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## Myco-protein from *Fusarium venenatum*: a well-established product for human consumption

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**Abstract** *Fusarium venenatum* A3/5 was first chosen for development as a myco-protein in the late 1960s. It was intended as a protein source for humans and after 12 years of intensive testing, *F. venenatum* A3/5 was approved for sale as food by the Ministry of Agriculture, Fisheries and Food in the United Kingdom in 1984. Today, myco-protein is produced in two 150,000 l pressure-cycle fermenters in a continuous process which outputs around 300 kg biomass/h. The continuous process is typically operated for around 1,000 h. One factor which has limited the length of production runs was the appearance of highly branched mutants in the population. Several factors affect the time of appearance of such mutants and a number of strategies for delaying their appearance have been investigated. After reduction of the RNA content, the fungal biomass is mixed with egg albumin and made into a variety of products. Consumption of these can lead to reduced blood cholesterol and to lower energy intake in a subsequent meal. *F. venenatum* myco-protein is now used in products available in six European countries and there are plans for it to be sold in France, the United States and Germany.

### Introduction and historical perspective

Concern that animal protein sources would be insufficient to meet man's requirements for protein has led to the search for suitable, high-protein, microbial substitutes. Initially this search focused on the use of various yeasts, including brewers' and bakers' yeast (*Saccharomyces cerevisiae*), *Torula* sp. and *Candida utilis*, and food yeast was able to provide a substantial contribution to human diets during both world wars. Later, this interest spread to the use of both bacteria and filamentous fungi, and to the use of a wide variety of waste products

as the primary substrate. Much of the research, however, has focused on the production of microbial protein for use in animal feed rather than for human consumption.

*Fusarium venenatum* A3/5 (ATCC PTA-2684, formerly identified as *Fusarium graminearum* A3/5, O'Donnell et al. 1998; Yoder and Christianson 1998) was first investigated as a potential protein source for human consumption during the late 1960s by the British company Rank Hovis McDougall (RHM). RHM was seeking to develop a microbial protein source which would be cheap and easy to produce from starch- or glucose-based media, but which would also be palatable. They found that filamentous fungi provided a suitably textured product and *F. venenatum* A3/5 was selected as the best fungus for further product development after 3 years of screening approximately 3,000 different fungi (Anderson and Solomons 1984).

In order to bring myco-protein from *F. venenatum* A3/5 onto the market, it was necessary for RHM to invest 12 years in researching the safety of the organism (as a potential plant pathogen) and of the final product (Edelman et al. 1983; Anderson and Solomons 1984; Solomons 1986), making myco-protein the most carefully tested food product on the European market. Myco-protein produced from *F. venenatum* A3/5 was approved by the Ministry of Agriculture, Fisheries and Food (MAFF) for sale in the United Kingdom in 1984, but toxicity testing has continued (Miller and Dwyer 2001), as have investigations into allergic reactions (Tee et al. 1993). Some strains of *F. venenatum* (e.g. NRRL 22198) are known to produce type A trichothecenes, including diacetoxyscirpenol (DAS), scirpentriol, 15-acetoxyscirpenol and 4-monoacetoxyscirpenol (O'Donnell et al. 1998). Isotrichodermin, isotricodermol, sambucinol, apotrichothecen, culmorin, culmorone and enniatin B have also been detected in cultures of *F. venenatum* (Miller and MacKenzie 2000). However, the strain used for myco-protein production, ATCC PTA-2684 (formerly NRRL 26139 = ATCC 20334), did not produce myco-toxins when grown in conditions which induced myco-toxin production in the strain NRRL 22198 (O'Donnell

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et al. 1998), and the growth conditions used for production are not suitable for mycotoxin production (Johnstone 1998). Nonetheless, mycotoxins are tested for at 6-h intervals during the production process, to ensure that myco-protein is mycotoxin-free. While the safety of the product was being investigated, a cultivation process was developed in which *F. venenatum* A3/5 is grown in continuous flow culture.

The process of development of myco-protein from *F. venenatum* has been reviewed by Edelman et al. (1983), Angold et al. (1989) and Trinci (1992).

