Organisation de Coopération et de Développement Economiques
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**17-Apr-2007**

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**English - Or. English**

**ENVIRONMENT DIRECTORATE**
**JOINT MEETING OF THE CHEMICALS COMMITTEE AND**
**THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

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**Cancels & replaces the same document of 06 April 2007**

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**Series on the Safety of Novel Foods and Feeds, No. 15**

**CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF THE CULTIVATED MUSHROOM Agaricus bisporus: KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS AND TOXICANTS**

**JT03225679**

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Series on the Safety of Novel Foods and Feeds

**No. 15**

# CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF THE CULTIVATED MUSHROOM *Agaricus bisporus*: KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS AND TOXICANTS

**Environment Directorate**

**Organisation for Economic Co-operation and Development**

**Paris 2007**

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**FOREWORD**

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based ***consensus documents,*** which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This consensus document addresses compositional considerations for new varieties of cultivated mushroom *Agaricus bisporus* by identifying the key food and feed nutrients and anti-nutrients. A general description of these components is provided. As well, there is background material on the production, processing and uses of *Agaricus bisporus* and considerations to be taken when assessing new *Agaricus bisporus* varieties.

Sweden served as the lead country in the preparation for this document.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

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# PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD, 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (*e.g.*, key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop consensus documents on phenotypic characteristics and compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD member countries.

These documents are a compilation of current information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, member countries have agreed that these consensus documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered.

A short, pre-addressed questionnaire is included <u>at the end of this document</u>. The information requested should be sent to the OECD at one of the addresses shown.

**THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT**

In 1990, a joint consultation of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review (OECD, 2000).

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (*i.e.*, species) or the specific food product. Critical components are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question. The comparison of key nutrients should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO, 1996). The comparator used to detect unintended effects should ideally be the near isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

## SECTION I — BACKGROUND

### A.  Natural history of *Agaricus bisporus*

Wild populations of *Agaricus bisporus* (Lange) Imbach, distinct from commonly cultivated strains, are now known from several regions of the world (Mozina *et al*., 1993; Kerrigan, 1995). Available isolates from these populations lay the foundation for our present knowledge about the species *A. bisporus*, and indicate that wild populations of the species are significant reservoirs of genetic resources presently unexploited by the commercial mushroom industry (Kerrigan and Ross, 1989).

An extensive investigation on the genetic diversity among 342 natural *A. bisporus* isolates from 12 locations around the world, confirmed earlier suggestions by Kerrigan *et al*. (1993a) that most local *A. bisporus* populations in the United States are made up of two ancestral elements, one indigenous and one being cultivar-like and introduced from Europe (Kerrigan *et al*, 1993a; Xu *et al*., 1997). The wide distribution of cultivar-like isolates has been hypothesised to represent recent escape of genotypes from mushroom cultivation, followed by introgression of these genotypes in the wild.

### B.  Domestication of *Agaricus bisporus*

Available records place the cradle of *Agaricus* cultivation in France. A French botanist, Tournefort, first described the primitive method used to grow the mushroom in 1707 (Joly, 1979; Chang and Miles, 2004). Later on, underground caves, where the climate conditions are well suited for growing mushrooms, became popular sites for the cultivation. *Agaricus* cultivation grew rapidly in France and spread to other European countries. By 1870, guidelines on cultural practices of *A. bisporus*, as well as vegetative inoculum ('spawn') was available in England (Robinson, 1870), and around ten years later mushroom cultivation appears to have started in the United States (Kerrigan *et al*., 1998).

Original cultivars were numerous but needed to be periodically replaced after a few culture cycles because the mycelium weakened or was overrun by pests or moulds (Sinden, 1981). As replacement strains were found more or less randomly in nature (spontaneously appearing on horse manure), the system did not guarantee best quality of the cultivated strains. Furthermore, the diminished use of horses in everyday life threatened the common availability of replacement strains.

The multiplication techniques developed around one hundred years ago resulted in a reduction in available genetic variability. Callac (1995) estimated that only seven ancestral European cultivar linages seem to be the origin of all the cultivated strains in the world. However, this situation is changing since wild strains from all the North hemisphere are now used in breeding strategies.

### C.  Modern methods of cultivation

Mushroom production involves 6 sequential steps, which consist of: phase I composting, phase II composting, spawning and spawn run, casing, pinning, and cropping. Different types of mushroom require different types of substrates. *A. bisporus* is a compost and, particularly, leaf-litter degrader, and is able to degrade the major polymers of woody plant materials; cellulose, hemicelluloses, and lignin (at least to some extent). This function is to a large extent taken care of by a set of secreted enzymes. The mushroom is cultivated on fermented compost, most commonly based on animal manure and cereal straw, which could be seen as microbial biomass and lignocellulosic residues. The intracellular and secreted proteins of *A. bisporus* actually produce a complete cellulase system, which enables it to grow on cellulose as the sole

carbon. The system is induced or repressed depending on the carbon source. Easily metabolised carbon, such as glucose, represses cellulase production, whereas more complex molecules induce cellulase production (Chang and Miles, 2004).

The process of mushroom composting, particularly with regard to *Agaricus*, takes place in two distinct phases. Phase I composting (compost preparation) aims at mixing and wetting the raw materials and at starting the composting process during which various micro-organisms break down the straw (Chang and Miles, 2004). During this phase nutrients may also be added (Kurtzman, 1997). There are two purposes of phase II composting (compost conditioning): 1) to eliminate insects, pests and spores of contaminating micro-organisms (pasteurisation) from the phase I composting substrate, and 2) to bring the substrate to a uniform temperature of about 50 to 55°C, which promotes decomposition of the substrates by thermophilic micro-organisms. Through this a more selective medium favouring the growth of the mushroom is produced (Chang and Miles, 2004).

The next step in the cultivation process is spawning, *i.e.* inoculation of the compost with *A. bisporus* mycelium (spawn) (Kurtzman, 1997). Spawning is generally done by mixing the mycelia throughout the compost, after which the spawn is allowed to grow and produce a thread-like network of mycelium throughout the compost (Vedder, 1978; Van Griensven, 1988). To promote mushroom formation of *A. bisporus*, the compost surface is covered with a surface layer called casing, which is usually a mixture of peat and limestone (Kurtzman, 1997, Volk and Ivors, 2001). Some fungi use light as their signal to form fruiting bodies, but for *Agaricus bisporus* micro-organisms in the casing layer provides the necessary signal to initiate the transition from the vegetative to the reproductive stage in which primordia or "pins", knots of mycelium that eventually develop into mushrooms, appear (Rainey *et al*., 1990; Rainey, 1991).

The mushroom crop grows in repeating 3- to 5-day cycles called "flushes" or "breaks". These flushes are followed by a few days when no mushrooms are available to harvest. The individual flushes tend to produce progressively fewer mushrooms. In commercial practice, three to five flushes are picked before the crop is removed to make room for the next. Commonly, mushroom farmers crop their mushrooms for 30 - 40 days. Most strains of *A. bisporus* (except for the portabella strains) are picked before the veil breaks and the stem elongates (Volk and Ivors, 2001).

There are several excellent reviews on cultivation of mushrooms available (Chang and Hayes, 1978; Vedder, 1978; Stamets and Chilton, 1983; Van Griensven, 1988; Chang and Miles, 2004).

**D.		Breeding of *Agaricus bisporus***

Modern breeding programs for *Agaricus bisporus* began as recently as about 30 years ago, simply because many techniques routinely used in the breeding of vegetable and cereal crops are not available or have not been adapted to *Agaricus* research. The main factor discouraging breeding work with the mushroom has been its reproduction, which was not understood until the early 1970s.

The majority of *A. bisporus* strains, including cultivated and many wild strains are predominantly secondarily homothallic (Raper *et al*., 1972), with a unifactorial mating system comprising multiple alleles (Imbernon *et al*., 1995). In this reproductive system, the majority of basidia produce only two basidiospores, each of which receives two of the four postmeiotic nuclei (Kerrigan *et al*., 1993b). Other agarics usually produce uninucleate basidiospores. Most often the two nuclei in the basidiospores of *A. bisporus* are non-sisters with respect to meiosis II (Summerbell *et al*., 1989) and carry compatible mating types. The bisporic spores, therefore, give rise to fertile heterokaryotic progeny (n+n) characteristically capable of fruiting (Evans, 1959; Kerrigan *et al*., 1993b). Virtually all parental heterozygosity is retained in offspring. Homokaryons are rarely produced by most strains and may never be formed by others (Kerrigan *et al*., 1992). Nuclear fusion (2n) occurs just prior to meiosis in the basidia lining the gills of the

mushroom. As meiosis in bisporic *A. bisporus* is accompanied by low recombination frequencies, it is likely that parental heterozygosity will be retained at a frequency much higher than that expected by chance (Summerbell *et al*., 1989; Allen *et al*., 1992). This of course has hampered breeding of *A. bisporus*.

