Useful role of fungi in food processing

Introduction to food-borne fungi

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Chapter 9

USEFUL ROLE OF FUNGI IN FOOD PROCESSING

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INTRODUCTION

The growth and metabolic activity of fungi (yeasts and moulds) in foods can have different effects. On the one hand, undesirable changes such as decay, spoilage and even toxin formation may occur; on the other hand, fungal activity has been exploited by man for the purpose of food production and processing. Practices of gathering fungal fruit bodies (mushrooms) as well as the application of moulds to prepare fermented foods go back several centuries. More recently, fungal mycelium as well as yeast cells have been cultivated to obtain protein-rich nutritious food for human and animal consumption. Fungi play a significant role in industrial fermentations to produce a variety of enzymes and other organic substances. Many of these are applied as food ingredients. Most recently, recombinant DNA techniques have become available to modify fungal properties. Some implications for the food industry will be mentioned.

FUNGAL FERMENTED FOODS

Fermentation is one of the oldest ways of food processing and is of great economic importance. The occurrence, manufacture, properties, and use of fermented foods is well-documented (Campbell-Platt 1987; Steinkraus 1997; Wood 1998). Some fermented products (cheese, beer, wine, soya sauce) have experienced an enormous scale-up of production, with the use of sophisticated inoculum; on the other hand, many fermented foods are still produced using age-old "traditional" techniques under simple or even primitive conditions.

For reasons of required product properties and economics, most food fermentations cannot be carried out profitably under sterile conditions. Fermented foods may therefore contain a variety of bacteria, yeasts and molds, originating from raw materials, inoculum and process contamination. In mixed-culture fungal fermented foods, stimulation by metabolites, by mutualistic degradations of substrate, or by release of lysis products; or inhibition by competition, formation of antibiotic substances or antimicrobial metabolites, are important determinants of the balance of microbial populations (Nout 1995). According to the physical nature of the substrate, fermentations can be distinguished in liquid and solid state fermentations. In liquid fermentations, an aqueous continuous phase serves as a medium for homogeneous distribution of micro-organisms and for heat- and mass transfer. Liquid fermentation is used for e.g. beverages and sauces manufacture.

Table 1 lists some fermented foods in which fungi play an essential role. In addition, their raw materials, representative fermenting micro-organisms, the type of fermentation system (liquid or solid) and the relative importance of groups of microflora for a successful fermentation are listed. In temperate climates, mould-ripened meat and cheese are dominated by *Aspergillus* and *Penicillium* spp. In addition, yeasts play a role in bakery products and in alcoholic beverages. In sub-tropical and tropical regions, fungal fermented foods predominate in East- and South East Asia. *Rhizopus, Amylomyces, Mucor, Neurospora* and *Monascus* spp. are found frequently as functional mycoflora. Yeast-fermented products from tropical areas include alcoholic snacks and beverages. A few selected fermented foods will be discussed in the next section.

Table 1. A selection of fungal mixed-culture fermented foods.

Moulds	Yeasts	Bacteria	Substrate	Type of fermentation	Product Name	Nature	Use	Origin
Actinomucor elegans			soybean curd	SSF-L	Sufu (Furu)	spreadable solid	flavouring protein food	China, Vietnam
Amylomyces rouxii	*Endomyces spp., Hyphopichia burtonii*	*Pediococcus pentosaceus Enterococcus faecalis*	uncooked rice	SSF	Ragi	solid tablet	inoculum for rice wine making	Orient
Amyl. rouxii	*Hyp. burtonii, End. fibuliger*	*Ped. pentosaceus Ent. faecalis*	cassava	SSF	Peuyeum	semi-solid mass	snack	Indonesia
Aspergillus oryzae A. soyae	*Zyg. rouxii, Torulopsis versatilis*	*Tetragenococcus halophila Ent. faecalis*	soya bean+ rice/barley	SSF	Miso	semi-solid paste	flavouring	Orient
A. oryzae A. soyae group	*Zyg. rouxii, Zyg. soyae Zyg. major, Hansenula spp., Torulopsis spp., Candida etchellsii, C. versatilis*	*Lactobacillus delbrueckii, Tet. halophila , Ped. damnosus*	soya bean+ wheat+ salt	SSF-L	Soya sauce	liquid	flavouring	Orient
A. oryzae	*Hans. anomala, Sacch. cerevisiae(saké)*	*Leuc. mesenteroides var. sake, Lb. saké*	rice	SSF-L	Saké	liquid	liquor	Japan
Monascus purpureus M. ruber, M. pilosius			rice	SSF	Ang-kak	granular solid	colouring, flavouring, health ingredient	China, Japan
Penicillium roqueforti	*Yarrowia lipolytica*	*Leuconostoc spp., Lc. Lactis, Lc. lactis biovar, diacetylactis , Lc. lactis ssp., cremoris*	milk curd	SSF	Roquefort-type blue-veined cheese	semi-solid cake	protein food flavouring	France
Pen. camemberti (P.. candidum, P. caseicolum, P. album)	*Candida spp., Kluyver-omyces spp, Saccharomyces spp., Torulopsis spp.*	*Brevibacterium linens Lc. lactis ssp. cremoris Lc. lactis*	milk curd	SSF	Camembert-type surface-ripened cheese	semi-solid cake	protein food flavouring	France
Pen. nalgiovense Pen. chrysogenum		*Micrococcus spp., Staphy-lococcus spp. Pediococcus spp.. Lactobacillus spp.*	meat (sausage)	SSF	Salami	solid	protein food	Europe
Rh. oligosporus R. chinensis, R. oryzae, Mucor indicus	*Trichosporon beigelii, Clavispora lusitaniae, C. maltosa, C. intermedia, Yar. lipolytica*	*Klebsiella pneumoniae Enterobacter cloacae Lactobacillus spp.*	mostly soybeans	SSF	Tempe	semi-solid cake	protein food snack	Indonesia
	Torlp. holmii, S. cerevisiae, Pichia saitoi, C. krusei	*Lb. plantarum, Lb. fructi-vorans. Lb. brevis var. lindneri, Lb. sanfrancisco*	rye or wheat	SSF	Sour-dough	semi-solid mass	inoculum for sourdough bread making	Europe
	Sacch. cerevisiae (S.. uvarum, (S. elegans, S.. bayanus)	*Oenococcus oeni Ped. acidilactici Lb. casei*	grape juice	L	Wine	liquid	liquor	Europe
	Sacch. cerevisiae (S. bayanus, (S. uvarum) Brettanomyces spp.	*Lactobacillus spp. Pediococcus spp.*	barley ; wheat	L	Lambic- Gueuze beer	liquid	liquor	Belgium