## The production process

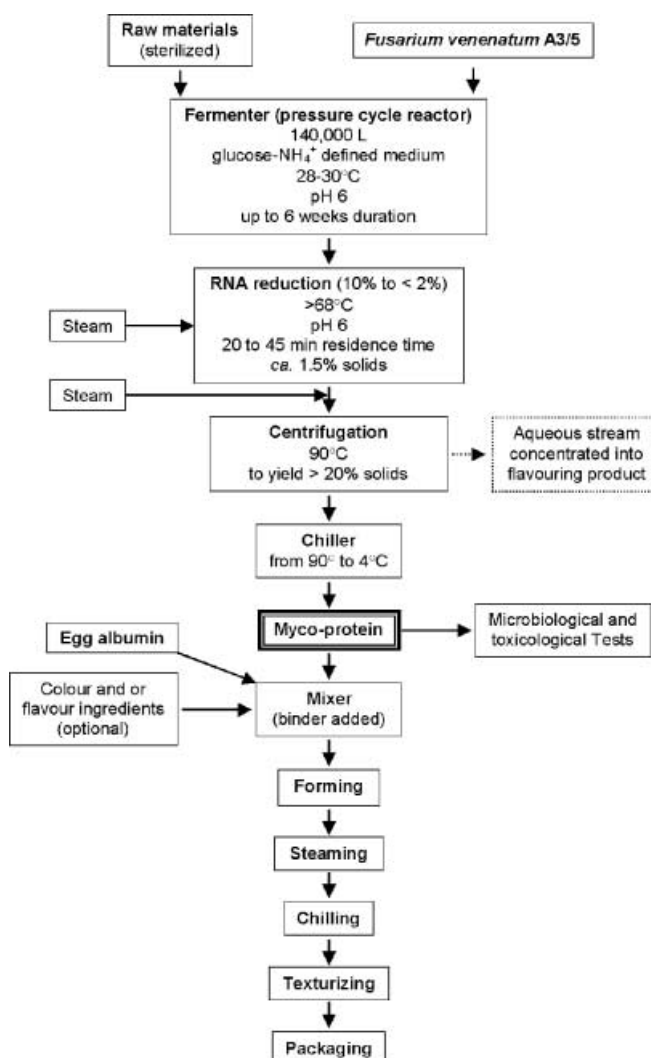
Mycoprotein from *F. venenatum* A3/5 is produced in 150,000 l pressure-cycle reactors (Fig. 1) in a continuous flow process (Trinci 1994). Since the product is the fungal biomass itself, a continuous flow process operated at a high dilution rate is the most economic production system (Pirt 1975; Edelman et al. 1983). Glucose is provided as the carbon source and ammonium as the nitrogen source, but the process is operated with all nutrients (including glucose) in excess. The CO<sub>2</sub> evolution rate, reflecting the biomass concentration, determines the flow rate (Rodger 2001). Both temperature (28–30°C) and pH (6.0) are controlled. Under these conditions, *F. venenatum* A3/5 has a specific growth rate of 0.17–0.20 h<sup>-1</sup> and 300–350 kg biomass/h can be produced.

The RNA content of the fungal biomass must be reduced in order to meet required safety standards (Edelman et al. 1983). This reduction was achieved by subjecting the biomass to a heat shock at 64–65°C (Towersey et al. 1977) in a separate stirred reactor and maintaining the biomass at this temperature for 20–30 min (Anderson and Solomons 1984). At this temperature, RNA is degraded into monomers which can diffuse out of the cells. Unfortunately, other cell components also diffuse out of the cells under these conditions and there is a net loss of biomass (a reduction of approximately 35–38%; Ward 1998) and proteinaceous material. Recently, Zeneca have patented a modification of this process in which the fungal biomass is rapidly heated to temperatures above 68°C (optimum 72–74°C) for 30–45 min (Ward 1998). The modified treatment results in less loss of biomass (30–33% loss) and greater retention of proteins.

After the RNA content of the cells has been reduced, the mycelial suspension is heated to 90°C before collection of the biomass by centrifugation and subsequent cooling (Marlow Foods Limited 1997). Knight et al. (2001) have demonstrated that heat preservation of processed myco-protein at 90°C does not significantly affect the fibrous nature or the acceptability of the product. Centrifugation removes the mononucleotides which were released by the RNA reduction treatment and concentrates the mycelia from approximately 1.5% (w/v) solids to a paste containing greater than 20% (w/w) solids



**Fig. 1** The myco-protein production plant at Marlow Food's Belasis site in Billingham, UK. (Photograph provided courtesy of Marlow Foods)



**Fig. 2** Diagrammatic representation of the process for producing myco-protein from *Fusarium venenatum* A3/5. The source of the new flavouring product (Quessent) is also shown (dotted arrow and box)

