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Fungi in the production of foods and food ingredients

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1. Introduction

The gathering of fungal fruit bodies probably represents the oldest use of fungi as food by man and a considerable volume of literature has been published regarding fungal consumption as food and the toxic, narcotic, and religous significance of fungi in many cultures (Rolfe & Rolfe 1928; Ramsbottom 1953; Allegro 1970). Mushrooms were prized by the ancient Egyptians and the Romans held them in high esteem. Records of the consumption of fungi in China date back to between 26 BC and 220 AD although there is evidence of use as early as 6000 years ago (Wang 1985). Mushrooms, however, are not the only direct source of fungal food. The 'native bread', a sclerotium of *Polyporus mylittae*, may weigh several kilograms and has been consumed by Australian aborigines (Willis 1967). Similarly, the stone fungus or 'tuckahoe' sclerotium of *Poria cocos* was believed to be roasted as a 'bread' and consumed by North American Indians and peoples in Eastern Asia (Weber 1929). Seeds of *Zizania aquatica* infected by the smut *Ustilago esculenta* have been reported to be consumed by people in Taiwan, and *U. maydis* is reportedly eaten in Mexico (Hawksworth *et al.* 1983).

Traditionally, fungal fruit bodies have often been associated with meat or consumed as meat-like products. Women gathering wild mushrooms in Malawi regard them as analagous to meat (Morris 1984) and Francis Bacon in his *Sylva Sylvarum* of 1627 described mushrooms as yielding 'so delicious a meat'. In many indigenous fermented foods, which are often processes of great antiquity (Stanton 1985, 1987), fungi contribute to the texture and flavour of the products. Many of these foods such as tempe, oncom, soy sauce, miso and katsuobushi have meat-like flavours and are consumed directly or used as seasonings and condiments. The association between fungi and meat is continuing with the interest in 'microbial farming' (Yin 1949) to produce mycoprotein foods which are currently being promoted as meat-like products.

Traditional fermented foods from the orient such as tempe, soy sauce and miso are becoming popular in the United States and Europe and this area of technology transfer presents major opportunities for the expansion of interest in fungi, particularly with the current interest in novel 'health' foods in parts of the developed world.

The direct exploitation of fungal metabolism to obtain useful components such as organic acids and enzymes for bioprocessing did not begin until the 1890s when the ability of moulds to saccharify starch was investigated. These processes, and the development of new ones for the production of

Table 1. The major species of cultivated mushrooms, substrates used, fruiting period and temperature, areas of production and world output figures

Species	Substrate(s)	Fruiting period§ (fruiting temperature)	Areas where cultivated	World production (thousand tonnes)
Agaricus bisporus/ *A. bitorquis*	Composted straw	6 weeks (14–18°C)	Widespread in temperate areas	750* (670)
Lentinus edodes	Logs of broad-leaved trees; sawdust blocks	5–6 years (12–20°C)	Temperate Eastern Asia, Europe, USA	180 (130)
Volvariella volvacea	Composted rice straw; cotton waste†	2–6 weeks (30–40°C)	South-East and Eastern Asia, Africa	65 (42)
Pleurotus spp.	Logs and various‡	10–14 weeks (10–33°C)	Eastern Asia, India, Europe, USA	40 (12)
Flammulina velutipes	Sawdust + rice bran	4–6 weeks (3–8°C)	Eastern Asia, Europe	40 (38)
Pholiota nameko	Logs of broad-leaved trees; sawdust + rice bran	4–8 weeks (10–25°C)	Eastern Asia	20 (15)
Auricularia spp.	Wood; sawdust + rice bran	Several years (23–28°C)	China, Taiwan	12 (5·7)
Tremella spp.	Logs of broad-leaved trees	Several years (20–27°C)	China, Japan, Taiwan	3 (1·8)

* Figure from Zadrazil & Grabbe (1983), those in parentheses for 1975 from Delcaire (1978).

† Also on sawdust + rice bran, banana leaves + sawdust, oil palm pericarp, waste sugar cane, water hyacinth.

‡ Sawdust, cereal straw, newspaper, banana pseudostems, grassmeal, oil palm pericarp, cotton cloth, cocoa pod waste.

§ May be shorter on certain substrates.

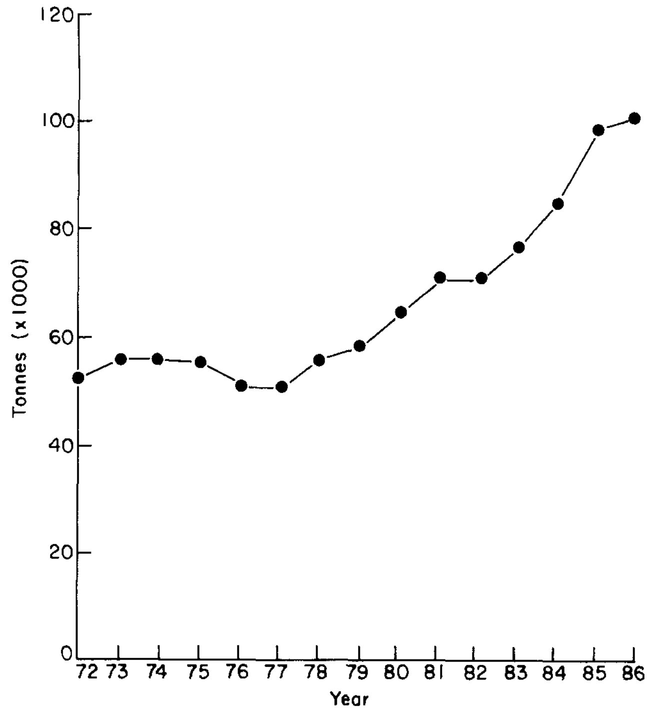

Fig. 1. Commercial production of mushrooms in the UK 1972–1986 (Source MAFF).

organic acids, in particular citric acid, led to commercial production and the search for other products for exploitation (Miall 1975). The technology gained from penicillin production helped in the exploitation of these processes, and today a large number of products can be produced from fungi. These include organic acids, lipids and enzymes which have applications in many industries, including the food industry, although in some cases cheaper alternative sources or processes other than from fungi are available.

