Useful role of fungi in food processing

Introduction to food-borne fungi

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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. Whereas undesirable changes such as decay, spoilage and even toxin formation can take place, 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 early application of moulds to prepare fermented food o back several millennia (Ko, 1988). More recently, fungal mycelium as well as yeast cells have been cultivated to obtain protein-rich nutritious food for human and animal consumption (Campbell-Platt and Cook, 1989). Fungi play a significant role in industrial fermentations to produce a variety of 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. The occurrence, manufacture, properties, and use of fermented foods is well-documented (Kosikowski, 1982; Rose, 1982; Steinkraus *et al.*, 1983; Wood, 1985; Beuchat, 1987; Campbell-Platt. 1987). Some fermented products (cheese, bee wine, soya sauce) have experienced a gigantic 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.

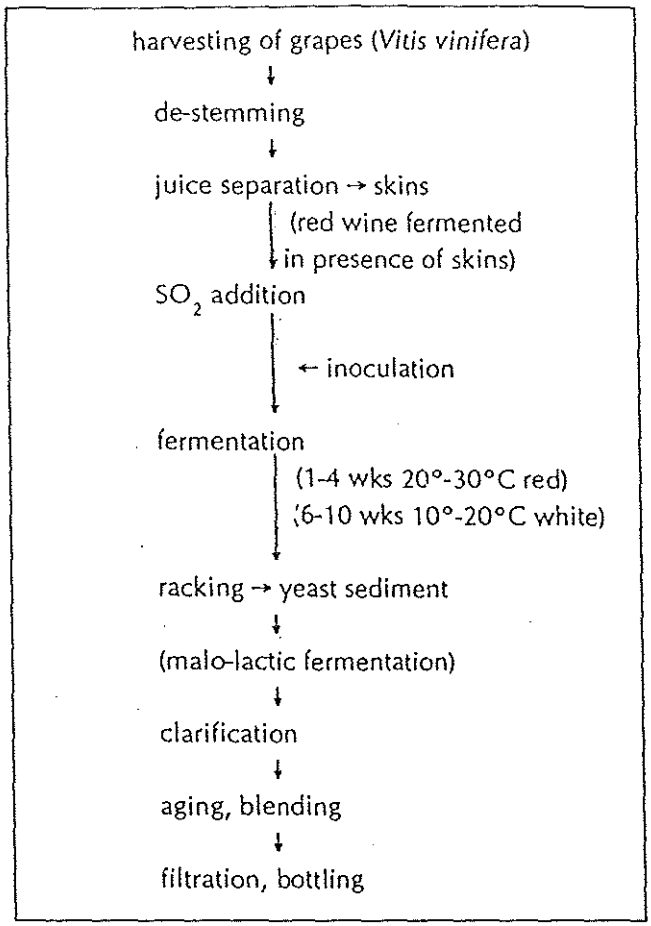
For reasons of product integrity and economics, most food fermentations cannot be carried out profitably under sterile conditions. Fermented foods may therefore contain a variety of bacteria, yeasts and moulds, originating from raw materials, inoculum and process contamination. According to the physical nature of the substrate, fermentations can be distinguished in liquid and solid substrate fermentations. In liquid fermentations, a watery 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. (Samson, 1993). In addition, yeasts play a role in bakery products and in alcoholic beverages. In subtropical 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.

Wine

The known 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 scheme of operations is summarized in Fig. 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 siphoning ("racking") in order to prevent off-odours from yeast autolysis. In high-acidity wines, lactic acid bacteria (*Leuconostoc oenos*) are inoculated to transform malic acid into lactic acid ("Malolactic fermentation") thus giving the wine a more mellow taste (Nout, 1992).

Figure 1. Flow-scheme of wine making.



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 (Androuet, 1971). The principle of Camembert production is outlined in Fig. 3. 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* slowly diffuse into the cheese and cause softening and flavour development. Usually the product is consumed at an age of 3-5 weeks (Kosikowski, 1982).