Identification in 1993 and 2003 of new varieties of the cultivated mushroom, *A. bisporus* (Lange) Imbach var. *burnetti* Kerrigan et Callac and *A. bisporus* (Lange) Imbach var. *eurotetrasporus* Callac et Guinberteau (which resulted in renaming of the traditional variety to *A. bisporus* (Lange) Imbach var. *bisporus*), characterised by tetrasporic basidia and by respectively a heterothallic and a primary homothallic life cycle, in combination with an increased understanding of the molecular genetics of *A. bisporus* has opened up the possibility to develop new strains of *A. bisporus* with alternative techniques (Callac *et al*., 1993, 2003).

## E.        Production of *Agaricus bisporus*

The cultivation of mushrooms world wide have increased rapidly over the past 30 years. Even though the actual production of *A. bisporus* increased from 900 000 tons in 1981 to just under 2 million tons in 1997 (a 2,2-fold increase), its share among cultivated mushrooms decreased during the same period from 71,6% to 31,8% (Chang and Miles, 2004). However, it is still the mushroom produced in largest quantities. It is cultivated in more than 100 countries all over the world. The major producers of *A. bisporus* mushrooms in 1999-2000 were China followed by the United States, the Netherlands, France, Poland and Italy (Table 1).

**Table 1.    World production of *Agaricus bisporus* in the year 1999-2000 (Chang and Miles, 2004).**

| Rank | Country | Production (tonnes) |
|------|---------|---------------------|
| 1 | China | 637,304 |
| 2 | United States | 391,000 |
| 3 | The Netherlands | 263,000 |
| 4 | France | 180,000 |
| 5 | Poland | 105,000 |
| 6 | Italy | 102,000 |

## F.        Consumption of *Agaricus bisporus*

The white button mushroom (*A. bisporus*) is the most preferred mushroom in Western Europe and North America (Chang and Miles, 2004). It is consumed fresh, cooked or conserved. However, the available data on *Agaricus* consumption is old. The highest per capita consumption in 1990 was reported in Germany, the Netherlands and Canada (Table 2).

**Table 2.   Annual consumption (kg per capita) of *Agaricus bisporus* in some high-consuming Western countries in 1990 (Moss and Mitchell, 1994).**

| Country | Fresh mushrooms | Preserved mushrooms | Total |
|---|---|---|---|
| Germany | 1.2 | 2.0 | 3.2 |
| The Netherlands | 2.5 | 0.4 | 2.9 |
| Canada | 1.4 | 1.3 | 2.7 |
| France | 1.0 | 1.3 | 2.3 |
| United Kingdom | 2.0 | 0.20 | 2.2 |
| USA | 0.90 | 0.90 | 1.8 |
| Italy | 0.70 | 0.40 | 1.1 |

## G.      Processing of *Agaricus bisporus*

Fruit bodies of *A. bisporus* are not only sold fresh but also processed by the industry before being offered on the market. The technological treatments, which include canning, freezing, and various drying processes, might alter the protein, carbohydrate and ash contents of the mushroom (Manzi *et al*., 2001).

A common preservation method for *A. bisporus* is canning. An important step in the canning process is blanching, which aims both to pre-shrink the mushrooms prior to sterilization and to inactivate the polyphenol oxidase (PPO), an enzyme responsible for browning of the mushroom (Biekman *et al*., 1997). Blanching may also influence the mineral content of *A. bisporus*, either directly or through EDTA (ethylene diamine tetraacetic acid), an additive in the blanching solution. These changes are probably caused by the elements in the mushrooms tissue binding with chelating agents such as EDTA. The mineral content may also be influenced by excessive amounts of ascorbic acid in the brine of canned mushrooms (Çoşkuner and Özdemir, 1997; Çoşkuner and Özdemir, 2000). Furthermore, during storage in brine, canned *A. bisporus* may lose some of its protein and fat contents along with some mineral elements, whereas Cu, Ca and Na increase, possibly due to the composition of the brine. Moreover, storage in brine could also lead to increased moisture and ash content (Çaglarlrmak *et al*., 2001).

Freeze-drying is usually considered to be the drying technique that gives the best quality dried products in terms of nutritional value as well as texture flavour and colour (Le Loch-Bonazzi *et al*., 1992). Important for dried mushrooms is the rehydratability of the dried product.

Although freezing might not influence nutritional and mineral content of fruit bodies to any large extent, as long as the product is kept frozen, the ice crystals formed in the frozen mushrooms may damage cellular structures and render processes possible after thawing.

## H.      Cooking of *Agaricus bisporus*

Manzi *et al*. (2001) noted that cooking procedures significantly increase nutrient concentrations by decreasing water contents. This finding was confirmed by Dikeman *et al*. (2005) who observed increased contents of some carbohydrates, acid hydrolysed fat, total dietary fibre and insoluble dietary fibre but not of crude protein and soluble dietary fibre in cooked mushrooms. The latter investigators also found reduced levels of chitin in *A. bisporus* after cooking (Dikeman *et al*., 2005). However, on a dry weight basis, a significant cooking-related loss of protein and fat could be observed in deep-frozen *Agaricus bisporus* (Manzi *et al*., 2001). The structural damage of the vegetative cells, occurring during the deep-freezing/thawing processes, is suggested to promote the nutrient loss. On the other hand cooking increased the dietary fibre content. This trend could not entirely be explained by the loss of water during cooking, since it can also be observed on a dry weight basis. According to the authors these results suggest the occurrence, during severe industrial treatment and/or cooking, of cross-linking reactions among

oligosaccharides, monosaccharides and proteins, leading to indigestible products analytically measured in the fibre fraction (Manzi *et al.*, 2001).

## I.        Appropriate Comparators for Testing New Varieties

This document suggests parameters that *A. bisporus* developers should measure when developing new strains. The morphological, agronomical and chemical data obtained in the analysis of the new mushroom variety should ideally be compared to those obtained from an appropriate near isogenic non-modified variety grown under identical conditions. The evaluation of the extent of equivalence may be enhanced by additional, valid comparisons between the genetically modified mushroom and commercial varieties. These additional data may be generated by the developer and/or compiled from the literature. In the case data are generated by the developer himself, it should be noted that the majority of strains cultivated in the world today originate from only seven ancestral European cultivar lineages, and that it can be useful to have data on both white and brown (including Portabella type of strains) cultivars. When using literature data, however, they have to be adequately assessed for their quality (*e.g.* in respect to type of material analysed, and analytical method used). Ranges, and when appropriate, mean values should be reported and considered for each parameter investigated. These data would indicate whether the GM lines fall within the natural range in phenotypic expression or critical component concentrations found in non-GM counterparts. The genetically modified and the non-genetically modified varieties tested should be grown under various methods and climates of cultivation.

Critical components include key nutrients, key anti-nutrients and key toxicants for the food in question. Key nutrients are those components in a particular product, which may have a substantial impact in the overall diet. These may be major constituents (carbohydrates, proteins, and lipids) or minor ones (minerals and vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the species, that is, compounds whose toxic potency and level may impact on human and animal health. Similarly, the levels of known anti-nutrients and allergens should be considered.

## J.        Traditional characteristics screened by *Agaricus bisporus* developers

The major objective of *Agaricus* breeders remains to create strains giving a good yield of white-capped fruitbodies that are tolerant against *Verticillium*, *Trichoderma*, *Pseudomonas*, and other pathogens/diseases. Secondary objects such as having strains with smooth fruit bodies of a suitable form and size are controlled at each step of the selection. Other objectives of mushroom breeding, such as improving the dependence of fruit body development on temperature, the rhythm of flushes, or taste are taking in account only at the end of the selection and eventually only at the time of the official description of novel commercial varieties. To a large extent the foundation for this hierarchy of interests is the areas where basic knowledge about *Agaricus* genetics is available. Thus, knowledge is available on reproduction modes, determinants of cap colour, tolerance against pathogens, form and structure of the cap, and stability of strains. As the understanding of *Agaricus* genetics develops, breeders' interests are likely to widen.

## SECTION II — NUTRIENTS IN AGARICUS BISPORUS

### A. Proximate analysis

The chemical composition of cultivated *A. bisporus* varies between different reports. Observed differences may to some extent be explained by the analytical methods being used (Weaver *et al.*, 1977; Cheung, 1997), but are mainly due to several other factors not being controlled. These factors include the genetic constitution - strain (Weaver *et al.*, 1977, Bakowski *et al.* 1986b), composition of the compost (Maggioni *et al.*, 1968, Bakowski *et al.*, 1986a, Kosson and Bakowski 1984), flush of the mushroom culture (Bakowski *et al.* 1986a), developmental stage of fruit body at harvest (Kosson and Bakowski 1984; Dikeman *et al.*, 2005), and what part of the mushroom that was analysed (Kosson and Bakowski 1984).