Abbreviations: SSF = solid-substrate; L = liquid; SSF-L = solid-substrate followed by liquid fermentation. Moulds: A.= *Aspergillus*; Amyl.= *Amylomyces*; Pen.= *Penicillium*; Rh.= *Rhizopus*. Yeasts: C.= *Candida*; End.= *Endomyces*; Hans.= *Hansenula*; Hyp.= *Hyphopichia*; S.= *Saccharomyces*; Torlp.= *Torulopsis*; Yar.= *Yarrowia*; Zyg.= *Zygosaccharomyces*. Bacteria: Ent.= *Enterococcus*; Lb.= *Lactobacillus*; Lc.= *Lactococcus*; Leuc.= *Leuconostoc*; Ped.= *Pediococcus*; Tet. = *Tetragenococcus*. Brackets: old names.

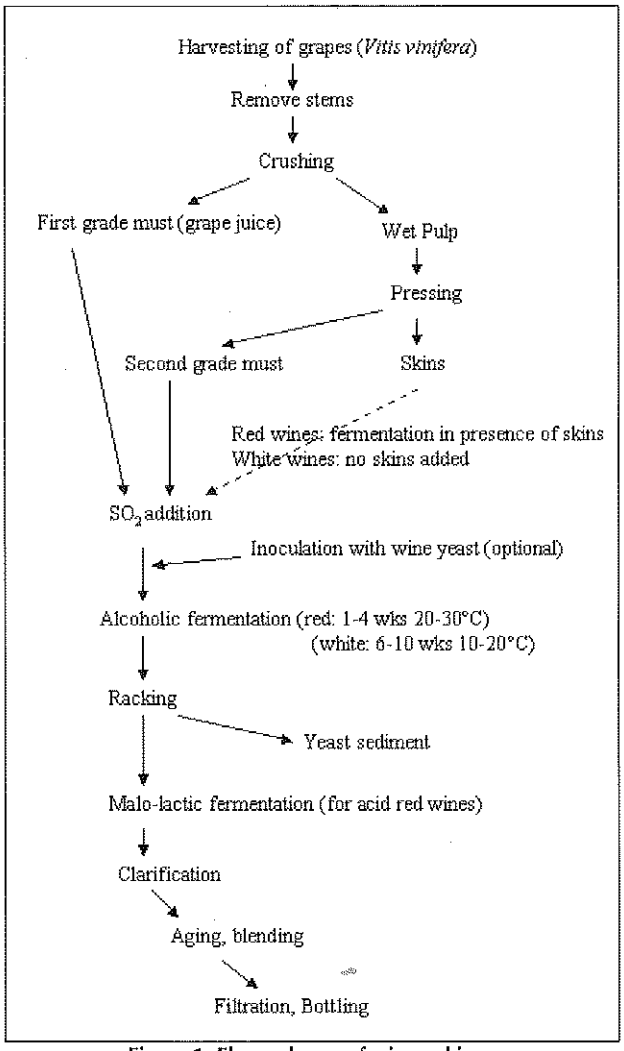

Figure 1. Flow-scheme of winemaking.

Wine

The wide variety of wines is not only due to the more than 5000 varieties of grape (*Vitis vinifera*) but particularly to the growing conditions (location, soil, climate) and fermentation conditions. The principle of winemaking is summarized in Figure 1.

Grapes must be free from mouldiness, except for the manufacture of the sweet "Sauterne" wines which require a "noble rot" of the mould *Botrytis cinerea*. Red wines are usually fermented "on the skins". Usually, 100-150 mg SO_2/litre is added to suppress excessive growth of epiphytic yeasts (*Candida, Hanseniaspora, Kloeckera, Metschnikowia, Pichia, Rhodotorula, Saccharomyces, Torulopsis, Trichosporon* spp.). This will enable good dominance of the selected wine yeast (*Saccharomyces cerevisiae*, often accompanied by *Torulopsis stellata*) (added at approx. 10^6/ml juice). It is important that epiphytic flavour producing yeasts are in balance with the functional yeasts for alcoholic fermentation. When all fermentable sugars have been exhausted, the alcoholic fermentation stops, and yeast is removed by syphoning ("racking") in order to prevent off-odours from yeast autolysis. In high-acidity wines, lactic acid bacteria (*Oenococcus oeni*) are inoculated to transform malic acid into lactic acid ("Malo-lactic fermentation") thus giving the wine a more mellow taste (Nout 1992).