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 strains kept for prolonged periods in culture collection had not lost the ability to produce CPA. Efforts are undertaken to obtain CPA-negative mutant strains, but up till present these are not yet

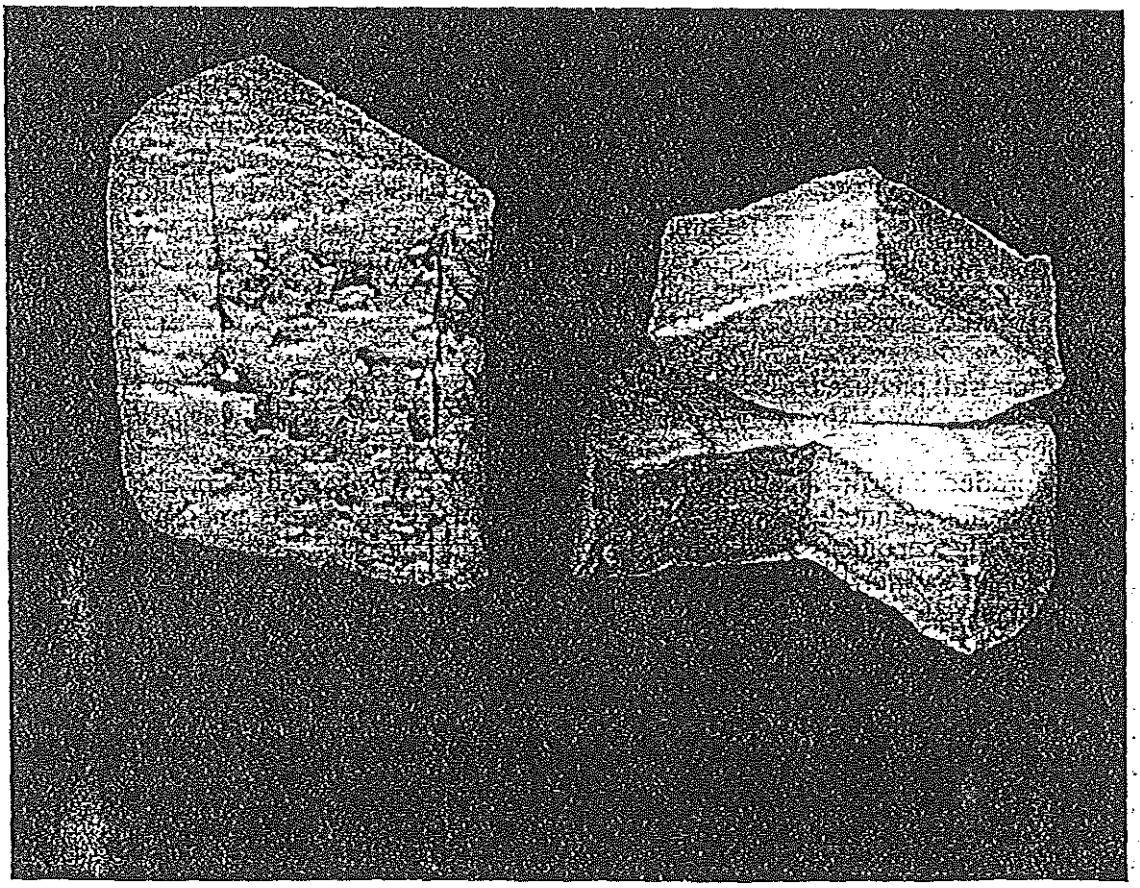


Fig. 2. Roquefort cheese (left) and Camembert cheese (right).