Representative data on proximate analysis of fresh *A. bisporus* are presented in Table 3. Usually, investigators have used standard methods of analysis such as those published by the Association of Official Analytical Chemists (AOAC) to determine moisture, crude protein, fat, fibre, carbohydrate, and ash content of the mushroom.

**Table 3.   Proximate composition of cultivated *Agaricus bisporus*.**

| Reference / Category | USDA 2005[a]* | Mattila *et al.*, 2002b[b]* | Manzi *et al.*, 2001[c]* | Dikeman *et al.*, 2005[d] | Kurasawa *et al.*, 1982[e] | Cheung, 1997[f] | CSTJ 2005[g]* | Range |
|---|---|---|---|---|---|---|---|---|
| Dry matter (% f.w.) | 7.6–8.8 | 7.7–7.8 | 7.2 | 5.5–7.0 | 7.8–8.4 | 7.6 | 6.1 | 5.5–8.8 |
| Protein[h] (g/100g d.w.) | 28.4–40.8 | 26.5–27.1 | 22.7 | 26.3–31.4 | 30.4–31.0 | 26.8 | 33.3 | 22.7–40.8 |
| Carbohydrate (g/100g d.w.) | 43.3–57.6 | 58.4–59.5 | 61.3 | | 55.1–55.2 | 61.0 | 48.7 | 43.3–61.3 |
| Total dietary fibre (g/100g d.w.) | 7.8–17.0 | 19.5–20.5 | 27.5 | 22.9–30.4 | | 18.2 | 32.8 | 7.8–32.8 |
| Fat (g/100g d.w.) | 1.3–4.5 (crude) | 4.0–4.3 (crude) | 4.6 | 4.7–5.8 (AHF) | 2.5–3.8 (crude) | 1.9 | 4.9 | 1.3–5.8 |
| Ash (g/100g d.w.) | 11.2–12.7 | 10.0–10.1 | 11.4 | | 10–12 | 10.3 | 13.1 | 10.0–13.1 |

*some values recalculated. Data originally given on a fresh weight basis have been recalculated to dry weight basis.

[a] different strains and growth stages *A. bisporus* (white mushroom, Crimini and Portabella).

[b] different *A. bisporus* strains (brown and white).

[c] from local market.

[d] different *A. bisporus* strains (white, Crimini, Portabella) and maturity (immature and mature).

[e] different *A. bisporus* strains (white and brown) from the market.

[f] from local market.

[g] average value of *A. bisporus* in Japan.

[h] the protein content have been calculated using the conversion factor 4.38 either by the authors or through recalculations, except for data form Mattila et al. 2002b where the protein content were evaluated by summing the amino acid residues.

As shown in Table 3 the dry matter is usually between 5.5 and 8.8%, but as high values as 13.7% has been reported (Weaver *et al.*, 1977). The dry matter content is influenced by irrigation (Barden *et al.*, 1990, Beelman *et al.*, 2003), type of compost and strain (Weaver *et al.*, 1977; Bakowski *et al.*, 1986a, Laborde and Delpech, 1991), but it may also vary within and between flushes (Laborde and Delpech, 1991, Bakowski *et al.*, 1986a). Brown strains of *A. bisporus* usually are higher in solids than white strains; Portabella mushrooms harvested fully mature with flat open caps being among the highest (Beelman *et al.*, 2003).

A common way of establishing the crude protein content of a sample has been to calculate it from the nitrogen content using a conversion factor. A factor of 6.25 has frequently been used, based on the assumptions that most proteins contain about 16% nitrogen, that they to approximately 100% are digestible and that only minor amounts of non-protein nitrogen are present in the analysed sample. However, a lower conversion factor may be more appropriate in the case of mushrooms due to the fact that these organisms contain significant amounts of non-protein nitrogen, for example in their chitinous cell walls. Therefore, a conversion factor of 4.38 is advocated for mushrooms, based on 70% protein digestibility (0.7x6.25). As some of the references presented in Table 3 originally used a conversion factor of N x 6.25, these data have been recalculated using the conversion factor for mushrooms.

The crude fat content is usually determined after solvent extraction, and includes free fatty acids, mono-, di-, and triglycerides, sterols, sterol esters, phospholipids and glycolipids. In *A. bisporus* the fat content is low, usually in the region 1.3–5.8 mg/100g dry weight (Table 3).

Carbohydrates constitute a heterogeneous chemical group and include polyhydroxy aldehydes, ketones, alcohols and acids, as well as their derivatives and polymers. Analytically 'carbohydrate' levels are usually calculated by difference. First moisture, protein, fat and ash are determined, and the remainder is named 'carbohydrates'. The carbohydrates can be given as total carbohydrate, which includes fibre, or as nitrogen-free carbohydrate (without fibre). Presumably most of the non-protein nitrogen in a mushroom is in the form of chitin contained in the fibre fraction although small quantities of other nitrogen compounds may also be present. The carbohydrate level reported in *A. bisporus* is between 43.3 and 61.3 g per 100 g dry weight (Table 3). Although, dietary fibre is included in carbohydrates, some investigators have also reported values on the amount of this chemical group.

Ash is what remains after the organic part of the mushroom has been oxidised through combustion. It is a measure of the total amount of minerals and salts in the mushroom.

*1)* ***Protein***

The protein content of *A. bisporus* is influenced by strain (Weaver *et al.*, 1997, Kosson and Bakowski 1984, Bakowski *et al.*, 1986b), compost composition (Crisan and Sands, 1978; Kosson and Bakowski 1984, Bakowski *et al.*, 1986a), flush number (Crisan and Sands, 1978; Bakowski *et al.*, 1986a), and time of harvest/developmental stage (Crisan and Sands, 1978; Kosson and Bakowski, 1984; Bakowski and Kosson, 1985; Burton, 1988). Protein levels are higher in the cup than in the stipe (Kosson and Bakowski, 1984). The protein level in *A. bisporus* is usually in the range 22.7–40.8 g/100g dry weight (Table 3), the mean of 60 reported values in the literature being 26.2 g/100 g dry weight (median 26.6, minimum 13.6, and maximum 40.8 g/100 g dry weight).[1] This level ranks below most animal meats but above most vegetables, fruits and other foods (Chang and Miles, 2004).

The measurement of crude protein is an indirect but easily performed approximation of total amino acids from the nitrogen level, although vulnerable to the presence of varying levels of non-protein amino

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1 .        No standard deviation has been calculated due to missing data in the original reports.

acids and other sources of nitrogen in the sample. Therefore, summing up the various amino acids quantified after acid hydrolysis will give more accurate data.

### (i) Amino acid composition

The amino acid composition of *A. bisporus* is given in Table 4. Essential amino acids are indicated in italics and make up between 32 - 43% of the total amino acid contents in *A. bisporus* (Weaver *et al.*, 1977; Mattila *et al.*, 2002b; USDA, 2005). The relative amount of free amino acids is quite high in *A. bisporus*. Oka *et al.* (1981) reported around 50%, and Maggioni *et al.* (1968) between 39% and 46% free amino acids.

The most abundant amino acid in *A. bisporus* is the non-essential amino acid glutamic acid. Not only does glutamic acid occur both as a free amino acid and integrated in proteins, but it is also often covalently linked to other small molecules such as N-(γ-L-glutamyl)-ethanolamine, N-(γ-L-glutamyl)-4-hydroxyaniline, and β-N-(γ-L-glutamyl)-4-hydroxymethylphenylhydrazine (agaritine). The most common essential amino acid is lysine, and the most rare amino acids are those containing sulphur, cysteine and methionine. Therefore, the sulphur amino acids are the limiting amino acids of *Agaricus* proteins.

The amino acid composition of *A. bisporus* fruit bodies is dependent on the strain (genetic factors), type of compost and its composition, the nutrient supplementation (nitrogen or fatty acids), the developmental stage/size of the fruit body, and the flush number (Maggioni *et al.*, 1968; Weaver *et al.*, 1977; Kosson and Bakowski, 1984; Bakowski *et al.*, 1985, 1986a, 1986b).