2. Production of fungal biomass

The production of fungal biomass as mushrooms probably represents the largest use of filamentous fungi in the food industry, but relatively few species of fungi are grown commercially. Fungal biomass can also be produced by cultivation of microfungi to produce mycoprotein products for use in a range of foods.

2.1 MUSHROOM CULTIVATION

Although many hundreds of species of edible mushrooms exist in the wild, less than 20 species are used extensively as food and only 8–10 species are regularly cultivated to any significant extent. Table 1 shows the major cultivated species of edible mushrooms together with substrates used for cultivation, production period and fruiting temperature, areas of cultivation and estimated world production figures. The total world production is now in excess of 1 million tonnes per annum (Zadrazil & Grabbe 1983) having increased from 250000 tonnes in 1960. Of these figures *Agaricus bisporus* with a small proportion of *A. bitorquis* form over 67% of the world's cultivated mushroom production. Currently there are some 400 mushroom growers in the UK. Production, almost exclusively *A. bisporus*, was approximately 100000 tonnes in 1986 and the six largest growers accounted for 57% of total production (Hinton 1987). The upward trend in mushroom production in the UK is shown in

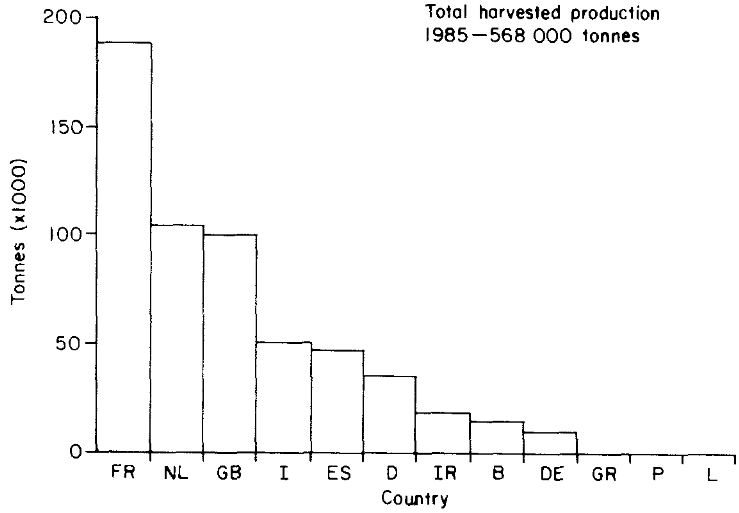

Fig. 2. Harvested production of cultivated mushrooms in the EEC countries in 1985 (Source Eurostat 1987). Key to countries: B, Belgium; D, Germany; DE, Denmark; ES, Spain; FR, France; GB, Great Britain; GR, Greece; I, Italy; IR, Ireland; L, Luxemborg; NL, Netherlands; P, Portugal.

Fig. 1. The value of UK production in 1987 was £223m at the retail level with a total retail value including imports of some £270m. Mushroom output in terms of value is now almost half the value of all protected crops in Britain (Hinton 1987). Within the European Community, France is the largest producer of mushrooms followed by the Netherlands, with the UK third (Fig. 2). The cultivation system appears to be much more intensive in Holland where in 1985 105 000 tonnes were produced on 100 ha of land compared with 100 000 tonnes on 500 ha in the UK.

Cultivation of *A. bisporus* is now well established in many regions of the world including South-East Asia, East Asia, the Indian subcontinent and some African and South American countries. Cultivation of *A. bitorquis* is becoming popular, particularly in Holland but poor consumer acceptability has not led to it being extensively cultivated in Britain. *Agaricus bitorquis* fruits at a higher temperature than *A. bisporus* and has an advantage in not being susceptible to the viruses which can damage crops of *A. bisporus*. New varieties of *A. bisporus* are becoming popular, particularly in France where the 'brown-cap variety' constitutes 30% of production. The brown variety has a firmer texture and longer shelf life but trials in the UK have shown poor consumer acceptability.

The majority of mushrooms are sold directly through retail outlets but packaging is often done by the producer rather than the retailer. Mushrooms may also be used in soups, creamed sauces, pies and as dried slices. Many manufacturers require guaranteed production for particular product lines because of the large capital investment in processing technology and for this reason some food companies run their own farms.