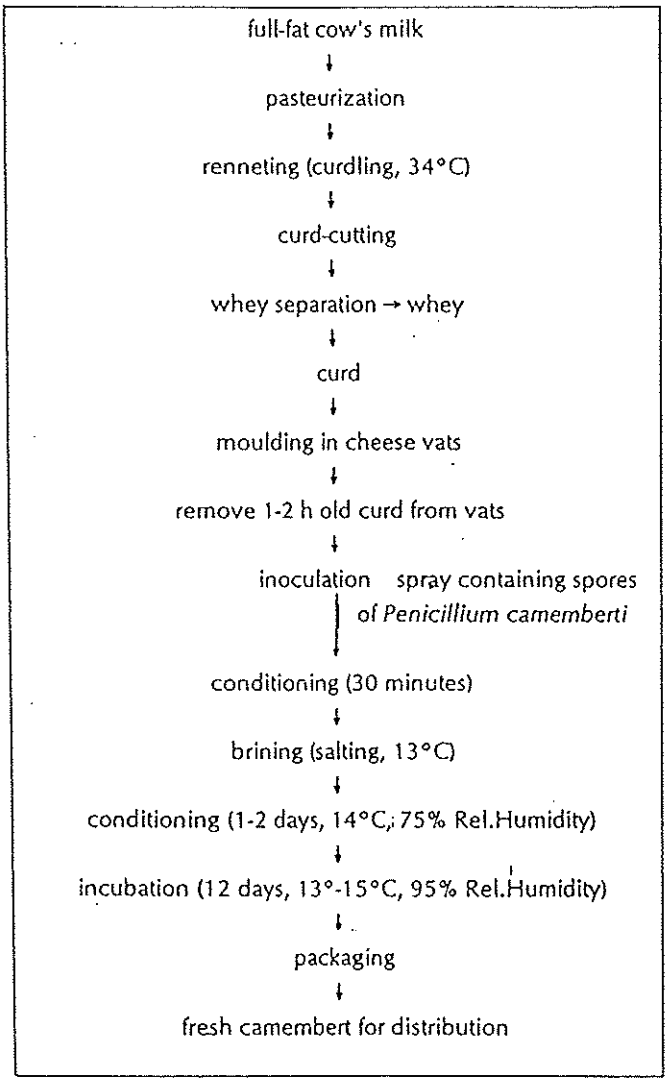
Table 1. A selection of fungal mixed-culture fermented foods (adapted from Nout, 1992).

Moulds	Yeasts	Bacteria	Substrate	Type of fermentation	Name	Nature	Use	Origin
Amylomyces rouxii	*Endomyces* spp. *Hyphopichia burtonii*	*Pediococcus pentosaceus* *Streptococcus faecalis*	uncooked rice	SSF	Ragi	solid tablet	inoculum	Orient
A. rouxii	*Hyp. burtonii* *End. fibuliger*	*Ped. pentosaceus* *Ent. faecalis*	cassava	SSF	Peuyeum	semi-solid mass	snack	Indonesia
Aspergillus oryzae *A. sojae*	*Z. rouxii* *Torulopsis versatilis*	*Ped. halophilus* *Ent. faecalis*	soya bean + rice/barley	SSF	Miso	semi-solid paste	flavouring	Orient
A. oryzae *A. sojae-group*	*Z. rouxii* *Z. sojae* *Z. major* *Hansenula* spp. *Torulopsis* spp. *Candida etchellsii* *C. versatilis*	*Lactobacillus delbrueckii* *Ped. halophilus* *Ped. damnosus*	soya bean + wheat + salt	SSF-L	Soya sauce	liquid	flavouring	Orient
A. o.	*Hans. anomala* *S. cerevisiae*(saké)	*Lc. mesenteroides* var. *saké* *Lb. saké*	rice	SSF-L	Saké	liquid	liquor	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
P. camemberti (*P. candidum*) (*P. caseicolum*) (*P. album*)	*Candida* spp. *Kluyveromyces* 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
P. nalgiovense *P. chrysogenum*		*Micrococcus* spp. *Staphylococcus* spp. *Pediococcus* spp. *Lactobacillus* spp.	meat (sausage)	SSF	Salami	solid	protein food	Europe
Rh. oligosporus *Rh. chinensis* *Rh. oryzae* *M[illegible] indicus*	*Trichosporon beigelii* *Clavispora lusitaniae* *C. maltosa* *C. intermedia* *Y. lipolytica*	*Klebsiella pneumoniae* *Enterobacter cloacae* *Lactobacillus* spp.	mostly soybeans	SSF	Tempe	semi-solid cake	protein food snack	Indonesia
	T. holmii *S. cerevisiae* *Pichia saitoi* *C. krusei*	*Lb. plantarum* *Lb. fructivorans* *Lb. brevis* var. *lindneri* *Lb. sanfrancisco*	rye or wheat flour	SSF	Sour-dough	semi-solid mass	inoculum	Europe
	S. cerevisiae (*S. uvarum*) (*S. elegans*) (*S. bayanus*)	*Leuc. oenos* *Ped. acidilactici* *Lb. casei*	grape juice	L	Wine	liquid	liquor	Europe
	S. cerevisiae (*S. bayanus*) (*S. uvarum*) *Brettanomyces* spp.	*Lactobacillus* spp. *Pediococcus* spp.	barley wheat	L	Lambic-Gueuze	liquid	liquor	Belgium

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

available (Leistner, 1990). 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 $<15°C$.