**Table 4.  Amino acid composition of various strains of cultivated *Agaricus bisporus* expressed as g/100g protein.**

| Reference / Amino acid | USDA, 2005*[a] | | | Mattila *et al.*, 2002b*[b] | | Weaver *et al.*, 1977[c] | | | | | | | | | Range |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **White** | **Crimini** | **Portabella** | **White** | **Brown** | **D26** | **324** | **310** | **340** | **318** | **348** | **322** | **321** | **341** | |
| Alanine | 6.4 | 7.5 | 4.2 | 7.6 | 7.6 | 9.7 | 8.6 | 11 | 9.1 | 11 | 9.5 | 11 | 8.8 | 9.1 | 4.2–11 |
| Arginine | 2.5 | 4.9 | 2.7 | 5.6 | 5.2 | 5.3 | 6 | 4.1 | 5.8 | 4.3 | 5.5 | 5 | 6.5 | 6 | 2.5–6.5 |
| Aspartic acid** | 6.3 | 9.1 | 6.2 | 13 | 13 | 11 | 11 | 11 | 11 | 10 | 11 | 11 | 10 | 10 | 6.2–13 |
| Cysteine | 0.39 | 0.24 | 0.48 | 1.1 | 1.1 | trace | trace | trace | trace | trace | trace | trace | trace | trace | trace–1.1 |
| Glutamic acid** | 11 | 17 | 11 | 21 | 23 | 14 | 19 | 16 | 18 | 15 | 17 | 18 | 17 | 19 | 11–23 |
| Glycine | 3.0 | 4.4 | 2.5 | 5.1 | 4.7 | 4.9 | 4.9 | 5.7 | 5.3 | 5.0 | 5.0 | 4.6 | 5.0 | 5.0 | 2.5–5.7 |
| Proline | 2.5 | 7.0 | 3.0 | 5.0 | 5.0 | 5.6 | 5.4 | 4.8 | 5.6 | 10 | 6.1 | 5.4 | 5.7 | 5.4 | 2.5–10 |
| Serine | 3.0 | 4.5 | 2.7 | 5.2 | 5.3 | 5.1 | 4.8 | 5.8 | 5.3 | 5.4 | 5.3 | 5.1 | 5.6 | 4.6 | 2.7–5.8 |
| Tyrosine | 1.4 | 2.2 | 1.7 | 14 | 14 | 2.6 | 2.5 | 2.9 | 2.3 | 2.5 | 2.3 | 2.1 | 2.2 | 2.1 | 1.4–14 |
| *Histidine* | 1.8 | 2.7 | 1.7 | 2.8 | 2.6 | 2.2 | 2.5 | 1.6 | 2.0 | 1.7 | 2.2 | 2.2 | 2.3 | 2.3 | 1.6–2.8 |
| *Isoleucine* | 2.5 | 4.0 | 2.0 | 4.4 | 4.1 | 4.7 | 4.3 | 4.6 | 4.3 | 4.3 | 4.1 | 4.0 | 3.9 | 4.0 | 2.0–4.7 |
| *Leucine* | 3.9 | 6.1 | 3.2 | 7.3 | 6.9 | 7.2 | 7.0 | 7.9 | 7.5 | 7.7 | 7.1 | 6.7 | 7.3 | 6.7 | 3.2–7.9 |
| *Lysine* | 3.5 | 10 | 2.5 | 6.8 | 6.1 | 12 | 11 | 8.1 | 8.4 | 7.7 | 9.8 | 11 | 11 | 11 | 2.5–12 |
| *Methionine* | 1.0 | 1.9 | 0.72 | 1.6 | 1.4 | trace | trace | trace | trace | trace | trace | trace | trace | trace | trace–1.9 |
| *Threonine* | 3.5 | 4.5 | 2.7 | 5.3 | 4.9 | 5.0 | 4.7 | 5.6 | 5.2 | 5.1 | 4.7 | 4.8 | 4.7 | 4.3 | 2.7–5.6 |
| *Tryptophan* | 1.1 | 2.2 | 1.2 | n.d. | n.d. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 1.1–2.2 |
| *Phenylalanine* | 2.8 | 3.9 | 2.2 | 5.1 | 4.7 | 4.6 | 4.4 | 4.7 | 4.5 | 4.7 | 4.3 | 4.2 | 4.3 | 4.1 | 2.2–5.1 |
| *Valine* | 7.5 | 4.6 | 6.2 | 5.8 | 5.8 | 5.5 | 4.8 | 5.9 | 5.1 | 5.8 | 5.3 | 5.3 | 5.1 | 5.2 | 4.6–7.5 |
| % essential a.a. of tot. a.a. | 43 | 41 | 39 | 34 | 32 | 41 | 38 | 38 | 37 | 37 | 38 | 38 | 39 | 38 | 32–43 |

n.d.: not determined due to degradation in acid hydrolysis; n.a.: not analysed

* data originally given on a fresh matter basis have been recalculated to g/100g protein.

** asparagine and glutamine cannot be differentiated from aspartic acid and glutamic acid respectively.

[a] Protein content calculated using the conversion factor 4.38; **White**: 40.8 g/100g d.w., **Crimini**: 32.5 g/100g d.w.& **Portabella**: 28.4 g/100g d.w.

[b] Protein content evaluated by summing the amino acid residues; **White**: 27.1 g/100g d.w. & **Brown**: 26.5 g/100g d.w.

[c] Protein content calculated by the authors using the conversion factor 6.25 (thus, the protein contents are not true values but are meant to illustrate differences among strain within a species); **D26**: 30.2 g/100g d.w., **324**: 25.6 g/100g d.w., **310**:19.4 g/100g d.w., **340**: 29.9 g/100g d.w., **318**: 38.8 g/100g d.w., **348**: 24.8 g/100g d.w., **322**: 37.1 g/100g d.w., **321**: 33.2 g/100g d.w. & **341**: 26.0 g/100g d.w..

*2)        **Fat***

Crude fat in *Agaricus bisporus* includes all classes of lipid compounds but the total levels are comparatively low, usually in the range 1.3–5.8% of the dry matter (Table 3).

The constituents of lipids in the cultivated mushroom *A. bisporus* have been investigated quite extensively (Abdullah *et al*., 1994). Most studies have been devoted to the fatty acids, which make up around for 0.15% of the fresh *A. bisporus* cap (Cruz *et al*., 1997). The fatty acid profile reveals a surplus of unsaturated over saturated fatty acids, with ratios usually in the region 3:1 – 4:1 (USDA, 2006; Mau *et al*., 1991; Cruz *et al*., 1997; Aktümsek *et al*., 1998; CSTJ 2005; Maggioni *et al*., 1968), although higher ratios have also been reported (Bonzom *et al*., 1999). The by far most predominant fatty acid in *A. bisporus*, whatever strain and developmental stage of the mushroom, is the unsaturated fatty acid linoleic acid (18:2) (Table 5). The data of Cruz *et al*. (1997) was obtained from mushrooms cultivated on horse manure. For most other data in Table 5, however, the conditions of cultivation are unknown. Controlling for the developmental stage of fruit bodies when comparing fatty acid profile is important, as opening of the cap has been reported to influence the fatty acid composition depending on the strain. On cap opening, linoleic acid decrease both in the cap and the stem portion of the mushroom (Hira *et al*., 1990; Cruz *et al*., 1997). Thus, the fraction of unsaturated fatty acids decreases with advancement of growth stage.

*A. bisporus* contains neutral lipids (mainly as glycerides, free fatty acids and sterols) and phospholipids. Among the phospholipids, phosphatidylcholine and phosphatidylethanolamine predominate (Holtz and Schisler, 1971; Bonzom *et al*., 1999). Other identified phospholipids are phosphatidylserine, phosphatidylinositol, phosphatidic acid, and cardiolipin. In *A. bisporus* the neutral and polar lipid fraction has been reported to occur in a ratio of 1:2 or 1:1 depending on strain (Holtz and Schisler, 1971).

*A. bisporus* also contains relatively large amounts of sterols. Unlike plant sterols, which usually contain sterols with one double bond, mushroom sterols are characterized by two double bonds (Parks and Weete, 1991). Sterols are natural components of cell membranes. Mattila *et al*. (2002a) reported a total amount of between 677 and 789 mg sterols/100g dry weight. In descending order of occurrence the sterols found in *A. bisporus* are ergosterol (602-654 mg/100 g dry weight; 83-89% total sterols), ergosta-5,7-dienol (47-94 mg/100 g dry weight; 7-12% total sterols), fungisterol (14-26 mg/100 g dry weight; 2-3% total sterols), and ergosta-7,22-dienol (15 mg/100 g dry weight; 2% total sterols) (Mattila *et al*. 2002a). Several other investigators have also identified ergosterol as the main sterol in *A. bisporus* (Koyama *et al*., 1984; Huang *et al*., 1985; Young, 1995).