The Shiitake mushroom (*Lentinus edodes*) has traditionally been cultivated on logs of broad-leaved trees in East and South-East Asia particularly in Japan where there are an estimated 230 000 growers (Zadrazil and Grabbe 1983). World production is approximately 180 000 tonnes (Zadrazil and Grabbe 1983) and in recent years this species has become more popular in the West, particularly in the USA where 'Steak and Shiitake' is now popular on restaurant menus (Burns 1987). The long production cycle of this mushroom (5–6 years) and the need for particular woodland management, probably limited the spread of *Lentinus* cultivation to the West. Shiitake is now being cultivated in the USA and in several European countries including Britain. Recent trials have been conducted in South Devon to evaluate the potential of Shiitake cultivation for increasing the utilization of broad-leaved woodlands (Burns 1987).

New methods of cultivation using compressed sawdust blocks containing rice bran, oatmeal or soybean meal have enabled a reduction in the production cycle for this species and made possible an intensification of production. Recently Pettipher (1988) has investigated the cultivation of *Lentinus edodes* on blocks consisting of seven parts hardwood sawdust and three parts oatmeal. Edible mushrooms were produced from colonised blocks of substrate in 9–15 days after transfer to an environment suitable for fruiting with an average yield of *ca* 135% fresh weight of fruit bodies per unit dry weight of substrate.

The paddy straw mushroom (*Volvariella volvacea*) fruits at a higher temperature than *Agaricus* or *Lentinus* and its cultivation is mainly restricted to South-East Asia although it has been cultivated in Nigeria and Madagascar (Wood & Smith 1987). The productivity of this species has been increased in recent years by protected cultivation on rice straw and by utilizing various waste products, e.g. cotton waste, banana leaves and sawdust, oil palm pericarp, water hyacinth (Wood & Smith 1987). Although regarded as a delicacy, the fresh mushrooms have a poor shelf life and are usually picked before maturity. Paddy-straw mushrooms which are sold in the West are canned, immature fruit bodies often with the volva still partly enclosing the cap.

The winter mushroom (*Flammulina velutipes*) is quite significant in terms of world production, but in Europe it has only recently been considered for cultivation, although it has been gathered in China and Japan for many centuries and grows in the wild in many parts of Europe. Because of its low temperature requirement for fruiting (3–8°C) this species may prove suitable for commercial development in the foreseeable future.

Oyster mushrooms (*Pleurotus* spp.) have attracted considerable interest for commercial development. The wide range of substrates on which they are capable of growing, together with the ease of substrate preparation have resulted in a sharp increase in world production (Table 1) (Hayes 1985; Wood & Smith 1987). Although *P. ostreatus* is the most widely cultivated species, a number of others have also been cultivated (Hayes 1985) and the range of fruiting temperatures in this group of species (15–33°C) should enable cultivation methods to be produced for most temperate and tropical areas. The exploitation of a number of *Pleurotus* species contrasts with other genera where only 1 or 2 species are grown commercially.

Traditionally, mushrooms have been considered a luxury food, but in parts of Africa they may form up to 10% of daily protein intake (Piearce 1981). Worldwide mushroom consumption in 1974 was highest in West Germany, followed by Canada, France, United Kingdom, Netherlands and the United States (Edwards 1976 cited by Hayes & Wright (1979)). This pattern may change in future years as the technology of mushroom culture is exploited in many developing countries. Mushroom cultivation is currently the most successful and economically viable process for the conversion of lignocellulosic wastes (Wood & Smith 1987) and its importance in this respect is likely to increase in future years.

2.2 MYCOPROTEIN

Moulds have been considered as potential sources of food and feed since World War II and a large number of species have been grown in liquid culture as possible sources of protein or flavour additives (Gray 1970; Litchfield 1979). Although growth rates and yields of filamentous fungi are lower than most bacteria and many yeasts, harvesting of mycelium from growth media is easier and levels of nucleic acids are lower.

In 1964 Rank Hovis McDougall plc (RHM) began a research programme to investigate the production of single cell protein (SCP) from carbohydrates, and a worldwide survey involving more than 3000 soil samples was undertaken to isolate moulds for potential protein production. This work resulted in the selection of a strain of *Fusarium graminearum* (ATCC 20334) from a soil sample taken near High Wycombe and this strain was used to develop the mycoprotein product. Some strains of *F. graminearum* are known to produce the mycotoxin zearalenone when grown on maize and this can produce an oestrogenic syndrome when fed to pigs (Moss 1987). After extensive toxicity tests and health screening trials on a range of mammals and poultry, the *Fusarium* mycoprotein was sanctioned as safe by the UK government in 1980 and approved for sale in the UK by the Ministry of Agriculture, Fisheries and Food in 1985. Human trials were conducted at the Massachusetts Institute of Technology to assess the acceptability and nutritional properties of mycoprotein. Mycoprotein

Cooking Instructions:
Serve hot: Remove pie from box. Place on a baking sheet in a preheated oven 190°C, 375°F, Gas mark 5 for 20 minutes.

INGREDIENTS: WATER, WHEATFLOUR, VEGETABLE MARGARINE (WITH COLOUR: ANNATTO), MYCO-PROTEIN, MUSHROOMS, SOYA FLOUR, SALT, VEGETABLE STOCK, MODIFIED STARCH, DEXTROSE, MUSTARD POWDER, XANTHAN GUM

NUTRITION	TYPICAL VALUES (COOKED AS PER INSTRUCTIONS)	
	PER 100g (3½ oz)	PER PIE
ENERGY	245 k.CALORIES 1015 k.JOULES	460 k.CALORIES 1925 k.JOULES
PROTEIN	7.4g	14.1g
CARBOHYDRATE AVAILABLE	20.8g	39.5g
TOTAL FAT	15.0g	28.5g
of which POLYUNSATURATES	4.7g	8.9g
SATURATES	4.2g	8.0g
DIETARY FIBRE	2.0g	3.8g
ADDED SUGARS	less than 0.1g	less than 0.1g
ADDED SALT	1.4g	2.7g

Produced for J Sainsbury plc Stamford Street London SE1 9LL

Fig. 3. Product containing mycoprotein now available to the consumer through a retail outlet. (Reproduced by kind permission of J. Sainsbury plc.)

was found to compare favourably with milk protein, have a high content of polyunsaturated fatty acids, high levels of dietary fibre and a low degree of allergenicity (Edwards 1986).