Mould-ripened Camembert cheese

This is one of the several surface-ripened cheeses. Originating from Normandy, France it was first prepared by Marie Harel in 1791. In 1890, M. Ridel developed the famous wooden box facilitating a world-wide exportation.

The principle of Camembert production is outlined in Figure 2. After production of very young cheese curd, this is sprayed with a fine mist containing *Penicillium camemberti* conidia. After customary brining and conditioning, mould growth starts at the cheese surface during the incubation period. The crust (rind) of Camembert cheese is thin and white. The various starter strains have colours ranging from greyish-blue to pure white. The interior of the cheese must be yellowish with a rather firm white centre. During ripening, proteolytic and lipolytic enzymes of *P. camemberti* diffuse somewhat into the cheese.

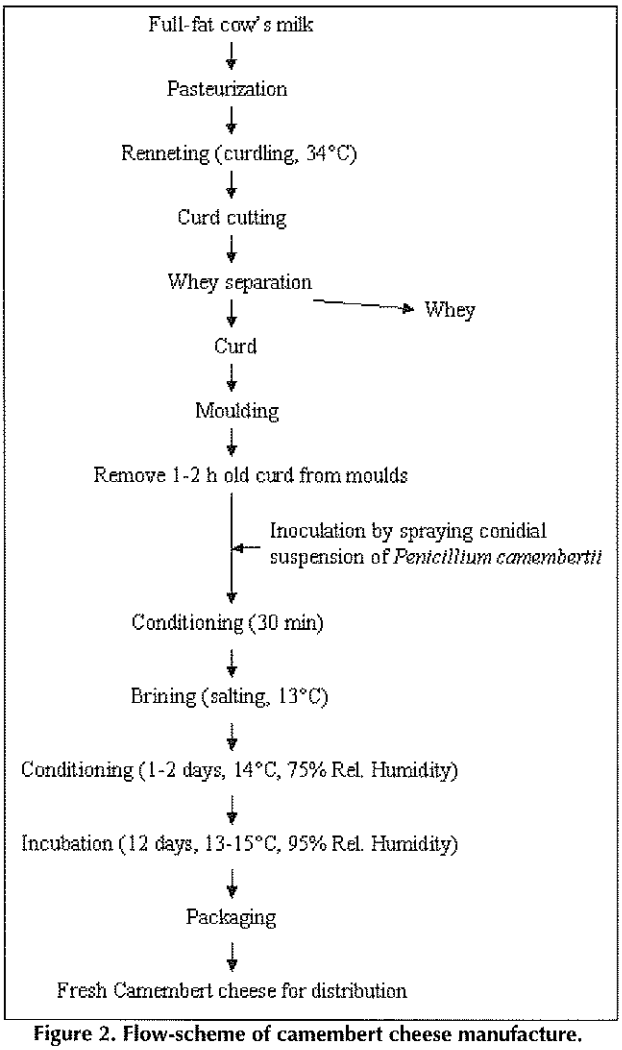

Figure 2. Flow-scheme of camembert cheese manufacture.

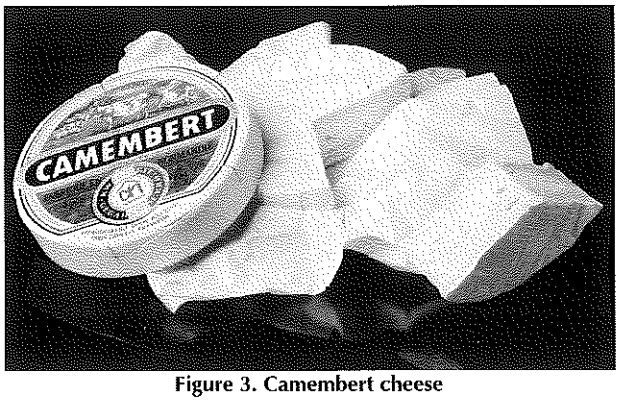

Figure 3. Camembert cheese

But, the characteristic softening is due to pH increase caused by the release of NH_3. Yeasts, particularly *Debaryomyces hansenii*, are often present in cheese and also contribute to pH increases due to their consumption of lactic acid (Wyder and Puhan 1999). Proteolytic reactions and aminotransferase activity contribute greatly to flavour development (Prieto *et al.* 1999; Sable and Cottenceau 1999). Usually the product is consumed at an age of 3-5 weeks. Tested in pure culture, all known *P. camemberti* strains are able to produce the mycotoxin cyclopiazonic acid (CPA). This appears to be a stable property, since old culture collection strains had not lost the ability to produce CPA. Efforts are undertaken to obtain CPA-negative mutant strains, and starters are selected on this criterium. The risk of poisoning is very small, however. Only very low levels of CPA could be detected in Camembert cheese. This is explained by its chemical instability in the presence of amines and its poor diffusion from the rind into the cheese. In addition, CPA is hardly produced at storage temperatures <15°C.

Sufu

Sufu, also written as fu-ru, is a strongly flavoured mould-fermented soybean curd with a spreadable consistency. (Su, 1986). Sufu is produced mainly in China both commercially and domestically, with annual production estimated at least 300,000 metric tons. Sufu is consumed as an appetizer and a side dish e.g. with breakfast rice or steamed-bread. The principle of the sufu making process is outlined in Figure 4 (Nout and Aidoo, 2000). All sufu is made from cubes of tofu (curd obtained by salt-coagulated soya bean milk). The tofu used for sufu making is firmed-up by pressing or by a short hot air treatment, and subsequently the cubes are surface-inoculated and incubated at high relative humidity at temperatures of 20-35° C, depending on locality and season. The types of moulds involved are mainly *Actinomucor* and *Mucor* spp. Large-scale factories use pure culture inoculants of e.g. *Actinomucor elegans*, whereas at a smaller production level, mouldy straw mats are used to inoculate the tofu cubes with a mixed flora of moulds and some bacteria.