**Table 1** The nutrient composition of myco-protein products (Quorn pieces and burgers) compared with the nutrients in skinless chicken, lean beef (tenderloin or ground), milk, tofu and tempeh (soybean cake fermented by fungal growth). All values are

given for 100 g raw product. Data for myco-protein products was obtained from Marlow Foods Ltd. Data for other products was obtained from the USDA Nutrient Database for Standard Reference, Release 13

	Quorn pieces	Quorn burger	Chicken (skinless)	Beef (lean)	Ground beef (lean)	Milk (2%)	Tofu	Tempeh
Calories (kcal)	92	117	119	160	264	50	76	193
Total fat (g)	3.2	4.6	3.1	7.8	20.7	1.9	4.8	10.8
Saturated fat (g)	0.6	2.3	0.8	2.8	8.3	1.2	0.7	2.2
Cholesterol (mg)	0	0	70	62	75	7.5	0	0
Total carbohydrate (g)	1.8	5.8	0	0	0	4.8	1.9	9.4
Sugars (g)	0.8	2.5	0	0	0	4.8	1.5	ca. 4.6
Fibre (g)	4.8	4.1	0	0	0	0	0.3	ca. 4.3
Protein (g)	14	12.8	21.4	20.8	17.7	3.3	8.1	18.6
Sodium (g)	0.3	0.5	0.8	0.05	0.07	0.05	<0.01	<0.01
Iron (mg)	0.7	0.4	0.5	9.8	6.2	0.02	4.3	1.6

(Marlow Foods Limited, personal communication). This paste forms the raw material from which a range of products for human consumption can be prepared.

In the preparation of the final products, the myco-protein paste is combined with a binding agent (egg albumin) and mixed to align the mycelia into a fibrous network with a texture comparable to meat. This is then shaped using standard food processing technology. End-products range from chunks and mince to sausages, burgers, fillets and steaks.

The production process is summarized in Fig. 2 and has been reviewed by Trinci (1992).

## Nutritional assessment

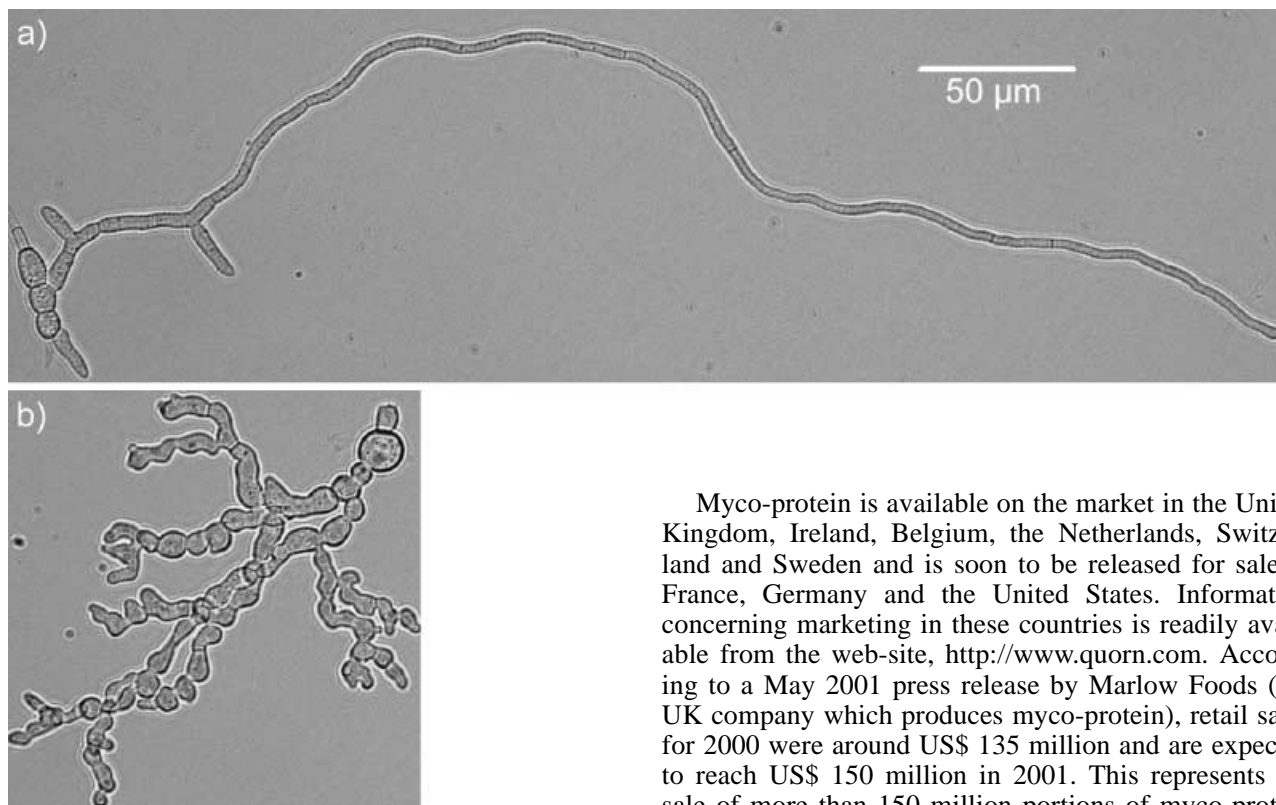
*F. venenatum* myco-protein contains approximately 44% (w/w) protein, on a dry weight basis, and the net protein utilization value is comparable to that of milk (Edelman et al. 1983). All essential amino acids are present. Although the concentration of sulphur-containing amino acids is relatively low (Anderson and Solomons 1984), the concentrations of essential amino acids in myco-protein are roughly comparable to those found in eggs (Miller and Dwyer 2001). Myco-protein also provides a source of dietary fibre (as chitin and glucans from the mycelial walls; Anderson and Solomons 1984), contains no cholesterol, and is low in saturated fats (Turnbull et al. 1990). In the processed forms available on the market in Europe, *F. venenatum* myco-protein compares favourably with comparable meat products and other vegetarian products (Table 1; Davies and Lightowler 1998).