Initial fermentation work was conducted using a pilot scale fermenter producing 50 tonnes of mycoprotein per year but in 1984 a new venture company, New Era Foods was set up between RHM plc and Imperial Chemical Industries (ICI) plc and mycoprotein production was scaled up using the 1000 tonne per year pilot pruteen fermenter at the ICI plant at Billingham on Teeside.

In current production methods, *F. graminearum* is grown using a continuous culture system at 30°C with a substrate of food-grade glucose syrup derived from wheat starch; ammonia is added as a nitrogen source. Potentially the glucose syrup could be derived from a wide range of starchy foods or food processing residues via chemical hydrolysis or possibly amylolytic enzymes. During fermentation the culture doubles in mass every 4–5 h. After fermentation, levels of RNA which have caused problems in acceptability of SCP from yeast and bacteria are degraded at 64°C by endogenous

RNAase from initial levels of 10% dry weight down to below 2%. The wet mycelium is formed into steamed sheets which are either frozen to −20°C or spray dried to form a powder. Each batch of mycoprotein is screened for a range of mycotoxins prior to any further processing (Edwards 1986).

Mycoprotein can be dried and rehydrated, canned or frozen and it can be incorporated into a range of foods including soups and fortified drinks, biscuits, chicken-, ham- and beef-flavoured protein, game pie and some fish products. Several products have already gone into production and have been on sale through a leading supermarket chain since 1985 (Fig. 3).

The potential for deriving food or feed from the processing of waste products has been established for several years in the Pekilo process which produces fungal protein for animal feed from paper pulp liquor using *Paecilomyces variotii* (Litchfield 1979). Ivarson & Morita (1982) have demonstrated that fungal biomass with a high protein content can be produced by *Scytalidium acidophilum* from acid hydrolysed waste paper at pH 1. This application is particularly attractive since contamination problems are minimized by the low pH and the waste paper is a readily available commodity which is expensive to dispose of by other means. With current interest in bioprocessing of waste products there may be an increasing use of microfungi which can be exploited for a range of food or feed products.

3. Fermented foods

Filamentous fungi have been associated with the production of fermented foods for at least 2000 years, particularly in South-East Asia where these foods are consumed by large numbers of people on a daily basis. Hesseltine (1965) provided a detailed insight into the relationship between fungi, food and fermentation and this key paper was the impetus for many subsequent studies on these foods. Stanton (1985, 1987) has reviewed fermented food production in the tropics, and Steinkraus (1983), Wood (1985) and Campbell-Platt (1987) discuss types of fermented foods and the micro-organisms involved, together with the biochemical and nutritional processes which take place during fermentation.

3.1 FERMENTED FOODS INVOLVING FUNGI

Table 2 lists the fermented foods which involve the use of filamentous fungi in their production. Although fermented foods such as mould-ripened meat and cheese are familiar in Europe and North America, the majority of fermented foods involving filamentous fungi are produced in East and South-East Asia. In Africa, fermented foods are produced almost exclusively using bacteria or bacteria and yeasts. The reason for this is not entirely clear but may relate to climatic and cultural differences, or to the limited use of rice and soybean in Africa.

The diversity of food products produced by fermentations involving moulds is considerable, ranging from mould-ripened cheese and meat, savoury staple foods such as tempe, oncom and bonkrek, flavouring products like soy sauce, miso, katsuobushi and sweet foods including tape and brem cake. The majority of moulds involved in these fermentations belong to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Amylomyces* and *Mucor*. Those of *Neurospora*, *Monascus* and *Actinomucor* are involved in fewer fermentations. Species of *Aspergillus* and *Penicillium* are typical of products from temperate areas (cheese, meat, soy sauce, miso) and *Rhizopus*, *Amylomyces* and *Mucor* of products in predominantly tropical regions (tempe, tape).

3.2 FERMENTATION STARTER CAKES

Starter cultures are important in the production of a number of fermented foods and pure cultures of selected moulds are used in many industrial processes such as cheese, miso and tempe production.