As vitamin $D_2$, also called ergocalciferol, is derived by photoirradiation-induced conversion from its precursor ergosterol, it is dependent on the action of sunlight or artificial ultraviolet light (see section IIA.6.) (Mattila *et al*, 2002a). Because of different conditions during *A. bisporus* cultivation, quite variable levels of sterols can be expected in the cultivated mushroom.

**Table 5.   Fatty acid composition (% of total amount) of fatty acids in cultivated *Agaricus bisporus*.**

| Reference / Fatty acid | USDA, 2006[a] | Mau *et al.*, 1991[b] | Cruz *et al.* 1997[c] | Koyama *et al*, 1984[d] | Aktümsek *et al.*, 1998[e] | CSTJ 2005[f] | Maggioni *et al.*, 1968[g] |
|---|---|---|---|---|---|---|---|
| 8:0 Caprylic acid | | | | | 1.4% | | |
| 10:0 Capric acid | 0.0 – 0.9% | | | | 0.8% | | trace |
| 12:0 Lauric acid | 0.0 – 1.9% | | | | 0.1% | | trace – 0.2% |
| 13:0 Tridecanoic acid | | | | | 2.1% | | |
| 14:0 Myristic acid | 0.0 – 1.7% | 0.3 - 0.4% | traces – 1.4% | 0.4% | 1.0% | 0.6% | 0.4 – 0.7% |
| 14:1 Myristoleic acid | | | | (0.5*) | 1.2% | | |
| 15:0 Pentadecanoic acid | | | | | 0.2% | 1.2% | 0.6 – 1.2% |
| 16:0 Palmitic acid | 12.1 – 19.2% | 12.4 - 12.6% | 11.4 – 16.9% | 9.4% | 12.6% | 12.5% | 13.2 – 18.0% |
| 16:1 Palmitoleic acid | | | | 0.7% | 3.1% | 0.8% | trace – 0.3% |
| 17:0 Eptadecanoic acid | | 0.5% | | | 0. 7% | 0.8% | 0.4 – 0. 9% |
| 18:0 Stearic acid | 3.4 – 4.9% | 3.6% | 5.1 – 9.0% | 3.0% | 2.3% | 4.5% | 2.9 – 3.3% |
| 18:1 Oleic acid | 0.0 – 3.4% | 1.8% | 3.2 – 7.6% | 0.9% | 4.9% | 2.3% | 1.4 – 2.6% |
| 18:2 Linoleic acid | 69.0 – 76.4% | 78.1 – 78.6% | 64.4 – 78.5% | 74.9% | 68. 8% | 73.4% | 70.5 – 78.3% |
| 18:3 Linolenic acid | 0.0 – 0.9% | | | n.d. | 0.7% | 0.1% | 0.5 – 0.7% |
| 20:0 Arachidic acid | | 2.8 - 3.0% | 1.5 – 2-1% | | 0.2% | 1.9% | |
| 22:0 Behenic acid | | | | | | 1.3% | |
| 24:0 Lignoceric acid | | | | | | 0.7% | |
| Not specified fatty acids | 0.0-8.6% | 0 | 0 | 10.2% | 0 | | 0.6 – 4.4% |

*preliminary identification

[a]. different *A. bisporus* strains and growth stages (white, Crimini and Portabella) .
   Trace amounts (<0.0005 g/100g) are rounded to 0.0 in the table but may be included in the amount total fatty acids.)
[b] different methods of cultivation, *i.e.* with or without compost fragmentation at casing.
[c] different strains(AMYCEL 2100 and LION C9) and different developmental stages (button, medium and flat).
[d] from local market.
[e] from local market.
[f] average value of *A. bisporus* in Japan.
[g] from flush 1 and 4, grown on horse manure with N-supplemented compost [$(NH_4)SO_4$ or urea + $(NH_4)SO_4$].

*3)* *Carbohydrates*

Mushrooms are known to contain fairly large amounts of carbohydrates. Including the fibre fraction, *A. bisporus* contains between 43.3 and 61.3 g total carbohydrates per 100g dry weight (Table 3); the mean carbohydrate content of 10 reported values in the literature being 55.4 g/100 g dry weight (median 56.4, minimum 43.3 and maximum 61.3 g/100 g dry weight)[2]. The carbohydrates include polysaccharides (such as glucans, glycogen and chitin), monosaccharides (such as ribose, fucose, glucose and mannose), disaccharides (such as trehalose and sucrose), sugar alcohols (such as mannitol and inositol) and sugar acids (such as galacturonic and glucoronic acids) (Crisan and Sands, 1978; Beelman *et al*., 2003). The fact that starch has been identified in *A. bisporus* (Dikeman *et al*., 2005) needs confirmation.

As carbohydrates are calculated by difference, data based on a conversion factor of 6.25 for proteins have been recalculated using the mushroom conversion factor 4.38 (true for result from Kurasawa *et al*., 1982 and CSTJ 2005). The carbohydrate content from Manzi *et al*. (2001) has been recalculated subtracting protein, fat and ash from the dry matter.

### (i) Dietary fibre

Most of the carbohydrates are structural polysaccharides of the cell walls and are indigestible for humans. Thus, they may be considered as dietary fibre (Beelman *et al*., 2003). The fibre content as reported in Table 3 is measured chemically and varies between 7.8 and 32.8 g/100g dry weight, the mean of 17 reported values in the literature being 24.4 g/100 g dry weight (median 25.4, minimum 7.8 and maximum 40.1 g/100 g dry weight) (Cheung, 1997; Manzi *et al*., 2001; Mattila *et al*., 2002b; Beelman *et al*., 2003; USDA 2005; Dikeman 2005; CSTJ 2005)[2]. As correction for non-protein nitrogen in chitin have been made only in some of the original studies, the calculated average should be taken only as an approximation.

Some compounds claimed to have advantageous and functional properties are present in the dietary fibre fraction. One of these is chitin, a structural polymer of the fungal cell wall occurring in the insoluble fibre fraction. In many strains the chitin fraction will increase as the mushrooms grow and mature (Beelman *et al*., 2003; Dikeman *et al*., 2005). Chitin is a nitrogen-containing polysaccharide that consists of monomers of N-acetyl-glucosamine. Around 30% of the total dietary fibre occurs as chitin, and may be detected in the form of glucosamine (Manzi *et al*., 2001). As chitin contains a significant amount of non-protein nitrogen, this nitrogen will contribute to an incorrect crude protein content if determined using the traditional conversion factor for proteins (N x 6.25) after Kjeldahl analysis. A conversion factor of (N x 4.38) is held more appropriate for mushrooms (Crisan and Sands, 1978). Chitin levels in *A. bisporus* vary between 1.8–8.3 g/100g dry weight (Manzi *et al*., 2001, Dikeman *et al*., 2005). β-Glucans, a polymer of glucose, are only found at low levels in the cultivated mushroom (Manzi *et al*., 2001, Dikeman *et al*., 2005).

### (ii) Sugars and sugar alcohols

Among sugars and sugar alcohols in *A. bisporus,* mannitol dominates (Beecher *et al*., 2001, Tseng and Mau, 1999). In *A. bisporus* it is the main form of storage carbon and may contribute with up to 50% of the fruit body dry weight (Rast, 1965). The mannitol level in the fruit bodies increase during growth and is highest in full grown mushrooms with flat caps (Rast, 1965, Wannet *et al*., 2000). In full grown mushrooms highest amounts occur in the stipe (42.3% of the dry weight) and the cap (33.6% of the dry weight), while lower levels are found in the lamella (8.6% of the dry weight) (Rast, 1965). As the level depends on the growth stage and what part of the mushroom that have been analysed, it is not surprising that different levels (Table 6) have been reported (Rast, 1965; Hammond and Nichols, 1976; Ajlouni *et al*., 1993). In plants it has been suggested that mannitol accumulates in

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2.      No standard deviation has been calculated due to missing data in the original reports.

response to environmental stress, such as to salt (Stoop and Pharr, 1994, Stoop *et al.*, 1996). A similar function may exist in *A. bisporus*. In non-stressed fruit bodies, the concentration of mannitol increase rapidly early during development, after which it remains relatively constant during fruit body maturation, whereas mushrooms grown under salt stress accumulate larger amounts of mannitol than non-stressed mushrooms (Stoop and Mooibroek, 1998). This observation confirms the notion that mannitol may act as an osmolyte in growing fruit bodies (Jennings, 1984).