Figure 3. Flow-scheme of camembert cheese manufacture.

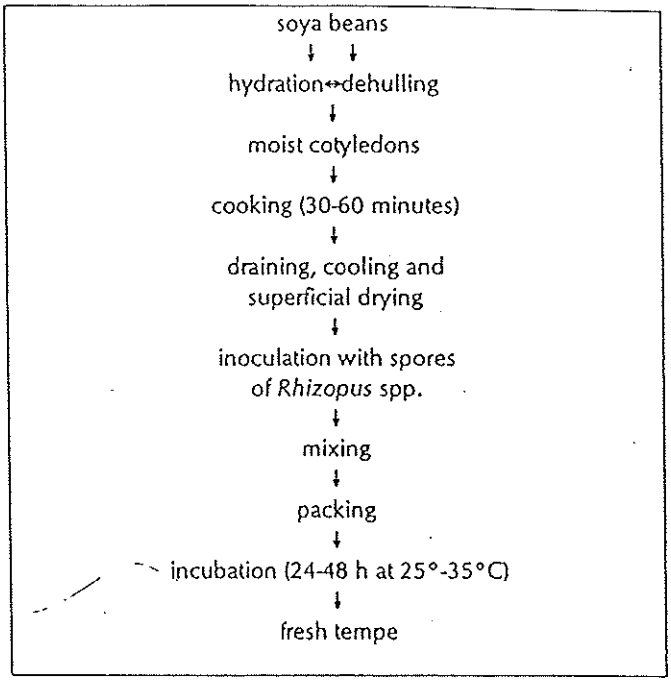


Tempe

Tempe originates from Java, Indonesia but 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.