Table 2. Fermented foods which involve the use of filamentous fungi in their production

Fermented Food	Substrate(s)	Filamentous fungi involved*	Distribution	Use(s)
Ang-kak	Rice, maize	*Monascus* spp., *M. purpureus*	EA, SEA, ME	Colouring
Arroz fermentado	Rice	*Absidia corymbifera*, *Aspergillus* spp., *Rhizopus microsporus*	SA	Seasoning
Bonkrek	Coconut press-cake	*Neurospora* spp., *Rhizopus oligosporus*	SEA	Food
Brem	Rice	*Amylomyces rouxii*, *Rhizopus* and *Mucor* spp.	SEA	Sweet snack
Cassava bread	Cassava	*Geotrichum candidum*	AS, CA	Staple food
Cheeses (mould ripened)	Milk	*Penicillium camembertii*, *P. roquefortii*	WW	Food or snack
Chicha	Maize	*Aspergillus* and *Penicillium* spp.	SA	Food
Chinese yeast	Soybean	Mucoraceous moulds	EA	Food
Filmjolk	Milk	*Geotrichum candidum*	EUR	Food
Gari	Cassava	*Geotrichum candidum*	AS	Staple food
Hama-natto	Soybean, occasionally with rice or wheat	*Aspergillus oryzae*	EA, SEA	Seasoning
Hot pepper sauce	Soybean, red pepper, rice, shoyu, beef, jujube flour (*Ziziphus* sp.)	*Aspergillus* spp.	CA, USA EA	Seasoning
Katsuobushi	Skipjack tuna fish (bonito)	*Aspergillus glaucus*, *A. ochraceus*, *A. repens*	EA, IS	Seasoning
Kawal	Leaves of *Cassia obtusifolia*	*Rhizopus* sp.	AN	Food or seasoning
Kenkey	Maize flour	*Geotrichum candidum*	AS	Staple food
Kurut	Ripened milk or yoghurt	*Penicillium* spp.	AN, IS, EA	Food
Meats (salami, ham, sausage) (mould ripened)	Meat and various ingredients	*Aspergillus* spp., *Penicillium camembertii*, *P. nalgiovense*	WW	Food flavouring
Meitauza	Soybean meal	*Actinomucor elegans*	EA	Food
Mien chien	Wheat gluten, glutinous rice	Various Deuteromycotina and *Syncephalastrum* spp.	EA, SEA, USA	Seasoning
Mirin	Glutinous rice, coconut milk	*Aspergillus oryzae*	EA, P	Flavouring
Miso	Rice, soybean, barley	*Aspergillus oryzae*, *A. sojae*	EA, NA	Seasoning
Ogi	Maize	*Aspergillus*, *Cephalosporium*, *Fusarium* and *Penicillium* spp.	AS	Staple food
Oncom	Peanut press-cake	*Neurospora intermedia*, *Rhizopus* spp.	SEA	Food
Poi	Breadfruit (*Artocarpus* sp.), unripe banana, taro (*Colocasia* sp.)	*Geotrichum candidum*	P	Food
Pozol	Maize, occasionally cocoa beans	*Geotrichum candidum* and other genera	CA	Food
Shoyu	Soybeans, wheat	*Aspergillus oryzae*, *Aspergillus*, *Mucor* and *Rhizopus* spp.	EA, SEA, NA	Seasoning
Sufu	Soybean whey curd	*Actinomucor elegans*, *Mucor* spp.	EA, SEA	Food or snack

Table 2. (*continued*)

Fermented Food	Substrate(s)	Filamentous fungi involved*	Distribution	Use(s)
Tape and lao chao	Glutinous rice, cassava, maize, millet	*Amylomyces rouxii*, *Mucor* and *Rhizopus* spp.	SEA, EA	Sweet food
Tempe	Soybean and others	*Rhizopus oligosporus*, *Rhizopus* spp.	SEA, NA, EUR, SA	Food
Torani	Rice	*Geotrichum candidum*	IS	Seasoning

* Many of these fermented foods also involve yeasts and bacteria. In some fermented foods the importance of moulds has not been established and further work is needed.

Key: AN, Africa North; AS, Africa South; CA, Central America; EA, East Asia; EUR, Europe; IS, Indian Subcontinent; ME, Middle East; NA, North America; P, Pacific Islands; SA, South America; SEA, South-East Asia; WW, world-wide.

Table compiled from Hesseltine (1965, 1983), Beuchat (1983), Steinkraus (1983), Wood (1985), Hesseltine & Wang (1986), Campbell-Platt (1987).

Starter cakes are produced in many countries in South-East Asia, East Asia and the Indian subcontinent for the production of various sweet alcoholic foods, beverages and sweets. The cakes consist of rice flour and sometimes various spices, together with micro-organisms including the moulds *Amylomyces rouxii*, *Rhizopus* and *Mucor* spp. The cakes are produced by small 'cottage industries' quite distinct from the production of fermented foods and beverages, and the preparation and properties of spices used as ingredients are often secret. The mould *Amylomyces rouxii* appears to reproduce entirely by chlamydospores and is not known to occur other than in the starter cakes and the associated fermented products. Growth rates of this fungus are high but exhibit considerable variation between strains. Growth of 20 strains from Indonesian starter cakes varied between 32 and 64 mm per day on agar media at 30°C.

Amylomyces is believed to have been selected by domestication from *Rhizopus* and recent taxonomic studies using DNA complementarity have confirmed a close affinity with this genus (Ellis 1985). Large-thick-walled chlamydospores are produced by *Amylomyces* and these probably ensure survival when the starter cakes are dried and stored prior to being used in food and beverage preparation.

3.3 TECHNOLOGY TRANSFER AND FOOD SAFETY

A number of fungal fermented foods have attracted attention from the West as being suitable for technology transfer particularly for markets interested in nutritious 'health' foods. Tempe in particular has become popular in the USA and a number of companies are producing the product, which amounted to 420 tonnes in 1980 with a value of 1·7 m dollars (Hesseltine 1983). Tempe is also being produced in some European countries and is currently available in health food outlets in Britain.