A reduction in the level of blood cholesterol was observed during periods of 3 weeks (Turnbull et al. 1990) and 8 weeks (Turnbull et al. 1992) for subjects eating myco-protein on a daily basis. Both studies also observed a reduction in low-density lipoprotein cholesterol. The increased intake of dietary fibre was thought to account for much of the decrease in cholesterol levels (Turnbull et al. 1992; Wheelock 1993). The fibre present in myco-protein was also thought to play a significant

role in reducing the energy intake of subjects who were fed myco-protein, relative to the same subjects when fed chicken (Turnbull et al. 1991, 1993; Burley et al. 1993). Turnbull et al. (1993) also demonstrated that subjects eating myco-protein were generally less likely to want to eat than those fed chicken for up to 3 h after eating the meal, but this finding was not repeated by Burley et al. (1993). In contrast, Lang et al. (1999) did not observe any effect of protein source on subsequent intake of food, for proteins of plant (soy protein) or animal (casein or gelatine) origin. The fibre content and/or the amino acid composition of myco-protein were thought to account for the slight reduction in blood glucose and insulin levels following consumption of myco-protein in milk-shakes, compared to levels after consumption of soy and milk proteins. Blood glucose and insulin levels have been implicated as factors involved in determining appetite and satiety. Wheelock (1993) suggested that myco-protein could play a significant role in enabling people to achieve a healthier diet, assuming that it replaces foods which are high in fat but low in fibre.

## Marketing

When Lord Rank of RHM first proposed the development of a fungal protein for human consumption, it was thought that myco-protein could provide a tasty, palatable and convenient source of protein for countries in which protein shortages occurred. Edelman et al. (1983) estimated that in order for the process to be economically viable for sale in poor countries, production would have to exceed 20,000 t per year. With the commissioning of the second 150,000 l production plant in 1996, total production capacity (assuming an output of 350 kg h<sup>-1</sup>) could exceed 6,000 t per year. Fortunately, the world protein shortages predicted in the 1960s have not materialized, and myco-protein has found an important market role in Europe as an alternative to meat for vegetarians and for those seeking healthier diets. Development and production costs have remained too high for myco-pro-



**Fig. 3** Mycelia of **a** *F. venenatum* A3/5 and **b** a highly branched mutant of A3/5 (A24-1) obtained from a glucose-limited chemostat culture after 18 h growth in modified Vogel's medium with glucose as the carbon source and  $\text{NH}_4^+$  as the nitrogen source. Scale bar represents 50  $\mu\text{m}$

tein to be readily available in poorer countries where cheap, palatable protein-rich foods are still needed.

The first myco-protein product was brought onto the market in 1985 as a savoury pie in one of the United Kingdom's major supermarket chains (Sainsbury's). Other supermarket chains were soon able to bring out their own co-branded myco-protein-based products, and in 1990 the first product available for home cooking was launched in the form of small chunks ("pieces") which are suitable for use in casseroles, stews, curries, etc. The range of both ready-made meals and home cooking products has continued to expand.