As *A. elegans* does not grow well at temperatures exceeding 25° C, other moulds e.g. *Mucor hiemalis* or *Rhizopus chinensis* are used at higher temperatures. After several days, the tofu cubes are covered with a dense layer of cottony mycelium. This intermediate product is referred to as pehtze or pizi. The pehtze has become a source of fungal proteolytic enzymes, and the soybean protein has been partially degraded.

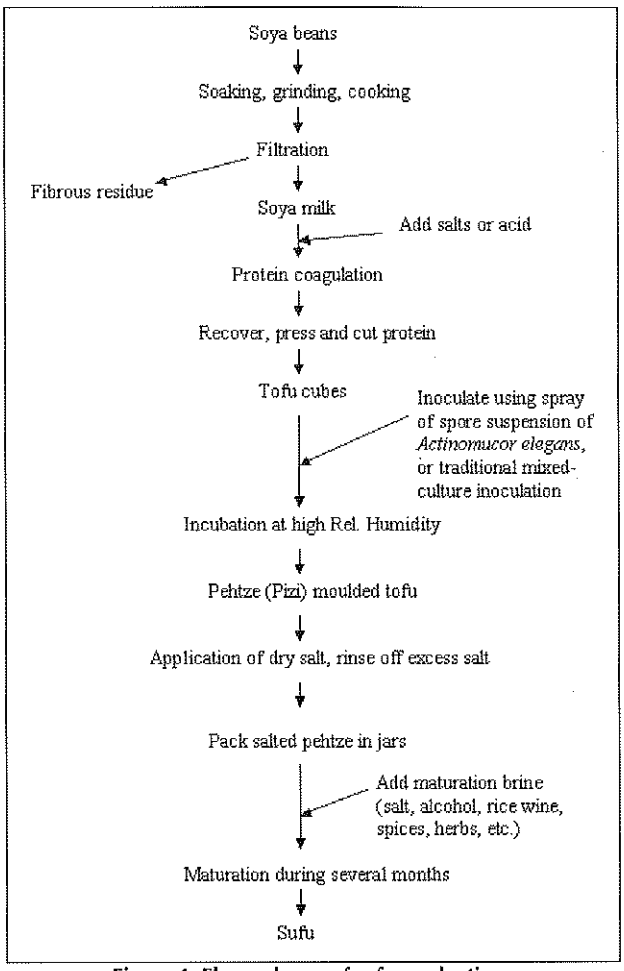

Figure 4. Flow-scheme of sufu production.

Figure 5. Red (left) and Grey (right) Sufu

The next steps, salting and brining, are aimed at preserving the product while allowing enzymatic maturation that will finally result in the soft consistency and the required strong smell and taste.
A first bulk treatment with solid salt has the aim to quickly increase the salt content to about 15%. Next, salted pehtze cubes are filled into jars or other containers and filled up with maturation brine or dressing mixture. The composition of the maturation brine strongly influences the properties of the final product. It always contains about 10-12% NaCl, and sometimes rice wine up to about 10% ethanol content. Very popular is red sufu, for which the maturation brine contains ang-kak (see Table 1). Ang-kak does not only provide red and orange pigments, but it also contains several active enzymes that contribute to the degradation and flavour of sufu. Although not much data is available on the bacteriology of sufu, the presence of *Tetragenococcus halophila* (refer to soya sauce) has been reported. After several months of maturation, the outside of the jars is cleaned and they are labelled and packed for distribution.

Tempe

Tempe originates from Java, Indonesia but is also popular in the Netherlands and it has gained considerable consumer markets in the U.S.A., Europe and Australia. It is a sliceable cake obtained by solid-substrate fungal fermentation of previously soaked and cooked leguminous seeds, cereals or other suitable material. The most common substrate is soya beans (Nout, 1992; Nout and Rombouts, 1990; Wood 1998). Tempe provides a cheap, nutritious, digestible and safe source of vegetable protein. It is not eaten fresh, but only after cooking (stewing) or frying in oil (crispy "tempe kripik"). The traditional manufacturing process is outlined in Figure 7. Soya beans are soaked and wet-dehulled (traditional process in Indonesia), or dry-dehulled and soaked (mechanized process in e.g. the Netherlands). During soaking a natural lactic fermentation takes place lowering the pH of the beans, rendering them favourable to mould growth and protecting them from pathogenic and spoilage-causing bacteria.

After boiling and cooling to ambient temperature, the beans are inoculated using traditional "usar" starters (*Rhizopus* spp. on a carrier of *Hibiscus* leaf: Nout *et al.*, 1992) or powdered mixed culture starters on a carrier of rice flour or cassava fibre. The major functional moulds, *Rhizopus oligosporus* and *R. oryzae* germinate rapidly at 37°C and their fast mycelial growth ensures their dominance over contaminating strains of e.g. *Aspergillus* spp. Within 30 hours the loose beans are "knitted" together to form a solid mass. *R. oligosporus* was observed to penetrate approximately 2 mm into cooked soya beans (Varzakas, 1998).