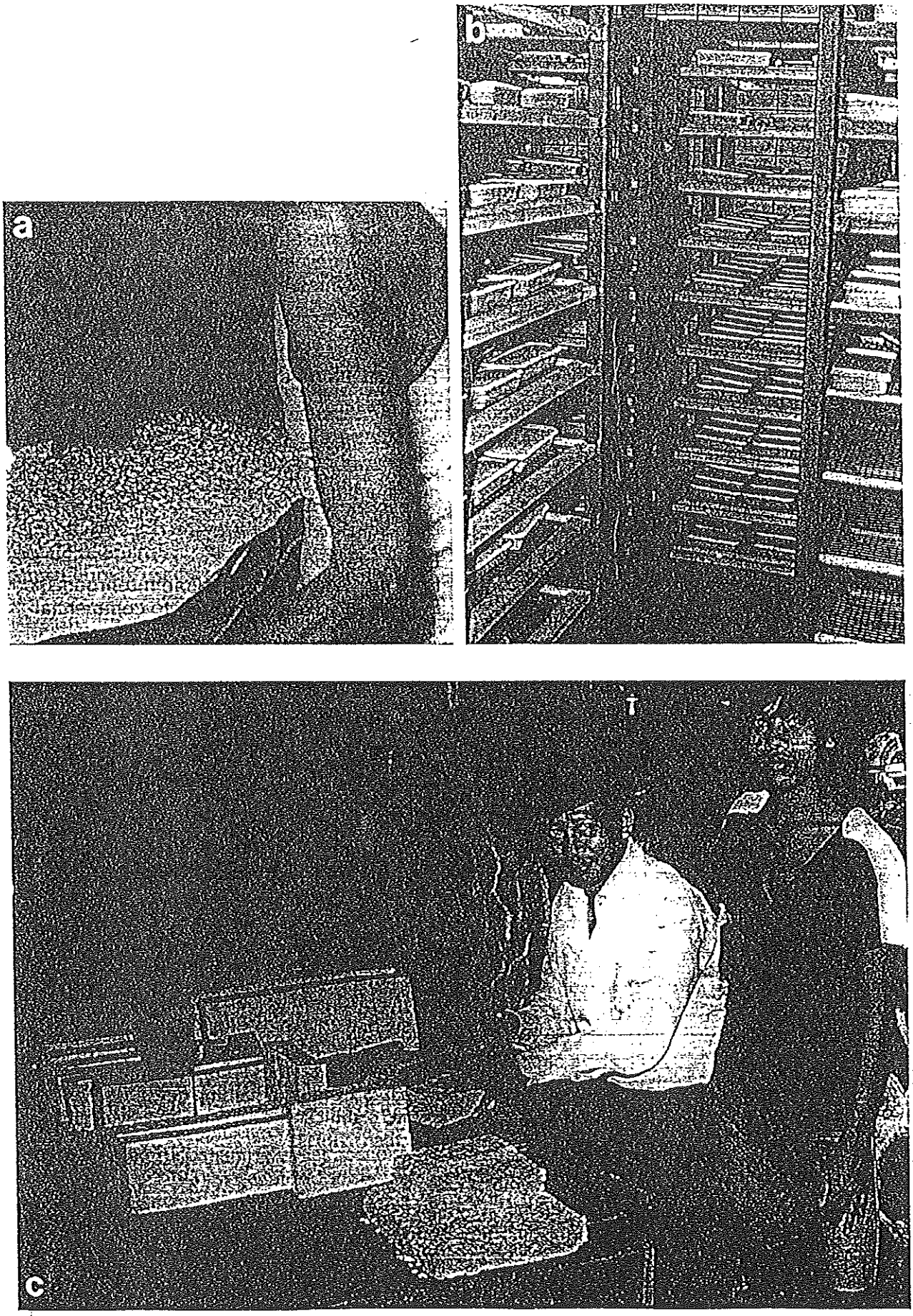
The most common substrate is soya beans (Nout, 1992; Nout and Rombouts, 1990). Tempe provides a cheap, nutritious, digestible and safe source of vegetable protein. It is not eaten fresh, but only after cooking (stewing) or frying in oil (crispy "tempe kripik"). The traditional manufacturing process is outlined in Fig. 4.

Figure 4. Flow-scheme of tempe production (from Nout and Rombouts, 1990).



Soya beans are soaked and wet-dehulled (Indonesia), or dry-dehulled and soaked (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 down, 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 35°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. 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. As a result, a shiny white tempe is produced. 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). Antinutritional factors e.g. phytic acid are also degraded, improving the bio-availability of phosphate and minerals.

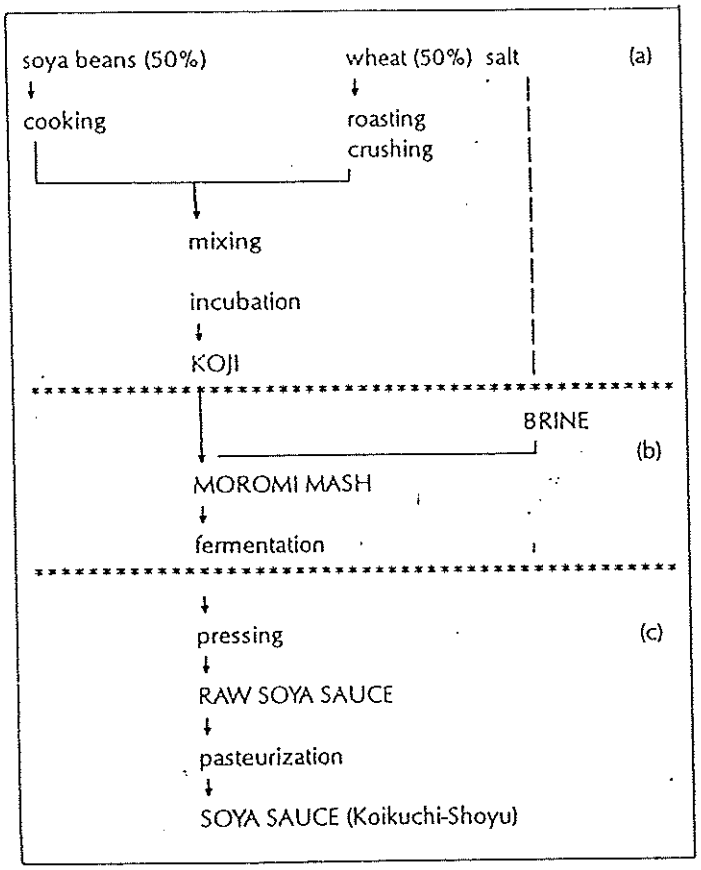
Fig. 5. a. Preparation of a bed of inoculated soyabeans prior to the fungal stage of the tempe fermentation (Malang, Indonesia). b. Incubation during tempe fermentation in perforated plastic boxes (450 g)(Groningen, The Netherlands). c. Sale of fresh tempe (Malang, Indonesia)