Since 1994, marketing of myco-protein has focused more on the choice of myco-protein as a food product for a healthy diet, rather than as a vegetarian product, and myco-protein is now the best-selling meat-alternative in the United Kingdom. A comparison of meals made with branded myco-protein pieces, chicken, or tofu in 1999 found that myco-protein was considered to be a suitable alternative to chicken in terms of appearance, flavour, texture and aroma (McIlveen et al. 1999). The general acceptance of myco-protein as a food can be seen in its inclusion as an ingredient in food tests not concerned with the acceptability or otherwise of the myco-protein itself (Griep et al. 1997, 2000).

Myco-protein is available on the market in the United Kingdom, Ireland, Belgium, the Netherlands, Switzerland and Sweden and is soon to be released for sale in France, Germany and the United States. Information concerning marketing in these countries is readily available from the web-site, <http://www.quorn.com>. According to a May 2001 press release by Marlow Foods (the UK company which produces myco-protein), retail sales for 2000 were around US\$ 135 million and are expected to reach US\$ 150 million in 2001. This represents the sale of more than 150 million portions of myco-protein annually.

### The problem of morphology

One of the advantages of a continuous flow process is the reduction in time required for decontamination, sterilization and batch growth, and myco-protein cultures are typically maintained for about 1,000 h. However, Solomons (1985) reported that morphological changes (a reduction in the average length of hypha per tip; Fig. 3) occurred in the population after approximately 1,000–1,200 h of cultivation (i.e. after 250–340 generations). This change was considered to be unfavourable for final product formation. Wiebe et al. (1991, 1994a, 1995) demonstrated that the appearance of highly branched morphological mutants is a reproducible and predictable event in glucose-limited chemostat cultures of *F. venenatum* A3/5 at high dilution rates and pH 5.8, and that the mutants rapidly displace the parent strain under these conditions (Table 2). Mutants isolated from glucose-limited chemostats at high dilution rates have a growth rate advantage over the parental strain in glucose-limited conditions, but not necessarily when glucose is in excess (Wiebe et al. 1992). In contrast, most of the highly branched mutants which have been isolated from the myco-protein production plant had a growth rate advantage relative to the parent strain in conditions of nutrient excess (Simpson et al. 1998). Several of these mutants also had growth rate advantages over A3/5 in nutrient-limited (glucose-, ammonium-, sulphate-, and/or magnesium-limitation) conditions.

**Table 2** Time of appearance (at a detectable level) of highly branched mutants of *Fusarium venenatum* A3/5 and ability of these mutants to displace A3/5 in continuous flow cultures with glucose as the carbon source under the conditions indicated. The ability of mutants to displace A3/5 is indicated by the number of plus signs. – indicates that the mutant does not displace A3/5

Growth conditions				Time of detection of highly branched mutants (generations)	Ability of highly branched mutants to displace A3/5	Reference
Nutrient limitation	Nitrogen source	Dilution rate (h <sup>-1</sup> )	pH			
Glucose	NH <sub>4</sub> <sup>+</sup>	0.19	5.8	107 ± 5	++++	Wiebe et al. 1994a
Glucose	NH <sub>4</sub> <sup>+</sup>	0.17	5.8	110 ± 18	++++	Wiebe et al. 1996
Glucose	NH <sub>4</sub> <sup>+</sup>	0.14	5.8	101–111	++++	S. Dewar, personal communication
Glucose	NH <sub>4</sub> <sup>+</sup>	0.10	5.8	>168	–	Trinci 1994
Glucose	NH <sub>4</sub> <sup>+</sup>	0.05	5.8	>159	–	Wiebe et al. 1994b
NH <sub>4</sub> <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	0.19	5.8	115	++++	Trinci 1994
Mg <sup>2+</sup>	NH <sub>4</sub> <sup>+</sup>	0.19	5.8	71	++++	Trinci 1994
Glucose	NH <sub>4</sub> <sup>+</sup>	0.19	4.5	193	–	Wiebe et al. 1996
Glucose	NH <sub>4</sub> <sup>+</sup>	0.19	4.5/6.6 (120 h)	151	++	Wiebe et al. 1996
Glucose	NH <sub>4</sub> <sup>+</sup> peptone	0.19	5.8	23	–	Wiebe et al. 1998
Glucose	NO <sub>3</sub> <sup>-</sup>	0.15	5.8	131	–	Wiebe et al. 2001
None	NH <sub>4</sub> <sup>+</sup>	0.16–0.20	6.0	250–340	++++	Solomons 1985