Concern over the occurrence of mycotoxins in food and feed has probably restricted the adoption of more fungal fermented foods into Western diets, although most are considered to be safe (Hesseltine & Wang 1986). Many of these foods use species of *Aspergillus* and *Penicillium* of which strains are known which are capable of producing mycotoxins. *Penicillium roquefortii* is used extensively in the ripening of blue-vein cheese and some strains of this species are known to produce mycotoxins including penicillic acid, mycophenolic acid, PR toxin, roquefortine and isofumigaclavine A and B (Law 1984; Moss 1987). Recent taxonomic studies suggest that *Aspergillus sojae* and *A. oryzae*, which are used in a number of fermented foods, are merely non-toxigenic domesticated varieties of *A. flavus* which itself includes some aflatoxin-producing strains (Wicklow 1984; Kurtzman 1985).

Species in the genus *Rhizopus*, which is important in the production of a number of fermented foods, were until recently not thought to produce any known mycotoxins. The identification of a new mycotoxin rhizonin A in *R. microsporus* (Wilson *et al.* 1984) together with the suggestion of toxins in a large number of other *Rhizopus* species (Rabie 1986) indicates that a more detailed examination of these fungi is required, particularly in relation to fermented foods (see Moss, this volume).

The factors which influence mycotoxin production and breakdown are not well understood, and nothing is known of how selection pressures involved in the domestication of many microfungi for fermentation may have steered metabolism away from the production of mycotoxins. Studies on these processes may not only suggest new approaches to the production of safe minimally preserved foods, but may indicate ways of reducing mycotoxin levels in foods through a better understanding of the organism and its environment.

4. Fungal components

Although fungal biomass and fermented foods represent a direct contribution of fungi to food and food ingredients, a number of components of fungal origin find applications in the food industry. Table 3 lists fungi which are reported to produce these components although few species are exploited commercially.

4.1 ORGANIC ACIDS

Organic acids produced by fermentation have many applications in industry and several are used extensively in the food industry, either as components of food products or in food processing. Citric acid is the most important, with some 75% of the world production of approximately 350 000 tonnes being used in the food sector (Milson 1987). Demand for citric acid is increasing steadily and production is being met by fermentation using *Aspergillus niger*, usually with glucose or molasses as the substrate. Fermentation can be on solid substrates (Koji method), surface grown on liquid substrates, or more recently submerged culture with yields of citric acid up to 70–90% of substrate dry weight. Yields of citric acid have been improved by selection of suitable natural strains although the mechanism of citric acid production by this fungus is still not fully understood (Kubicek 1987). Most of the world production is centred on Europe and the USA although some is manufactured in Japan, China, India and South America (Milsom 1987). Citric acid is used in the food industry to adjust flavour in various soft drinks, wines, sweet foods and vegetable juices. It forms an important stabilizer in jellies and as a component of soft drink powders and tablets. In many foods citric acid is used to reduce oxidation of flavours and colours.

Malic, fumaric and tartaric acids are also used in the food industry and can be produced by filamentous fungi but currently these are available more cheaply using chemical means or in the case of malic acid as a by-product of wine residues. Gluconic acid is a slow acting acidulent used as a component of baking powders, bread mixes and effervescent powders. Fermentation is competitive with electrochemical synthesis and although *Penicillium* spp. were initially used, most of the production of 50 000 tonnes per annum uses *A. niger*. Only a small proportion of this is used in the food industry with most being used in cleaning agents (Milsom 1987).

Lactic acid is produced commercially either by bacterial fermentation or by chemical methods and is an important ingredient of some sweets, cakes and milk powders for baby foods. Lactic acid can be produced by fermentation using *Rhizopus* or *Mucor* spp. (Ward *et al.* 1936; Bigelis 1985) and the simpler recovery and greater purity of product may make this process viable in the future, particularly if the chemical methods become more expensive. Succinic acid is used as a flavouring agent and erythrobic acid and its esters as water- or fat-soluble antioxidants in canned products and meat. Although both acids can be produced by fungal fermentations they are currently synthesized more cheaply by chemical means.

4.2 LIPIDS

The vast majority of fats and oils are obtained from plant products although some are derived from the petrochemical industry. Ratledge (1978) has reviewed the range of micro-organisms capable of synthesizing lipids and under suitable conditions lipid content of filamentous fungi may approach 60% of dry weight with some 80% composed of triglycerides (Bigelis 1985). Many moulds contain a larger proportion of polyenoic acids than yeasts (Ratledge 1978). making them attractive for commercial exploitation, particularly if levels of polyunsaturated fatty acids could be increased. Species of *Mucor* and *Mortierella* are potentially exploitable since they accumulate γ-linolenic acid as a significant proportion of their unsaturated fatty acids (Bigelis 1985). Recent developments by a company in the UK have led to commercial production of γ-linolenic acid using *Mucor javanicus*. It is believed that a single run in the large-scale fermenter yields enough to supply the UK market for many months.

4.3 ENZYMES

Kilara (1985) has estimated that some 75 000 tonnes of industrial enzymes were produced in 1985 with an approximate value of *ca* US$ 600m. Of these, some 50–60% were thought to be proteases and 30% carbohydrases. By 1990 it is estimated that worldwide sales will reach US$ 1500m. Together with organic acids, fungal enzymes form the major use of fungal components for the processing and preparation of foods. Amylolytic enzymes, α-amylase and glucoamylase are produced by a wide range of fungi (Table 3), although *Aspergillus oryzae* and *A. niger* are the most widely used sources because they are generally regarded as safe (GRAS) (Taylor & Richardson 1979). Both α-amylase and glucoamylase are used in the saccharification of starch particularly in the bread-making, confectionery, ice-cream, soft drink and brewing industries and in many other areas of the food sector. Glucoamylase from *A. niger* is used in starch processing for the production of maltose and dextrose syrups (Sheppard 1986).