**Table 6. Mannitol content (% dry weight) in the stipe, cap, and gills of cultivated *Agaricus bisporus*.**

|  | stipe | cap/pileus | gills | whole mushroom |
|---|---|---|---|---|
| Ajlouni *et al.*, 1993 | 19 – 28 | 30 | 10 | 26 |
| Hammond and Nichols, 1976 | 33 – 52 | 34 – 49 | 12 – 18 |  |
| Rast, 1965 | 24 – 42 | 21 – 34 | 9 –11 |  |

*A. bisporus* also contain appreciable amounts of the disaccharide trehalose, usually at fairly constant levels around 1–3% of the dry weight (Hammond and Nichols, 1976, 1979, Ajlouni *et al.*, 1993). However, slightly lower levels have also been reported (Rast, 1965). Occasionally, trehalose levels as high as 18% of the dry weight has been detected in fruit bodies developing between flushes (Hammond and Nichols, 1979).

Very low levels of the organic acids such as fumaric acid, succinic acid, and citric acid, and somewhat higher levels of malic acid occur in fresh and stored *A. bisporus* (Le Roux and Danglot, 1972).

### 4)      *Nucleic acids*

Only a few studies have measured the nucleic acid content of *A. bisporus*. Nucleic acids occur as RNA and DNA, as well as their precursors. On a dry weight basis mushrooms have been reported to contain 0.17% DNA and 2.49% RNA (Li and Chang, 1982), and 0.11% 5´-nucleotides (Tseng and Mau, 1999). Some of the latter compounds are important for the flavour.

### 5)      *Mineral and trace elements*

Mushrooms probably contain every mineral present in their growth substrate (Crisan and Sands 1978). Therefore, differences in mineral and trace element concentrations in cultivated *A. bisporus* may to a large extent depend on the method of cultivation and the type of compost being used (Vetter, 1989; Ünal *et al.*, 1996; Tüzen *et al.*, 1998; Spaulding and Beelman, 2003). However, the level of minerals and trace elements in mushrooms may also be dependent on strain (Spaulding and Beelman, 2003).

When comparing mineral and trace element levels, it is important to compare the same part of the mushroom. According to Vetter (1994) sodium is the only mineral element occurring at higher levels in the stipe than in the cap. Other minerals and trace elements are generally found in the stipe at lower or equal levels to those in the cap (Vetter, 1989; Zródlowski 1995; van Elteren *et al.*, 1998). However, Muñoz and colleagues found higher levels of Bi, Cr, Cu, Fe and Pb in stalks than in caps (Muñoz *et al.*, 2005).

The uptake of mercury and selenium is much lower in cultivated *A. bisporus* than in wild relatives. Several hypothesis have been suggested to explain this observation but none of these have hitherto been supported by solid scientific data.

Heavy metal contents in mushrooms grown on non-contaminated composts are usually low. It should be noted, that in some studies it has been observed that washing and peeling of mushrooms

may reduce the heavy metal content in the consumable parts (Źródlowski 1995). To what extent this reduction is dependent on the air and soil quality is unknown.

The most common minerals in mushrooms are in general potassium, phosphorous, sodium, calcium and magnesium (Crisan and Sands, 1978, Chang and Miles, 2004). Observed levels are reported on a dry weight basis in Table 7. Additional data on contents of B, Ba, Co. Cr, Ga, Li, Mo, Sr, Ti and V are available elsewhere (Vetter, 1989).

**Table 7. Mineral contents (on a dry weight basis) in cultivated *Agaricus bisporus*.**

| Reference / Mineral element | USDA 2005[a]* | SLV 2004[b]* | Mattila *et al.,* 2001[c] | Haldimann *et al.,* 1995[d] | Ünal *et al.,* 1996[e]* | Dar 1996[f] | CSTJ 2005[g]* | Various references[h] | Range |
|---|---|---|---|---|---|---|---|---|---|
| Na (g/kg dw) | 0.66–0.78 | 0.64 | 0.42–0.44 | | 0.35–1.0 | | 0.98 | 0.85–0.96 | 0.35–1.0 |
| K (g/kg dw) | 42–58 | 50 | 46–47 | | 18–23 | 21–27 | 57 | | 18–58 |
| Mg (g/kg dw) | 1.2–1.3 | 1.5 | 1.3–1.4 | | | 1.0–1.4 | 1.6 | | 1.0–1.6 |
| Ca (g/kg dw) | 0.40–2.3 | 0.32 | 0.13–0.25 | | 0.32–0.49 | | 0.49 | | 0.13–2.3 |
| P (g/kg dw) | 11–16 | 14 | 13 | | 8.0–17 | 12–14 | 16 | | 8.0–17 |
| Fe (mg/kg dw) | 52–68 | 42 | 28–48 | | 90–138 | 80–146 | 49 | | 28–146 |
| Zn (mg/kg dw) | 68–143 | 64 | 47–66 | | 467–642 | 54–77 | 66 | | 47–642 |
| Se (mg/kg dw) | 1.2–3.4 | 0.38 | 1.4–3.2 | 1.3–5.7 | | | | 0.45–1.2 | 0.38–5.7 |
| Cu (mg/kg dw) | 42–65 | | 29–35 | | 85–110 | 77–90 | 52 | | 29–110 |
| Mn (mg/kg dw) | 6.2–18 | | 5.1–5.5 | | | 24–26 | 6.6 | | 5.1–26 |
| Pb ($\mu$g/kg dw) | | | 35–180 | 50–490 | | | | | 35–490 |
| Cd ($\mu$g/kg dw) | | | 36–96 | 40–280 | | | | | 36–280 |
| As (mg/kg dw) | | | | 0.19–1.5 | | | | | 0.19–1.5 |
| Hg ($\mu$g/kg dw) | | | | <80–130 | | | | | <80–130 |
| Cr ($\mu$g/kg fw) | | | | | trace | | | | trace |
| Ag (mg/kg dw) | | | | | | | | 0.15–0.62 | 0.15–0.62 |

\* Original data recalculated to dry weight basis using the dry matter content stated in the article.

[a] different *A. bisporus* strains and growth stages (white, Crimini and Portabella).

[b] from local stores.

[c] different *A. bisporus* strains (white and brown).

[d] different *A. bisporus* strains (white and brown).

[e] different composts, *i.e.* horse manure or broiler chicken manure or a mix of 70% horse manure + 30% wheat straw.

[f] different developmental stage, *i.e.* pinhead, button, cup and open, lacking information on *A. bisporus* origin.

[g] average value of *A. bisporus* in Japan.

[h] Vetter 2003; Falandysz *et al.*, 1994; and Piepponen *et al.*, 1983.

*6)*      *Vitamins*

As shown in Table 8, cultivated *A. bisporus* seems to be a good source of the B-complex vitamins, and of niacin and folate (Beelman *et al*., 2003; Mattila *et al*. 2001). For these vitamins, one portion of mushrooms may contribute with close to or more than 10% of the recommended daily intake according to the Nordic Nutrition Recommendations (NNR 2004).

On the other hand, *A. bisporus* contains very low levels of vitamin A, vitamin D, vitamin E, and thiamine (Anderson and Fellers, 1942). The low level of thiamine has been suggested to result from anti-nutritive thiaminases in the mushroom degrading thiamine (Wittliff and Airth, 1970a, 1970b; Wakita, 1976). The low level of D vitamins in *A. bisporus* cultivated indoors contrasts with the comparatively high levels in wild mushrooms. Levels of vitamin $D_2$ are low in spite of relatively high concentrations of the precursor ergosterol. Mattila *et al*. (1994) found 0.21 μg vitamin $D_2$ (ergocalciferol)/100 g fresh weight, which is one to two orders of magnitude lower than in mushrooms such as *Cantharellus tubaeformis*. These findings are also supported by Teichmann (2005) who found the levels of vitamin $D_2$ in *A. bisporus*/white, *A. bisporus*/brown and *A. bisporus*/Portabella to range between 5.5-6.9, 3.1-3.8 and 4.4-10.1 μg vitamin $D_2$ /100 g dry weight, respectively. Whereas the levels of vitamin $D_2$ in *Chantarellus tubaeformis*, *Chantarellus cibarius* and *Boletus edulis* ranged between 209.7-225.7, 138.5-164.0 and 65.2-81.8 μg vitamin $D_2$ /100 g dry weight, respectively (Teichmann, 2005). The reason for the low vitamin $D_2$ levels in cultivated mushrooms seems to be that conversion of ergosterol to ergocalciferol (vitamin $D_2$) requires sunlight (or artificial ultraviolet light). Studies have shown that the concentration of vitamin $D_2$ in *A. bisporus* might be increased by as much as 467% by post-harvest UV-irradiation (Mau *et al*., 1998). No vitamin $D_3$ (cholecalciferol) has been detected in *A. bisporus*.

