990). Moreover, staphylococcal enterotoxins are not very heat resistant when present in tempe, and since tempe must be cooked or fried before consumption, the risks of food-borne infection or intoxication are small indeed. Soybean tempe has never been incriminated as a cause of food-borne disease. However, tempe 'bongkrek', made from coconut presscake in Central Java, Indonesia, may enable the multiplication of *Pseudomonas cocovenenans* which produces the toxins bongkrek acid and toxoflavin (Fig. 4). Tempe 'bongkrek' has caused several fatal poisonings. Interestingly, Ko (1985) established that a large *Rhizopus oligosporus* inoculum size, or incorporation of 2% NaCl are adequate to prevent the growth and toxin production of *P. cocovenenans* in this type of tempe.

In ensiled animal feed, fungal spoilage by *Penicillium roqueforti* and *Aspergillus fumigatus* is common. All of 34 *P. roqueforti* strains isolated produced P.R.-toxin, and 6 of 13 isolates of *A. fumigatus* produced fumitremorgens, verruculogen and TR2-toxin, in laboratory media (Gedek *et al.*, 1981). In practice however, there is little evidence of accumulation of such toxins in silage or of poisoning of cattle (Nout *et al.*, 1993).

In fermentations involving non-cooked raw materials, the combined effect of water activity, salt concentration, acidity, anaerobiosis, temperature and microbial competition must be optimized.

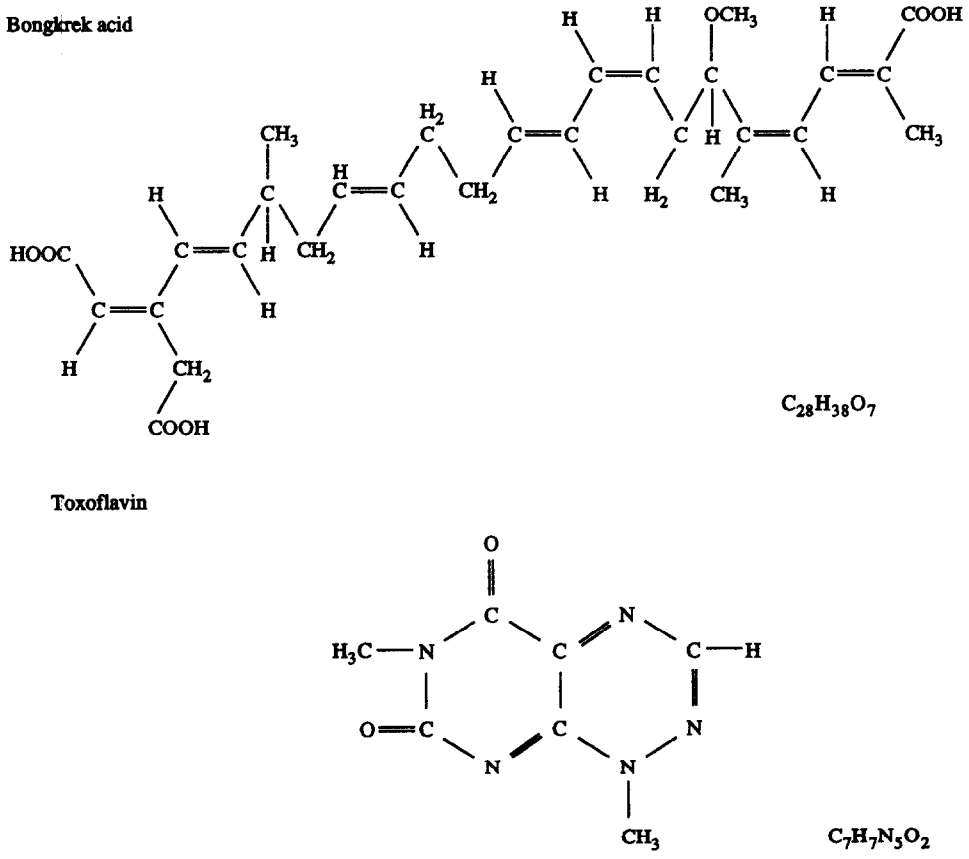


Fig. 4. Toxins of *Pseudomonas cocovenenans*.