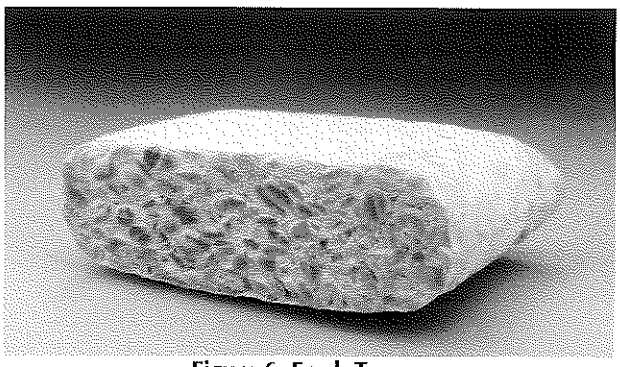

Figure 6. Fresh Tempe

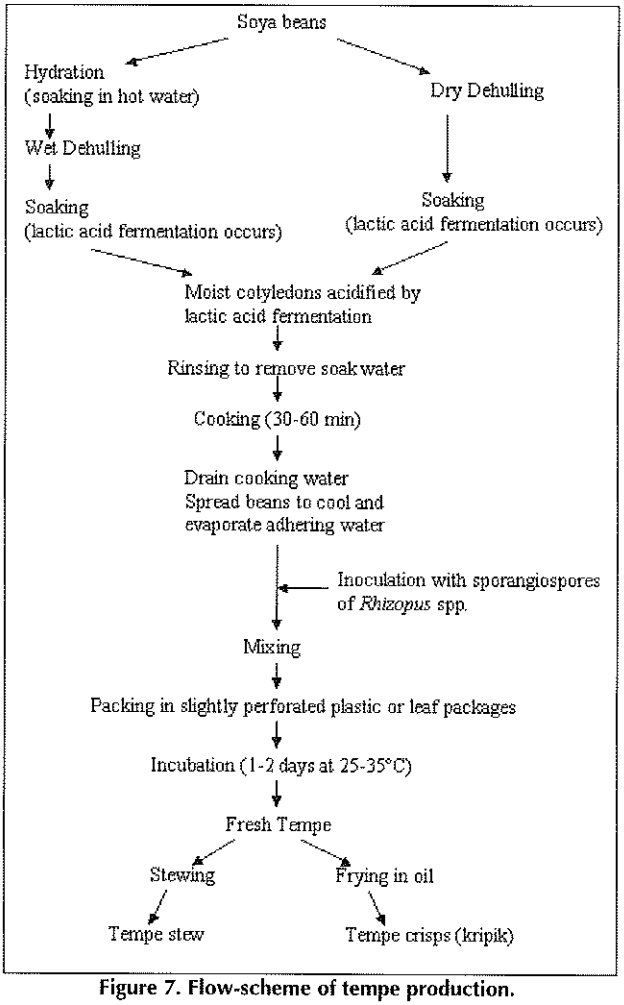

Figure 7. Flow-scheme of tempe production.

As a result of packaging in sparsely perforated leaves or polythene sheet, micro-aerobic conditions prevail, enabling mycelial growth but suppressing the formation of black sporangia. Sporulation is also inhibited by formed NH_3 (Sparringa and Owens, 1999a) that is retained in the package. As a result, a shiny white tempe is produced. Although the cooked soya beans are slightly acid, their pH will increase due mainly to the production of NH_3 and to some extent the consumption of lactic acid (Sparringa and Owens, 1999b). The enzyme activity of the *Rhizopus* spp. includes proteolytic, lipolytic, carbohydrate degrading enzymes and phosphatases. By their action, part of the polymeric substrate is solubilized enabling easy digestibility (Chango *et al.*,

1993) and a dramatic increase of low-molecular weight water-soluble components from 7% in cooked beans to 27% in fresh tempe (Kiers *et al.*, 2000). The degradation of non-starch polysaccharides (arabinogalactans and pectic substances) was correlated with the decrease of firmness of the beans during the first 24 hours of fermentation (De Reu *et al.*, 1997). Antinutritional factors e.g. phytic acid are also degraded, improving the bio-availability of phosphate and minerals. During the tempe manufacturing process, the levels of flatulence-associated galacto-oligo saccharides is significantly reduced; this is caused mainly by leaching during soaking and cooking, and to some extent to the fungal fermentation (Ruiz-Teran and Owens, 1999). Several fat-soluble vitamins and provitamins are formed during the fermentation (Denter *et al.* 1998). Health-promoting effects of tempe are attributed to polyhydroxylated isoflavones that can be formed from the soya bean isoflavones genistein and daidzein (Klus and Barz, 1998; Wuryani, 1995). Recent process innovations include semi-continuous processing lines (Nout, 2000) for tempe and fermentation at controlled temperatures in rotating drum bioreactors for tempe-like food ingredients (Han *et al.* 1999).

Soya sauce

Soya sauce is of Chinese or Japanese origin. There are about 3,600 companies in Japan which produce soy sauce or shoyu and of these the five largest produce about half of the total annual production of 1.2 million metric tons (Nout and Aidoo 2000).There are many different types, but Koikuchi-Shoyu is the best representative of fermented soya sauces (Fukushima 1989; Yokotsuka and Sasaki 1998). It is a clear deep-brown liquid with the following approximate composition: 22°Be, 17% w/v NaCl, 1.6% w/v total Nitrogen, 1% w/v formol Nitrogen, 3% w/v reducing sugars, 2.3% v/v alcohol, and pH 4.7. In principle, the manufacturing process (Figure 8) consists of 3 phases: koji making, brine fermentation and refining. Koji making is a solid state mould fermentation of a mixture of previously steam-cooked soya beans and roasted and crushed wheat. This is inoculated with an inoculum of conidiospores of *Aspergillus oryzae* or *A. sojae* and is incubated at 25°C during 2-3 days to obtain dense growth and greenish-yellow sporulation indicating that a high level of fungal enzymes has been produced. These enzymes include peptidases, proteinases, glutaminase, amylase, pectinases and cellulases needed for subsequent hydrolysis of the polymeric protein and carbohydrate matter of the raw material.