**Table 8.  Vitamin content (expressed per kg dry weight) in cultivated *Agaricus bisporus*.**

| Reference<br><br>Vitamin | USDA, 2006 [a]* | CFC, 2002 [b]* | CSTJ, 2005 [c]* | Mattila *et al.*, 2001 [d] | Ünal *et al.*, 1996 [e]* | Sapers *et al.*, 1999 [f]* | Udipi and Punekar, 1980 [g]* | Range |
|---|---|---|---|---|---|---|---|---|
| Retinol (Vit A ) (µg) | | | 0 | | | | | 0 |
| Thiamin (Vit $B_1$) (mg) | 8.8–12 | 11 | 9.8 | 6 | 8.1–12 | | 11 | 6–12 |
| Riboflavin (Vit $B_2$) (mg) | 53–64 | 46 | 48 | 42–51 | 49–61 | 53 | 26 | 26–64 |
| Niacin (mg) | 476–511 | 526 | 492 | 430–530 | 580–911 | 347 | 507 | 347–911 |
| Vitamin $B_6$ (mg) | 11–14 | | 18 | | | | | 11–18 |
| Pantothenic acid (mg) | 170–198 | | 252 | | 223–304 | | | 170–304 |
| Folate (mg) | 1.8–2.5 | | 4.6 | 4.5–5.9 | 6.4–10*** | | | 1.8–10 |
| Vitamin $B_{12}$ (µg) | 5.3–13 | | 0 | 6.0–8.0 | | | | 0–13 |
| Vitamin C (mg) (ascorbic acid) | 277 | 263 | 164 | 170–210** | 635–952 | | 171 | 164–952 |
| Vitamin D (µg) | | | 98 | | | | | 98 |
| α-Tocopherol (Vit E) (mg) | 1.3–2.3 | 36 | 0 | | | | | 0–36 |
| Vitamin K (µg) | 0.0 | | 0 | | | | | 0 |
| Carotenoids (mg) | | 1.3 | 0 | | | | | 0–1.3 |

*  Original data recalculated to dry weight basis using the dry matter content stated in the article, **as dehydroascorbic acid, *** folic acid.

[a] different *A. bisporus* strains and growth stages (white, Crimini and Portabella). No data on vitamin C in Crimini and Portabella.

[b] button mushroom.

[c] average value of *A. bisporus* in Japan.

[d] different *A. bisporus* strains (white and brown).

[e] different composts, *i.e.* horse manure or broiler chicken manure or a mix of 70% horse manure + 30% wheat straw.

[f] from commercial packing plant.

[g]  from The Agricultural College, Pune, or from local market.

## B.      *Agaricus bisporus* **as feed**

In order to explore whether by-products from cultivation and production of *A. bisporus* can be used as animal feed, the lower part of stipes were dried by various methods (freeze-dried, 80ᵒC, or 120ᵒC) and ground to powder. The potential usefulness of these products as feed was assessed by comparing their chemical composition with that of freeze-dried samples of commonly consumed mushroom parts (caps and upper parts of the stipe) (Maeda *et al.*, 1993). Drying mushrooms at 120ᵒC seemed to be the most effective treatment to shorten drying time and increase palatability of mushrooms for dairy cattle. Addition of the stipe powder to silage resulted in retained fermentation quality up to an inclusion rate of 5% mushroom material. The pepsin-pancreatin digestibility of the lower part of stipes was around 50% as compared to 80% for caps and the upper part of stipes., The percentage of soluble nitrogen was around 30% of total nitrogen for stipes as compared to 55% for caps. The crude fat and crude protein content of stipes were half of that in the consumed parts of mushrooms (Table 9). Of the minerals investigated, the phosphor content was lower in the by-product than in the consumed part, and the calcium content fifteen times higher in the stipe than in the cap.

**Table 9. Chemical composition of the commonly consumed part of *A. bisporus* (the cap) as opposed to the by-product (the lower part of the stipe).**

| Composition (% of dry matter) | Harvested part (cap) | By-product (stipe) |
|---|---|---|
| Crude protein | 45.3 | 24.6-25.7* |
| Crude fat | 2.7 | 1.0-1.1* |
| Neutral detergent fiber | 41.0 | 44.5-45.2* |
| Acid detergent fiber | 12.8 | 20.4-21.3* |
| Hemicellulose | 28.2 | 23.9-24.2* |
| Cellulose | 11.8 | 14.1-14.3* |
| Lignin | 0.9 | 2.4-3.3* |
| Silica | 0.1 | 3.7-3.9* |
| Neutral detergent insoluble nitrogen | 38.5 | 64.8-68.2* |
| Acid detergent insoluble nitrogen | 12.7 | 22.5-30.8* |
| Crude ash | 11.9 | 18.3-18.5* |
| K | 4.61 | 3.31-3.33* |
| Ca | 0.02 | 0.32-0.33* |
| Mg | 0.16 | 0.18-0.19 |
| P | 1.40 | 0.72-0.76* |
| Na | 0.12 | 0.14 |

*dried at 80 or 120°C, or freeze-dried.

## SECTION III — ANTI-NUTRIENTS IN *AGARICUS BISPORUS*

### A.        Lectins

Lectins are carbohydrate-binding proteins that are neither enzymes nor antibodies, but which may contain a second type of binding site specific for a non-carbohydrate ligand (Barondes, 1988). Therefore, many lectins have agglutinating activity. Four different isoelectric forms of *A. bisporus* lectins (ABL) have been described (Sueyoshi *et al*., 1985), possibly differing in glycosylation. They are all tetrameric, composed of four identical subunits (16 kDa), and have a total molecular weight in the region around 60 kDa, and have similar specificities for cell-surface carbohydrate receptors. They contain around 4% carbohydrate in the form of glucose, mannose, galactose and glucosamine (Presant and Kornfeld, 1972; Sueyoshi *et al*., 1985). Crenshaw *et al*. (1995) isolated and characterized a cDNA clone encoding a lectin gene from *A. bisporus*. Southern blot analysis indicated that at least two lectine genes were present. Deduction of the complete amino acid sequence of ABL has lead to the identification of three potential *O*-glycosylation sites at Ser[5], Thr[12] and Ser[85]. The ABL amino acid sequence has been shown to resemble the sequences of saline-soluble fungal lectins in a family of proteins with pesticidal properties (Trigueros *et al*., 2003). Interestingly, ABL was recently observed to bind to the isolated glucogalactomannan from the cell walls of *Verticillium fungicola*, an *A. bisporus* pathogen causing the "dry bubble" disease (Bernardo *et al*., 2004).

ABL is not present in the vegetative mycelium of the mushroom. It appears during maturation of the fruit body, indicating that the synthesis of this lectin is developmentally regulated (Bernardo *et al*., 2004). The ABL mainly binds Galβ1-3GalNAc (Thomsen-Friedenreich antigen, TDF) and holds a particular binding nature different from that of other T-disaccharide specific lectins (Irazoqui *et al*., 1999). One of the isoforms of the ABL lectin has recently been crystallized and its three-dimensional structure determined by X-ray diffraction (Carrizo *et al*., 2004, 2005). Binding studies with mono- and disaccharides showed that the lectin has two distinct binding sites per monomer, apparently active independent from the binding sites on the other tetrameres. The specificity of the binding is remarkable as it is able to distinguish two monosaccharides that differ only in the configuration of a single epimeric hydroxyl (Carrizo *et al*., 2005). As TDF is over-expressed in epithelial cancer cells, the ABL binding has been studied in detail with the intention to develop anti-TDF antibodies with fine carbohydrate-binding for treatment of tumours (Irazoqui *et al*., 2000). The observation that ABL, in contrast to most lectins, which stimulate cell proliferation, is a reversible non-cytotoxic inhibitor of epithelial cell proliferation, has made the *A. bisporus* lectin interesting as a potential agent for cancer therapy (Yu *et al*., 1993).

## SECTION IV — TOXICANTS IN *AGARICUS BISPORUS*

### A. Allergens

Allergenicity due to consumption of *A. bisporus* is relatively rare. Such cases have been reported in Germany and India (Pelzer and Freygang, 1997; Pelzer, 1999; Hegde *et al*., 2002). The latter case was a woman that experienced anaphylaxis (facial oedema and generalised urticaria) minutes after consumption of *Agaricus bisporus* curry (Hegde *et al*., 2002). The allergen was identified as the low molecular weigh compound mannitol.