a
b
c

Soya sauce

Soya sauce is of Chinese or Japanese origin. Only in Japan, more than 2.10^6 tons are produced annually (1986). There are many different types, but Koikuchi-Shoyu is the best representative of fermented soya sauces (Fukushima, 1989). 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 (Fig. 6) consists of 3 phases: (a) koji making, (b) brine fermentation and (c) refining. Koji making is a solid-substrate mould fermentation of a mixture of previously steam-cooked soya beans and roasted and crushed wheat.

Figure 6. Flow-scheme of soya sauce process (adapted from Fukushima, 1989). (a) Koji making process; (b) brine fermentation process; (c) refining process.



This is inoculated with conidia of *Aspergillus oryzae* or *A. sojae* and is incubated at 25°C during 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. The second phase (maturation of the moromi mash) takes place in a salt bath (22-25% w/v NaCl) so that spoilage microorganisms cannot develop, but only enzymatic degradation takes place. Nevertheless, during the first 2 months at 15-20°C, halophilic lactic acid bacteria (*Pediococcus halophilus*), 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 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- and colour development and for inactivation of the fungal enzymes. After aseptic bottling, the product is distributed.

FUNGAL BIOMASS AND BIOTRANSFORMATIONS

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

Edible mushrooms

From the wide variety of edible mushrooms, only a few species have developed into commercial commodities. Table 2 lists the most important species and their conditions of growth. The total commercial mushroom production was estimated at more than 1 million tons (1983). *Agaricus* spp. account for approximately 70% of the total production (Campbell-Platt and Cook, 1989). 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 intervals 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).

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 pro-

Table 2. Some important commercialized types of edible mushrooms (adapted from Campbell-Platt and Cook, 1989).

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
Volvariella volvacea	Paddy straw mushroom	Composted rice straw; various agro by-products	2-6 weeks 30-40°C
Pleurotus ostreatus *Pleurotus sajor-caju*	Oyster mushroom	Saw-dust, straw, leaves, paper, cotton waste, etc.	10-14 weeks 10-35°C

tein 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 non-toxigenic strain of *Fusarium graminearum* has been used to produce a texturized protein which has been marketed as "Pruteen" and "Quorn" (Sadler, 1990). The product has found 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. graminearum*) 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 sugar cane 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. This type of conversion is not commercialized at present: for human consumption purposes the raw materials are often not sufficiently "food-grade", whereas for animal feed purposes the required nitrogen sources and processing would render the final product too expensive compared with alternative protein sources.

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 tons 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 tons/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 tons/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 tons/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 roqueforti* and *Mucor* spp. are applied in detergents, and in food processing e.g. accelerated cheese ripening, bread making, and tenderization of meat. Carbohydrases include amylolytic enzymes (α-amylase, glucoamylase) produced by *Aspergillus oryzae* and *A. niger* and are applied in e.g. bread making, 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).

Other compounds

A wide variety of valuable substances can be produced using fungi, including amino acids, polysaccharides, vitamins, pigments and flavour components (Campbell-Platt and Cook, 1989; Vandamme, 1993). A few selected examples of recent interest include:

a. 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);

b. 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);

c. 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 recombinant DNA constructs, all *Aspergillus* spp.

Yeasts, especially *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, have very good potential as host organisms. Various recombinant 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. soft drinks) is expressed in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*.

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