Criteria used for the selection of *Aspergillus* starter strains include (1) flavour and colour of the final product, (2) ability to sporulate well, (3) high rate of growth and enzyme production, (4) length of conidiophore, (5) genetic stability and (6) inability to produce mycotoxins (Aidoo *et al.* 1994). The second phase (maturation of the moromi mash) takes place in a salt bath (22-25% w/v NaCl) so that spoilage micro organisms cannot develop, but only enzymatic degradation takes place. Nevertheless, during the first 2 months at 15°-20°C, halophilic lactic acid bacteria (*Tetragenococcus halophila*), and in the following months at 30°C, salt-tolerant yeasts (*Zygosaccharomyces rouxii*) will develop after some time of ripening. They are part of the traditional process, and their metabolites add essential flavour components such as HEMF (4 hydroxy -2 (or 5)- ethyl - 5 (or 2) - methyl - 3 (2H) furanone) (Hayashida *et al.*, 1997) to the product. Nowadays they are added as inoculum to ensure their activity. This phase takes 6-8 months in total, after which the soya sauce is harvested by pressing. Continuous pasteurization (70-80°C) guarantees shelf-life, but is also essential for flavour- (Ishihara *et al.*, 1996) and colour development and for inactivation of the fungal enzymes. After aseptic bottling, the product is distributed. Melanoidins (plant melanins of molecular weight of about 5600) extracted from soy sauce were shown to inhibit the growth rate of HCT15 cells derived from human colon carcinoma, and AGS cells from human gastric carcinoma (Kamei *et al,*. 1997).

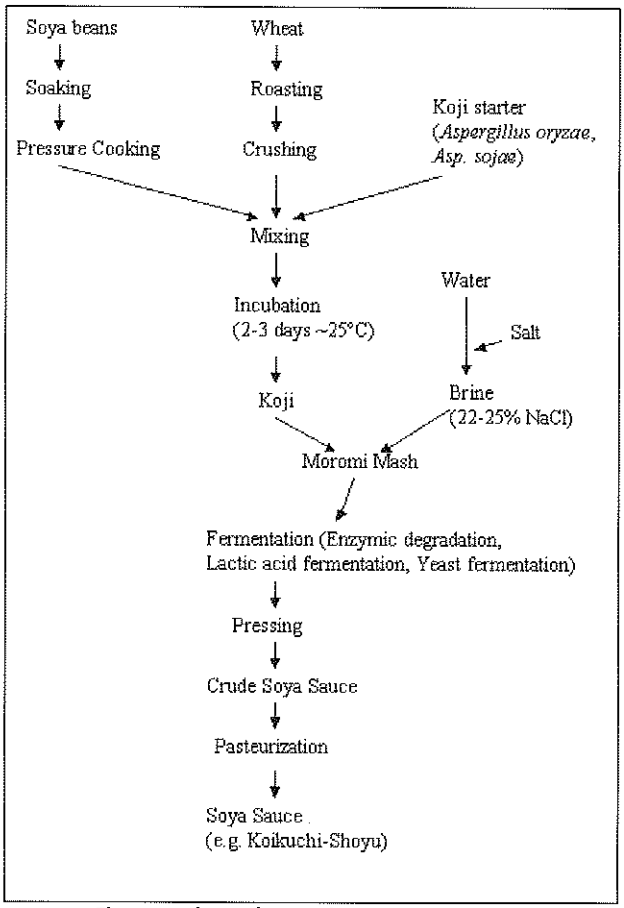

Figure 8. Flow-scheme of soya sauce process.

Table 2. Some edible mushrooms.

Species	Common name	Substrate	Fruiting conditions
Agaricus bisporus *Agaricus bitorquis*	Button mushroom (champignon)	Composted straw; horse manure	6 weeks 14-18°C
Lentinus edodes	Shii-take	Wood logs Saw-dust	5-6 years 12-20°C several weeks
Pleurotus ostreatus *Pleurotus sajor-caju*	Oyster mushroom	Saw-dust, straw, leaves, paper, cotton waste, etc.	10-14 weeks 10-35°C
Ustilago maydis	Maize mushroom ("huitlacoche", "caviar azteca")	pre-harvest maize cobs	several weeks at 25-35° C
Volvariella volvacea	Paddy straw mushroom	Composted rice straw; various agro by-products	2-6 weeks 30-40°C

FUNGAL BIOMASS and BIOTRANSFORMATIONS

Edible fungi can be grown for consumption purposes, either as their fruiting bodies (mushrooms), as mycelium (mycoprotein) or as yeast cells (single cell protein).

Mushrooms

From the wide variety of edible mushrooms, only a few species have developed into commercial commodities. Table 2 lists some species of interest and their conditions of growth. The total commercial mushroom production is estimated at 1-2 million tonnes. *Agaricus* spp. account for a majority of the total production. The nearly-white *A. bisporus* is known best, but the more virus-resistant *A. bitorquis* and brown-capped varieties of *A. bisporus* gain increasing interest.