Cellulases and pectinases appear to be less important than amylases in food processing but find applications in the production of specialist products such as digestive aids and baby foods and are potentially of major importance in the hydrolysis of cellulosic substrates, although this has yet to be realized commercially in the food industry. Pectinases and β-glucanase find applications in the wine and brewing industries (Montenecourt & Eveleigh 1985).

Many fungi produce proteolytic enzymes which are finding applications as alternative sources to those from animals and plants, although commercial interest in microbial sources only began in the 1960s with the use of bacterial proteases in the detergent industry (Adler-Nissen 1986). The proteases of *Mucor miehei, M. pusillus* and *Endothia parasitica* are finding use in the dairy industry as alternatives to using calf rennet in the production of cheese, and are thought to be used in 60% of the cheese produced in the USA (Law 1984). Initial problems encountered with the use of fungal proteases in cheese production included the need for higher inactivation temperatures and the development of bitterness due to increased proteolysis compared with calf rennet. Some improvements have occurred by selection of suitable fungi with lower inactivation temperatures (Adler-Nissen 1986).

Fungal proteases of *P. roquefortii* and *P. camembertii* can be used to accelerate cheese ripening and proteases of *A. oryzae* are used in breadmaking, meat tenderization and chill proofing of beer (Löffler 1986). Lipases may also find applications in the dairy industry and those from *Mucor miehei* and *A. niger* are potential flavour enhancers and modifiers for cheese manufacture and for production of modified butter fat (Sheppard 1986).

Other fungal enzymes which are used to a lesser extent include endo- and exo-nucleases of *A. oryzae* and *P. citrinum* for the production of flavour enhancing mononucleotides (Nakao 1979). Glucose oxidase of *A. niger* and *Penicillium* spp. may have potential in immobilized enzyme reactors for the commercial production of gluconic acid (Milsom 1987).

4.4 OTHER COMPONENTS

Components such as amino acids, polysaccharides, vitamins and pigments are produced by a wide range of fungi but at present most of these are derived from bacteria, yeasts or from natural plant

Table 3. Filamentous fungi reported to produce components which have applications in food production and processing. The majority of species have not been commercially exploited

Components	Fungi
Organic acids	
Citric	*Aspergillus niger*
Erythrobic	*Penicillium* spp., *Penicillium notatum*
Gluconic	*Aspergillus niger*, *Gliocladium* sp., *Gonatobotrys* sp., *Penicillium chrysogenum*, *P. funiculosum*, *Scopulariopsis* sp.
Fumaric	*Rhizopus arrhizus*, *R. nigricans*
Lactic	*Rhizopus* spp., *Rhizopus oryzae*, *Mucor* spp.
Malic	*Aspergillus flavus*, *A. oryzae*, *A. parasiticus*, *A. wentii*, *Aureobasidium pullulans*, *Paecilomyces variotii*, *Penicillium* spp.
Succinic	*Aspergillus fumigatus*
Tartaric	*Aspergillus griseus*, *A. niger*, *Penicillium notatum*
Lipids	
γ-Linolenic acid	*Mucor genevensis*, *M. javanicus*, *M. ramannianus*, *Mortierella* sp.
Enzymes	
α-Amylase	*Aspergillus foetidus*, *A. niger*, *A*, *oryzae*
Catalase	*Aspergillus niger*
Cellulase	*Aspergillus niger*, *Penicillium* spp., *Penicillium lilacinum*, *Trichoderma reesei*, *T. viride*
Dextranase	*Penicillium funiculosum*, *Trichoderma reesei*
α-Galactosidase	*Aspergillus* sp., *Mortierella* sp.
β-Galactosidase	*Aspergillus niger*
Glucoamylase	*Aspergillus awamori*, *A. niger*, *A. oryzae*, *Rhizopus arrhizus*, *R. delemar*, *R. niveus*
β-Glucanase	*Aspergillus niger*, *Penicillium emersonii*, *P. funiculosum*
Glucose oxidase	*Aspergillus niger*, *Penicillium glaucum*, *P. notatum*
Lactase	*Aspergillus niger*, *A. oryzae*
Lipase	*Aspergillus* spp., *Aspergillus niger*, *Geotrichum candidum*, *Mucor miehei*, *Mucor* spp., *Penicillium roquefortii*, *Rhizopus* spp., *R. delemar*, *R. rhizopodiformis*,
Nuclease	*Acrocylindrium* sp., *Aspergillus oryzae A. quercinus*, *Monascus purpureus*, *Neurospora crassa*, *Phoma cucurbitacearum*
Pectinase	*Aspergillus* spp., *Aspergillus niger*
Pentosanase	*Aspergillus* sp.
Protease	*Aspergillus niger*, *A. oryzae*, *A. saitoi*, *Endothia parasitica*, *Mucor* spp., *M. miehei*, *M. pusillus*, *Penicillium dupontii*, *P. notatum*, *Rhizopus* spp. *Rhizopus chinensis*, *Trametes sanguinea*
Polysaccharides	
Elsinan	*Elsinoë leucospila*
Pullulan	*Aureobasidium pullulans*, *Tremella mesenterica*
Scleroglucan	*Claviceps purpurea*, *Corticium* sp., *Helotium* sp., *Plectania occidentalis*, *Sclerotinia* spp., *Sclerotium glucanicum*, *S. rolfsii*, *Stereum sanguinolentum*, *Stromatinia* spp.
Others	*Armillaria mellea*, *Leptosphaeria albopunctata*, *Monilia fructigena*, *Rhinocladiella elatior*, *R. mansonii*
Amino acids	
Alanine	*Fusarium*, *Mucor* and *Rhizopus* spp., *Ustilago maydis*
Glutamic acid	*Cephalosporium* sp.
Lysine	*Aspergillus ustus*, *Gliocladium* sp., *Sphacelotheca sorghi*, *Ustilago maydis*
Tryptophan	*Claviceps purpurea*
Vitamins	
Ascorbic acid	*Aspergillus awamori*, *A. fumaricus*, *A. glaucus*, *A. japonicus*, *A. luchuensis*, *A. niger*
Pro-vitamin A	*Blakeslea trispora*, *Choanephora cucurbitarum*, *Dacrymyces deliquescens*, *Phycomyces blakesleeanus*
Pro-vitamin D_2	Many fungi
Polyols	
Mannitol	*Aspergillus* spp.
Pigments	
Lycopene	*Blakeslea trispora*
Xanthophylls	*Dacrymyces deliquescens*
Various	*Monascus anka*, *M. purpureus*