In this respect, predictive modelling of the behaviour of toxinogenic microorganisms such as *Clostridium botulinum* (Lund *et al.*, 1990) is a useful tool. In addition, the use of competitive fermentation starters has been successfully used to suppress the multiplication of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* in meat and cheese model systems (Earnshaw *et al.*, 1989).

Functional flora

Most starter organisms used in commercial practice are considered to be non-pathogenic. Exceptions may be *Staphylococcus saprophyticus* and *S. xylosus* which are part of certain meat curing inocula (Hammes, 1988). As the latter organisms have also been isolated from human infections, their pathogenicity merits further study.

The toxinogenicity of fungal starters, however, has recently been of concern. In particular, *Penicillium* spp. used in cheese (*P. roqueforti*, *P. camembertii*) and in meat (*P. chrysogenum*, *P. nalgiovense*) are, in principle, able to produce mycotoxins. *P. roqueforti* may produce roquefortine C and A, and mycophenolic acid in test media. *P. camembertii* produces some cyclopiazonic acid in the rind of camembert cheese if this is stored without refrigeration. At present, non-toxinogenic strains of these fungi are not known and a project is underway to obtain non-toxinogenic mutant strains (Leistner, 1990).

In cured meat, fungal starters contribute to the aroma, the quality of the skin, and product safety by suppressing wild strains and their metabolites. Non-toxinogenic strains of *P. nalgiovense* and *P. chrysogenum* (white mutant) are marketed as meat curing starters. More than 50% of *P. nalgiovense* and *P. chrysogenum* isolated from fermented meats are toxinogenic when tested on laboratory media (Leistner & Eckardt, 1979). Although very little information exists on the production and chemical stability of mycotoxins in complex food systems such as meat, some national food laws require that no toxinogenic fungi should be cultivable from fungal fermented products. Surely, this is no guarantee that mycotoxins are absent!

By-products of fermentation

Ethyl carbamate

A substance occurring in a variety of fermented foods is ethyl carbamate (urethane) (Fig. 5), a carcinogenic and mutagenic compound which

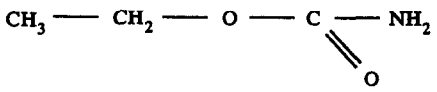


Fig. 5. Ethyl carbamate (urethane).

Table 3. Occurrence of ethyl carbamate in fermented foods[a]

Product	Number of samples	Average level (ppb)	Range (ppb)
Cheese	16	ND[b]	
Tea	6	ND	
Yoghurt	12	0·4	ND–4
Cider	8	0·6	ND–4
Bread	30	1·7	ND–8
Malt beverages	69	1·8	ND–13
Bread, toasted	9	5·2	2–14
Soy sauce	12	18	ND–84
Wine	6	18	7–40
Sake	11	52	3–116

[a] Literature data.
[b] ND = Not detectable.

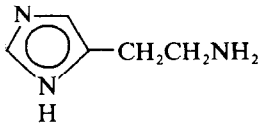
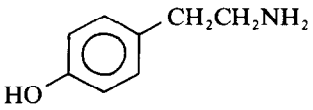
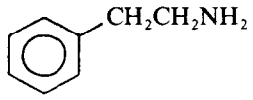
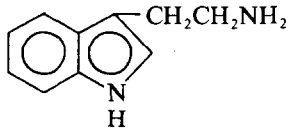
results from the esterification of ethanol with carbamic acid (Canas *et al.*, 1989). The latter can be formed from several precursors including naturally occurring citrulline, as well as yeast metabolites from L-Arginine and L-Asparagine, e.g. urea and carbamylphosphate. In addition, vicinal diketones, and HCN liberated from cyanogenic glycosides act as precursors. Heat and light enhance the formation of ethyl carbamate. Table 3 summarises literature data on its occurrence in foods and beverages (Hasegawa *et al.*, 1990). In most countries there is no legislative limit, but a level of 10 ppb was suggested by FAO/WHO for soft drinks, and 30–400 ppb was suggested for various alcoholic beverages by the Canadian Government.