After colonization of the substrate by the fungal mycelium has taken place, fruiting is initiated by changing the environmental conditions (aeration, humidity, temperature). Fruiting bodies are produced in a number of successive "flushes" with intermezzo's of 1-2 weeks.

After 4-5 flushes have been harvested, the yield decreases and the cycle is re-started with fresh substrate. The old colonized substrate is used as protein-rich animal feed ingredient. In wheat straw, the initial lignin content was halved after 12 weeks of growth of *Pleurotus* spp., and consequently the digestibility of the remaining cellulose was increased significantly for ruminants (Moyson and Verachtert, 1991).The large fruiting bodies of the exotic basidiomycete, *Ustilago maydis* are collected and consumed as mushrooms. They are especially popular in Latin America where they are known as "Huitlacoche" or "Caviar Azteca" (Valverde *et al.*, 1995).

Single-cell protein (fodder yeast, mycoprotein)

Various yeast strains (*Candida utilis, C. tropicalis, Yarrowia lipolytica, Kluyveromyces lactis*) can be grown at high cell yield on industrial by-product substrates e.g. cheese whey, waste water from potato starch industries, wood sulfite liquor, and hydrocarbon residues. Although several industrial processes have been patented, the single cell protein thus obtained is presently not competitive compared with other (e.g. soya) proteins.

Fungal mycelium of e.g. *Penicillium chrysogenum* and *Aspergillus niger* is produced in large quantities as a by-product of the fermentative production of antibiotics, enzymes, organic acids, etc. The mycelium of non-toxigenic fungi is an interesting food ingredient since it has a relatively high crude protein content (approx. 12% on fresh weight basis). The mycelium is used as an ingredient in animal feed.

Likewise, the mycelium of a strain of *Fusarium venenatum* (previously *F. graminearum*) is used for industrial-scale production of a texturized protein which is marketed as "Quorn" (Wiebe *et al.*, 1996).

Figure 9. Maize mushroom (*Ustilago maydis*)

The product finds application as meat substitute in savoury pies, soups, etc. The use of microbial protein for human or animal consumption is limited by its nucleic acid content. The WHO recommends a maximum level of nucleic acid (NA) of 2% in foods. Whereas bacterial protein contains rather high levels of NA, mycoprotein (*F. venenatum*) contains about 6-13%. The RNA content can be reduced to below 2% by a heat-shock ("curing") treatment. After fermentation, the mycelium is briefly held at 64°C to activate intracellular RNAses that convert cellular RNA into monomer nucleotides which can diffuse out of the cells. Simultaneously, this curing achieves pasteurization.

Protein-enrichment of starchy foods and feeds

Because of their ability to degrade carbohydrate matter, fungi are useful in the upgrading of the nutritive value of industrial and agro-processing by-products e.g. starch containing sweet potato residues (Yang *et al.*, 1993) or cellulosic sugarcane bagasse (Moo-Young *et al.*, 1992). For that purpose added cheap sources of nitrogen e.g. $(NH_4)_2SO_4$ and urea can be converted into protein thus enriching the food. Final products (after a few days fermentation) may contain approximately 30% w/w crude protein on a dry matter basis, when food-grade fungi e.g. *Aspergillus niger, Rhizopus* spp. and *Neurospora sitophila* are used.

Detoxification of mycotoxins

Several fungi are able to metabolize and detoxify mycotoxins. Patulin in apple juice could be degraded >90% during alcoholic fermentation with *Saccharomyces cerevisiae*; Ochratoxin A in barley malt was partly (50-80%) degraded by *Saccharomyces* spp. during beer brewing; Aflatoxin B1 in groundnut meal could be almost fully degraded (>95%) during 7 days solid-substrate fermentation with a selected *Aspergillus* strain (Bol and Knol, 1991). Although these fermentation processes may be relatively time consuming and expensive, biological detoxification of mycotoxins has the advantage over chemico-physical processes that the treatment takes place under mild conditions and the quality characteristics of the product e.g. nutritive value, can be better maintained.

FOOD INGREDIENTS and ADDITIVES OF FUNGAL ORIGIN

Organic acids

Organic acids produced by fermentation can be distinguished in 2 groups: (i) produced through the tricarboxylic acid pathway and (ii) produced directly from glucose. In group (i), **citric acid** is the most important organic acid produced by fermentation. Its annual production is estimated at 400,000 tonnes which are made mainly with *Aspergillus niger*, but also *Yarrowia lipolytica* is used. Surface as well as submerged culture systems are employed for the conversion of cheap carbon sources (molasses) and n-alkanes. Citric acid is extensively used in the food industry as an acidulant and flavouring substance. **Itaconic acid** can be made with *Aspergillus terreus* using e.g. molasses or wood hydrolysates, and finds application in the chemical industry (polymers, surface-active compounds). **Malic acid** is made in a 2-stage process: first, fumaric acid is produced from sugars using (immobilized) *Schizophyllum commune*, and subsequently the fumarase activity of *Aspergillus wentii* converts fumaric acid into malic acid. **Tartaric acid** (50,000 tonnes/year) is produced with *Aspergillus griseus* and *A. niger*, and is applied in the food industry as an acidulant. In group (ii), **gluconic acid** (50,000 tonnes/year) is made mainly with bacteria, but also *A. niger* and *A. foetidus* are used in submerged and solid-substrate ("koji") processes. Gluconic acid and its δ-lactone are applied in foods (acidulant) and in the medical field. **Lactic acid** (30,000 tonnes/year) is made mainly with lactobacilli, but also with *Rhizopus oryzae*, and finds wide applications in the food industry as acidulant, preservative agent, baking powder, etc. (Mattey, 1992).