Information compiled from Ratledge (1978), Kang & Cotrell (1979), Nakao (1979), Ninet & Renaut (1979), Taylor & Richardson (1979), Law (1984), Bigelis (1985), Kilara (1985), Montenecourt & Eveleigh (1985), Wood (1985) Adler-Nissen (1986), Löffler (1986), Sheppard (1986), Kubicek (1987), Milsom (1987).

products. Some interest has been shown in the development of fungal exocellular polysaccharides since these may be useful in the food industry as dispersants, components of calorific foods and for producing packaging films with low oxygen permeability (Kang & Cottrell 1979). However, exocellular polysaccharides such as xanthan and dextran from bacterial sources are currently more popular and the potential for fungal polysaccharides may be limited to certain specialized applications.

Vitamin D_2 is obtained from yeast but could be obtained from many filamentous fungi which contain the provitamin ergosterol (Weete 1980). Carotenoids are potential precursors of vitamin A as well as important colouring agents and *Blakeslea trispora* and *Phycomyces blakesleeanus* accumulate carotenoids in large quantities, although the market for these components is at present quite small with world supplies being obtained from plant sources (Ninet & Renant 1979).

5. The future

The field of food biotechnology should see several interesting developments in the years ahead. Fungi have had a long association with man in relation to food and food ingredients and this is likely to become more important as we move towards the next century. The consumption of mushrooms is becoming increasingly popular and Hinton (1987) has estimated that in the UK alone consumption will rise by some 30% by the year 2000. Shiitake (*Lentinus edodes*) and oyster mushrooms (*Pleurotus* spp.) are becoming more popular and the transition to using modified substrates and waste products for cultivation should enable more widespread low cost technology. Other species of mushroom may become popular, particularly if they can be adapted to grow on new substrates or can be used to produce new foods or ingredients. Some 'jelly fungi' (*Tremella* spp.) are consumed in sweet dishes in parts of China (Wood & Smith 1987).

The idea of growing moulds for food is not new but the development of a range of products based on the favourable nutritional composition and textural properties of fungi opens up new perspectives for the use of microfungi in food. Providing consumer barriers can be overcome, these foods should become increasingly important, particularly if production of mycoprotein can utilize low-cost substrates.

Many fermented foods are gaining popularity in the west and miso and tempe have become well established, particularly in the USA. Hesseltine & Wang (1986) cite figures of 30 USA outlets producing 420 tonnes per annum of tempe and nine factories in the USA and two in Canada producing miso, with the largest miso factory producing some 450 tonnes per annum. These figures are small compared to production of these foods in the non-western world but demonstrate how technology is being transferred to produce fungal foods in new markets. Many other non-western fermented foods remain to be studied and adapted to new markets to produce a range of nutritious sweet or savoury foods or food ingredients.

Although approximately 64 000 fungal species are recognized (Hawksworth *et al.* 1983) only a small fraction of these have been explored for their industrial potential. Some 350 species names have been proposed in the genus *Aspergillus* and 96 in *Penicillium* (Hawksworth *et al.* 1983) but very few of these find industrial applications in relation to the food industry and the production of food (Table 3), with *A. niger* and *A. oryzae* probably being the most important. The range of products produced by these two species of fungi is quite significant and suggests that many other fungi represent a large untapped resource. Hesseltine (1981) has drawn attention to the valuable role which taxonomists can play in steering biotechnologists towards related groups of fungi, and this approach may prove valuable in the search for related products or novel new ones.

The potential market for fungal products can only be assessed in relation to the cost of alternative sources which may come from plants, animals, other micro-organisms or via chemical processes. In the production of mycoprotein or fungal components the supply of low-cost substrates would appear to be an increasingly important factor. The search for new microfungi and improvement of established strains, together with the advances in DNA technology are developments which are likely to have an important influence on the contribution of filamentous fungi to the food industry in future years.

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