The mechanism of ethyl carbamate formation is poorly understood. Research with wine and stone fruit (cherry, plum) fermentations indicate that reducing the level of precursors by enzyme treatment, selection of yeast strains and control of fermentation conditions, and treatment of the pH adjusted fermented pulp with $CuSO_4$ could be useful in keeping the ethyl carbamate levels to a minimum.

Biogenic amines

Biogenic amines are a group of mildly toxic compounds which can be formed in fermented foods, mainly by decarboxylation of amino acids (Table 4). Approximately 1000 ppm is supposed to elicit toxicity. From a 'good manufacturing practice'

Table 4. Major biogenic amines

Biogenic amine	Formula	Precursor
Ethylamine C_2H_7N	$CH_3CH_2NH_2$	Ala
Putrescine $C_4H_{12}N_2$	$H_2N(CH_2)_4NH_2$	Orn
Histamine $C_5H_9N_3$		His
Cadaverine $C_5H_{14}N_2$	$H_2N(CH_2)_5NH_2$	Lys
Tyramine $C_8H_{11}ON$		Tyr
Phenylethylamine $C_8H_{12}N$		Phe
Tryptamine $C_{10}H_{14}N_2$		Try

point of view, levels of 50–100 ppm histamine, 100–800 ppm tyramine and 30 ppm phenylethylamine, or a total of 100–200 ppm are regarded as acceptable. Biogenic amines are especially associated with lactic fermented products, particularly wine, cheese, fish and meat and very low levels also occur in fermented vegetables (Fig. 6). The major biogenic amine producers in foods are Enterobacteriaceae and Enterococci. Most functional lactic acid bacteria do not produce significant levels of biogenic amines. Presence of free amino acids, low pH of the product, high NaCl concentrations, and microbial decarboxylase activity correlate with higher levels of biogenic amines (Ten Brink *et al.*, 1988). In meat products, species of Enterobacteriaceae were associated with cadaverine, and lactobacilli with tyramine formation. Also sauerkraut may contain varying levels of biogenic amines, due to the large variations in the naturally selected microflora. In cheese, Enterobacteriaceae, heterofermentative lactobacilli and *Enterococcus faecalis* were associated with considerable production up to 600 ppm of biogenic amines including phenylethylamine.

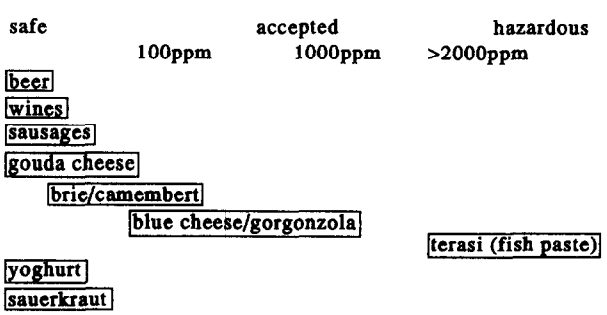


Fig. 6. Biological amines in fermented foods (literature data: sum of concentrations of individual amines in ppm).

Pasteurisation of cheese milk, hygienic practice and selection of starters with low decarboxylase activity are measures to avoid the accumulation of these undesirable products.

CONCLUDING REMARKS

Due to the competitive activity and the metabolites of starter microorganisms, many fermented foods are a less likely vehicle for food infection or intoxication than fresh foods. On the other hand, they are often not as stable as canned or frozen foods, and good hygienic practice during their manufacture strongly contributes to their durability and safety. The following risk factors are of importance:

(a) the use of previously contaminated raw materials;
(b) lack of pasteurisation;
(c) the use of poorly controlled natural fermentations, or of sub-optimum fermentation starter cultures;
(d) inadequate storage or maturation conditions enabling survival of pathogens, or growth and toxin production;
(e) consumption without prior heating.

How can these risks be minimized? Obviously, it is essential to ensure the wholesomeness of raw materials. Food fermentation cannot be used as a tool to produce first quality products from second quality raw materials.

In addition, further optimisation of starter cultures either by conventional selection and mutation, or by recombinant-DNA manipulations can result in increased levels of safety of fermented foods. In particular, selection of starters which are not toxinogenic, which antagonize pathogenic microorganisms, which produce broad-spectrum bacteriocins, or which have detoxifying ability should have priority.

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