Lipids

Fungal lipid content may be sometimes as high as 60-80% of biomass dry weight. However, plant and animal oils and fats are cheaper to produce, so it is only for specialty products that fermentation is of economic interest. In particular, the ability to accumulate polyunsaturated fatty acids is of interest from nutritional point of view. Several moulds are commercially used to produce γ-linolenic acid. On cheap sources of nitrogen and carbon (rape meal, starch, molasses), *Mucor javanicus* and *M. rouxii* can be grown in submerged cultures at γ-linolenic acid yields of 0.33 g/L medium. In such cases, the lipid content is 7-11% of the biomass dry weight, and γ-linolenic acid represents 17-37% of lipid weight (Lindberg and Hansson,1991).

Enzymes

Within the range of enzymes produced by fermentation, the majority are proteases and carbohydrases. Proteolytic enzymes obtained with *Aspergillus oryzae, Penicillium roquefortii* and *Mucor* spp. are applied in detergents, and in food processing e.g. accelerated cheese ripening, breadmaking, and tenderization of meat. Carbohydrases include amylolytic enzymes (α-amylase, glucoamylase) produced by *Aspergillus oryzae* and *A. niger* and are applied in e.g. breadmaking, brewing and confectionery. Other carbohydrases are cellulases (Persson *et al*,. 1991) made by *A. niger, Penicillium* spp. and *Trichoderma reesei*; pectinases made by *Aspergillus* spp.; and ß-glucanase made by *A. niger* and *Penicillium* spp.; these enzymes are applied to improve digestibility of fibrous foods, and filterability of fruit

juices and beer, etc. Other important enzymes include lipases produced by *Mucor* spp. and *A. niger*, applied for dairy flavour development; RNAses made by *A. oryzae* applied to prepare nucleotides acting as flavour enhancers; glucose oxidase made by *A. niger* which has many food and medical applications; and phytase made by *A. niger, A. oryzae* and *A. ficuum* which is applied to degrade the anti-nutritive factor phytate in foods and feeds, thereby improving the bio-availability of phosphate and minerals (Zyta, 1992).

Others

A wide variety of valuable substances can be produced using fungi, including amino acids, polysaccharides, vitamins, pigments and flavour components (Vandamme 1993). A few selected examples of recent interest include:the production of natural food-grade red, orange and yellow **pigments** (ankaflavin, monascorubrin and monasein) by *Monascus purpureus* and *M. barkari* (Yongsmith *et al,.* 1993); the formation of heat-stable, characteristic, antigenic **EPS (extracellular polysaccharides)** by most fungi has led to the development of a new generation of immuno-assays (latex agglutination tests and ELISA) for the detection of fungal contamination of foods and raw materials, even after they have undergone thermal processing (e.g. pasteurized fruit juices, jams, etc.) (De Ruiter *et al.,* 1993); the production of the major mushroom **flavour** component 1-octen-3-ol by submerged cultivation of *Pleurotus* and *Morchella* spp. In mushrooms, linolenic acid is converted into a precursor which is oxidized to 1-octen-3-ol upon exposure to O_2 during homogenization of mushroom tissue. After harvesting the fermentor-grown mycelial pellets, they are subjected to shear stress causing cell disruption. This initiates the oxidative conversion. The homogenate is pressed, the obtained juice is freeze-dried and contains approximately 1200 ppm of 1-octen-3-ol. It is applied in dehydrated soups, gravies, etc. (Schindler and Seipenbusch, 1990).

GENE EXPRESSION

Conventional strain improvement (by selection, crossing or mutagenesis) has given rise to production strains with improved process characteristics but the application of recombinant DNA technology offers considerable scope for process improvement and the development of new techniques. Presently, for many of the food-grade fungal species transformation protocols have been developed. In principle, it is technically possible to (1) regulate the expression of a target gene, (2) alter the gene copy number, (3) replace or delete a gene, and (4) introduce a gene from another (heterologous) source. The attraction of fungi, particularly moulds, as expression hosts is that some of them are food-grade, there is much industrial experience with their cultivation and proteins (enzymes) are secreted to high concentrations. Moulds of industrial importance include *Aspergillus niger, A. oryzae* and *Trichoderma reesei.* Nowadays, the production of calf chymosin (rennet for cheese-making), phytase (degrading the anti-nutritive factor phytate in food and feed) and proteases (baking, detergents) is carried out at a commercial level using r-DNA constructs, all *Aspergillus* spp.

Yeasts, especially *Saccharomyces cerevisiae* and *Kluyveromyces lactis,* have very good potential as host organisms. Various r-DNA constructs have been reported (Lang-Hinrichs and Hinrichs 1992) amongst which the following are of particular interest: (1) optimization of brewer's yeasts by expression of amylolytic enzymes, (2) improvement of baker's yeast by e.g. increasing it's freezing resistance, and (3) improvement of distiller's yeasts by enabling their growth on cheap agro-industrial by-products, e.g. cheese whey, starch, cellulose and hemicellulose. At a commercial level, calf chymosin (cheese rennet) is expressed in *Kluyveromyces lactis,* glucose oxidase of *Aspergillus niger* is expressed in *Saccharomyces cerevisiae,* and thaumatin (powerful sweetener for e.g. softdrinks) is expressed in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*

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