**Table 3** The time of appearance (at detectable levels) of highly branched mutants of *F. venenatum* A3/5, of two evolved variants of A3/5 (A23-S and A24-S) and two diploid strains (D1 and D2)

Strain	Derivation	Time of detection of highly branched mutants in population (generations)	Reference
A3/5	Wild-type	107±5	Wiebe et al. 1994a
A23-S	Evolved in glucose-limited chemostat at $D=0.19\text{ h}^{-1}$	124	Wiebe et al. 1994a
A24-S	Evolved in glucose-limited chemostat at $D=0.19\text{ h}^{-1}$	156	Wiebe et al. 1994a
D1	Diploid	540	Naylor et al. 1999b
D2	Diploid	594	Naylor et al. 1999b

Several strategies have been suggested to prevent or delay the appearance of highly branched mutants in *F. venenatum* A3/5 populations, including changing the selection pressure. For example, at low dilution rates ( $D \leq 0.10\text{ h}^{-1}$ ), the appearance of morphological mutants is significantly delayed (Table 2, Wiebe et al. 1994b; Trinci 1994). However, slow dilution rates would not be suitable for the production of a growth-rate-related product such as biomass. The appearance of morphological mutants was also delayed by reducing the pH from 5.8 to 4.5 (Table 2, Wiebe et al. 1996). The branch frequency was not significantly affected by the reduction in pH and the highly branched mutants which did appear did not displace the parental strain, as would be expected at pH 5.8. Changes in the nitrogen source have also been shown to affect the ability of morphological mutants to displace the parental strain (Table 2). When nitrate replaced ammonium as the nitrogen source (Wiebe et al. 2001) or when ammonium cultures were supplemented with mycological peptone (Wiebe et al. 1998), highly branched mutants appeared but did not increase in the population.

Another strategy for delaying the appearance of highly branched mutants is to periodically change the selection pressure (Trinci et al. 1993; Trinci 1994; Wiebe et al. 1996). The selection pressure can be varied by, for

example, changing the limiting nutrient or the pH (Table 2). Glucose-limited chemostat cultures at high dilution rates which have had intermittent steady states (for example because of mechanical failure of a pump) also show delayed appearance of highly branched mutants. Localized variations in conditions within the 40,000 l production plant formerly used for myco-protein production, from which highly branched mutants have been isolated, probably account for the longer culture times (without the appearance of highly branched mutants) attained in it compared to those observed in small-scale chemostat cultures.

Wiebe et al. (1994a; Naylor et al. 1999a) have demonstrated that it is also possible to evolve A3/5 in chemostat culture to obtain strains which are unaltered in their morphological phenotype, but are more morphologically stable than A3/5 in chemostat culture (Table 3). Similarly, a more stable morphology can be obtained by using a diploid of A3/5 in place of the haploid (Table 3; Naylor et al. 1999b).

The evolution of *F. venenatum* A3/5 and the impact on morphology has been reviewed by Trinci (1992, 1994) and Trinci et al. (2001).

## Perspectives

Some of the research carried out to assess the nutritional value of myco-protein used freeze-dried myco-protein which was then added to cookies or milk-shakes (Turnbull et al. 1992; Turnbull and Ward 1995), demonstrating that myco-protein would be suitable for incorporation into products in this manner. Freeze-drying destroys the fibrous nature of the product (Edelman et al. 1983), putting the product into the same market as single cell proteins, and myco-protein has not been marketed in this manner. However, Rodger (2001) noted that a range of yoghurt and ice-cream products, in which the myco-protein acts as a fat replacer, are under development, and also describes the potential use of myco-protein in breakfast cereal or puffed snacks. Further, a patent has appeared for an alternative (non-protein) use of *F. venenatum* in the production of flavouring compounds for use in the food industry – a myco extract, perhaps comparable to yeast extract (Fig. 1; Cordell et al. 1999). This flavour product was due to be introduced to the market in September or October 2001 under the trade name Quessent (Marlow Foods, personal communication).

Myco-protein from *F. venenatum* has become a well-established product in those countries in which it is available and remains the only source of myco-protein for human consumption on the market today. Even processes such as the Pekilo process developed in Finland in the 1970s, which were designed to produce myco-protein for animal feed, have not survived – although filamentous fungi which are grown for other purposes (such as the production of citric acid) may still be used as animal feed supplements. Patents for the production of myco-protein from other fungi growing on a variety of substrates continue to be filed (for example: Yang 1997; Yang et al. 1997; Zhao 2000).

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