Other forms of mushroom allergy are related to the cultivation and could be induced by either the compost/culture, and be independent of the mushroom species cultivated, or induced by mushroom tissues, very often spores. Extrinsic allergic alveolitis or hypersensitivity pneumonitis is a disease of the first type occurring in people working with *A. bisporus* and other cultivated mushrooms. Most likely mushroom lung develops in workers that have worked in sheds in which spawning takes place and where the compost, spawn, and organisms living in the media are mechanically mixed. The *Agaricus* related occupational allergy is rare and mainly manifested as asthma and dermatitis. Basidiocarp and spores have been identified as allergen sources that could elicit occupational asthma (Venturini *et al*., 2005). The disease may be identified with provocation tests (Kamm *et al*., 1991). Hand eczema, induced by delayed-type hypersensitivity, and airborne occupational allergic reactions leading to contact dermatitis have been reported in *A. bisporus* workers harvesting mushrooms (Korstanje and van de Staak, 1990; Kanerva *et al*., 1998). The agent responsible for the contact dermatitis has not been identified.

### B. Biogenic amines

Biogenic amines (histamine, tyramine, putrescine, cadaverine, etc.) is a group of toxins generally produced by carboxylase-positive micro-organisms from free amino acids in foods when the food in question is stored under unsuitable conditions. Kalac and Krizek (1997) found none of the biogenic amines in fresh or freshly stewed *A. bisporus*. However, after storage of the mushrooms for 48 h at 20°C, 368 mg putrescine and 37 mg cadaverine per kg dry matter (moisture content of fresh mushrooms approximately 90%) was found in intact fruiting bodies. Neither histamine nor tyramine was found. Putrescine levels were lower in sliced mushroom and stewed sliced mushroom, whereas cadaverine was not found at all. Storage of the mushrooms at 6°C produced no biogenic amines.

### C. Phenylhydrazines

Agaritine (β-N-[γ-L-(+)-glutamyl]-4-hydroxymethylphenyl-hydrazine) was isolated and characterized from the press-juice of *A. bisporus* during the early 1960s (Levenberg, 1961). Mushrooms that contain agaritine also contains a highly active enzyme that catalyses the cleavage of agaritine to 4-(hydroxymethyl)phenylhydrazine and/or the 4-(hydroxymethyl)benzenediazonium ion (Levenberg, 1961). Thus, agaritine is not the single phenylhydrazine in the mushroom. Both the presumed precursors for biosynthesis of agaritine, 4-(carboxy)phenylhydrazine and β-N-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine, and the enzymatic degradation product of agaritine, 4-(hydroxymethyl)-benzenediazonium ions have been identified in *A. bisporus* (Ross *et al*., 1982a; Chauhan *et al*., 1984,

1985). 4-(Hydroxymethyl)phenylhydrazine, which is assumed to be formed on agaritine degradation, has never been found in the mushroom as it is very unstable. Like most synthetic hydrazine derivatives, both agaritine precursors mentioned above and the 4-(hydroxymethyl)benzenediazonium ion has been shown to induce tumours in experimental animals (Toth, 2000). Although agaritine has never been shown to induce tumours in experimental animals, the compound could be regarded as an indicator of the likely presence of potentially toxic phenylhydrazines.

### (i) Agaritine

Several factors, including strain-type of *A. bisporus*, the quality of ingredients in the mushroom bed and the cultural practice employed in production, the flush of the cropping cycle, and the maturity at harvest may all interact to influence agaritine content of *A. bisporus* fruit bodies. Also, handling of the fruit bodies after harvest (storage time and conditions during storage) have a bearing on the agaritine content. Table 10, therefore, shows the amount of agaritine in fresh samples of *A. bisporus* obtained either directly from mushroom growers or purchased at the local market.

As shown in Table 10, the range in agaritine content reported in fresh *A. bisporus* is between 80 and 1 730 mg/kg fresh weight, but usually in the region 200 to 500 mg/kg (Andersson and Gry, 2004). The difference in agaritine content reported may to some extent be explained by different parts of the fruit body being studied by different investigators. Agaritine occur throughout the fruit body at fairly similar levels, but the stipe base and hymenium contain significantly deviating agaritine levels, lower levels in the stipe base and higher levels in the hymenium (Soulier *et al.*, 1993).

Rather small or no differences in mean agaritine content between strain types is apparent in the data of Table 10. However, in the study of Speroni *et al.* (1983), one strain (PSU-351 with brown colour phenotype) had significantly higher agaritine levels than the remaining seven strains, 5 100 mg agaritine per kg mushroom (on a dry weight basis) as compared to 1 700-2 800 mg/kg for other strains. It was hypothesised that PSU-351, which was only recently isolated from nature, contains higher agaritine levels because it had not yet lost its inherited ability to inhibit growth of certain fungi by producing agaritine. It is possible agaritine functions *in vivo* as an antimycotic agent. Also no relationship could be found between mushroom colour and agaritine level.

**Table 10. Agaritine content of 'fresh' cultivated *Agaricus bisporus*.**

| Mushroom sample (g = grower; m = market) | Content (mg/kg fresh weight)** | | Reference |
|---|---|---|---|
| | Average | Range | |
| 2 fresh samples (m)* | 440; 720 | 170-1 170 | Ross *et al.*, 1982b |
| 14 fresh samples of different strains (g) | 880 | 330-1 730 | Liu *et al.*, 1982 |
| 2 fresh samples of different strains (g) | 304±6.0; 438±2.5 | - <br> - | Fischer *et al.*, 1984 |
| 11 fresh samples (m) | 368±45 | 94-629 | Fischer *et al.*, 1984 |
| 1 fresh sample* | 228 | - | Hashida *et al.*, 1990 |
| 2 fresh samples of different strains (g)* | ~ 180 | 80-250 | Sharman *et al.*, 1990 |
| 5 fresh samples of different strains (m)* | | 160-650 | Stijve *et al.*, 1986 |
| 1 fresh sample within a Nordic project (g)* | 340 | - | Andersson *et al.*, 1994 |
| 2 fresh sample (m)* | 212; 229 | - | Andersson *et al.*, 1999 |

*size and form of mushroom not given; **the dry matter is approximately 10% of the fresh weight.

As there is an ongoing discussion on whether or not the agaritine levels are higher in young mushrooms than in old (smaller vs. larger) (Andersson and Gry, 2004), it is important to register age and size of analysed mushrooms. It should be noted that very few of the studies in Table 10 have controlled for the growth stage of the mushroom.

### (ii) Other phenylhydrazines

Compared to agaritine, the levels of other phenylhydrazines are low in *A. bisporus*. 4-(Carboxy)phenylhydrazine occurr at around 11 mg/kg fresh weight (Chauhan *et al*., 1984), β-N-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine at 16-42 mg/kg fresh weight (Chauhan *et al*., 1985; Toth *et al*., 1997), and the 4-(hydroxymethyl)benzenediazonium ion at 0.6-4 mg/kg fresh weight (Ross *et al*., 1982a; Toth *et al*., 1997).

## SECTION V — FOOD USE

### A. Identification of *Agaricus bisporus* food products

More than 2 million tons of *Agaricus bisporus* are produced annually worldwide, all destined for human consumption. The mushrooms are either sold fresh or processed by industry to easily stored products (dried and canned mushrooms) or products useful for the food industry (freeze-dried mushrooms). Although mushrooms contain protein, vitamins and minerals, their main role in the human diet is to contribute flavours and enhance the total quality of the dish.

### B. Identification of key nutrients, anti-nutrients and toxicants and suggested analysis for food use

The key constituents suggested to be analysed with appropriate methodology in new varieties of *A. bisporus* intended for human consumption are shown in Table 11. As all food products of *A. bisporus* used by consumers and food industry are derived from the fresh fruit bodies of the mushrooms, it is considered sufficient, in most circumstances, to analyse key constituents only in the fresh mushrooms. It will not be necessary to perform separate analyses of key constituents in commodities such as dried, freeze-dried or canned fruit bodies of *A. bisporus*.

**Table 11. Suggested constituents to be analysed in fresh fruit bodies of cultivated *Agaricus bisporus*.**

| Constituent | Fruit bodies |
|---|:---:|
| Proximates | X |
| Amino acids | X |
| Fatty acids | X |
| Mannitol | X |
| Trehalose | X |
| Vitamin $B_6$, riboflavin ($B_2$), niacin, pantothenic acid and folate | X |
| Agaritine | X |

Although it would be nice to have information also on the lectin content of *A. bisporus*, this mushroom constituent could not be suggested in the absence of quantitative data on lectin levels.

# SECTION VI – FEED USE

It is highly unlikely that *A. bisporus*, or any other mushroom, will ever be grown to produce animal feed. In the rare cases when by-products of *A. bisporus* cultivation and mushroom processing (mainly stipes) may be used as animal feed, which most probably will be locally in the neighbourhood of the mushroom farms, the verification that the animal feed will have the expected composition would be established by the comparative assessment of composition of fruit bodies required for genetically modified *A. bisporus*. Thus, no additional studies on the chemical composition of by-products of *A. bisporus* cultivation and processing are suggested.

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