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CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF OYSTER MUSHROOM (Pleurotus ostreatus): KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS AND TOXICANTS

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Consensus Document on Compositional Considerations for New Varieties of OYSTER MUSHROOM [*Pleurotus ostreatus*]: Key Food and Feed Nutrients, Anti-nutrients and Toxicants

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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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 document addresses compositional considerations for new varieties of oyster mushroom (*Pleurotus ostreatus*) by identifying the key food and feed nutrients, anti-nutrients and toxicants. A general description of these components is provided. In addition, there is background material on the production, processing and uses of oyster mushroom, and considerations to be taken into account when assessing new varieties of this mushroom. Constituents to be analysed, related to mainly food use as well as feed use, are suggested.

Sweden served as the lead country in the preparation for the document, and the draft has been revised on a number of occasions based on the input from other member countries and stakeholders.

The Task Force endorsed this document, which is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries and elsewhere. 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 currently available 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, OECD 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. Comments and suggestions can be sent to:

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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 (FAO/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. Any difference identified would then be assessed against 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.

INTRODUCTION

In 2005, the OECD published a Consensus Document of the Series on Harmonisation of Regulatory Oversight in Biotechnology addressing the biology of *Pleurotus* species (oyster mushrooms) (OECD, 2005). Korea served as lead country in preparation of the document, which included general information and the description of the taxonomy, natural distribution and agronomic practices of *Pleurotus* spp.; lifecycle and growth, sexual reproduction and crosses, genetics of *P. ostreatus*; as well as pests and diseases that can affect these mushrooms.

The first Consensus Document of the Novel Foods and Feeds Series that focused on the compositional considerations for a mushroom species was issued two years later, however dealing with another cultivated mushroom, *Agaricus bisporus* (OECD, 2007).

The present Consensus Document on compositional considerations for oyster mushroom (*Pleurotus ostreatus*) was needed to usefully complement, for food and feed safety aspects, the 2005 biology document designed as a tool for environmental safety assessment of the same species.

SECTION I – BACKGROUND

A. Taxonomy, nomenclature and occurrence

1. It is well recognised that the taxonomy of the genus *Pleurotus* is confusing as many species have been given several names (synonyms) and can be divided into subspecies. Guzmán (2000) reviewed the taxonomy of the *Pleurotus* genus, scrutinizing more than 230 publications and concluded that it was necessary to revise several described species as well as describe new species based on modern methodology. Over the last two hundred years more than 1000 names have been proposed in the genus but it is agreed that the number of species is much more limited. Furthermore, the species can be divided into several sections or subgenera. Further discussion on the taxonomy and natural distribution of the wild *Pleurotus* mushrooms can be found in the OECD consensus document on the biology of *Pleurotus* species (oyster mushrooms) (OECD, 2005).

2. Oyster mushrooms belong to the genus *Pleurotus* (Quel.) Fr., and today at least 70 species have been identified. *Pleurotus* was first recommended as a tribe within the genus *Agaricus* by Fries (1821) but was proposed as a separate genus by Quelet (1886). *Pleurotus ostreatus* (Jacq: Fr.) Kummer, the oyster mushroom, is the most cultivated species among oyster mushrooms and the type species of the genus *Pleurotus*. To clearly distinguish the type species from other oyster mushrooms it is called *P. ostreatus* in this document. Mating compatibility studies have shown that *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are synonyms or subspecies taxa for *P. ostreatus*.

3. *Pleurotus ostreatus* is in nature found in temperate zones of the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer, 1986) because it forms fruit-bodies at relatively low temperature compared to other *Pleurotus* species. The macroscopic morphologic features of this wood-rotting fungus and the microscopic characteristics of the spores, basidia, cheilocystidia and pleurocystidia of the mushroom is summarized by OECD (2005). Figure 1 show *P. ostreatus* growing on a tree.

4. Other *Pleurotus* species growing in warmer climates have been found to be as easily cultivated as *P. ostreatus*. Therefore, species such as *P. sajor-caju, P. cystidiosus*, *P. eryngii*, *P.tuber-regium, P. pulmonarius, P. citrinopileatus/P. cornucopiae, and P. djamor/P. flabellatus* are cultivated in various regions of the world, and there is scattered information available on the composition of these species.

5. In nature *P. ostreatus* can be found living on a large number of plants, including species of the genera *Abies*, *Acacia*, *Acer*, *Alnus*, *Betula*, *Carpinus*, *Carya*, *Castanea*, *Laurocersus*, *Liquidambar*, *Liriodendron*, *Lupinus*, *Magnolia*, *Malus*, *Morus*, *Nyssa*, *Ostrya*, *Pandanus*, *Picea*, *Pistacia*, *Populus*, *Pseudotsuga*, *Quercus*, *Salix*, *Tilia*, *Ulmus* and *Wisteria* (Farr et al., 1989). The broad host plant spectrum makes it easier to understand that the species can be cultivated on substrates containing different lignocellulosic materials. Although seen on dying trees, *P. ostreatus* is thought to be primarily a saprophyte, but behaves as a facultative parasite at the earliest opportunity. Occasionally, it grows on composting bales of straw and for example, on the pulp residues from coffee production.

Figure 1. Macroscopic feature of *P. ostreatus*

Source: OECD, 2005

B. Cultivation of *P. ostreatus* and other oyster mushrooms

6. *P. ostreatus* was first cultivated in the USA in 1900 and is now cultivated throughout the world. As indicated by the broad host plant spectrum, *P. ostreatus* and other oyster mushrooms can thrive in and on many lignocellulosic substrates, including, but not limited to, most hardwoods, wood by-products (e.g. sawdust, paper, and pulp sludge), cereal straws, maize, maize cobs, coffee residues (e.g. coffee grounds), hulls, stalk and leaves, banana fronds, and waste cotton, to mention a few (OECD, 2005). Oyster mushrooms (*Pleurotus* spp.) are now regarded as one of the three most important edible cultivated mushrooms together with the cultivated mushroom (*Agaricus bisporus*) and shiitake (*Lentinula edodes*).

7. The oyster mushrooms have many advantages as cultivated mushrooms: rapid mycelial growth, high ability for saprophytic colonization, simple and inexpensive cultivation techniques, and several kinds of species available for cultivation under different climatic conditions. In addition, they are low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals. These nutritional properties make these mushrooms good dietary foods. In addition, oyster mushrooms and products from them are consumed for medicinal purposes (Cohen et al., 2002; Kues and Liu, 2000). Owing to these attributes during recent years, the production and consumption of this mushroom has increased tremendously.

8. The oyster mushroom strain to be cultivated is aseptically inoculated onto a growing bed in glass jars or polypropylene plastic bags. The growing bed is prepared from a mix of water, lime and grain spawn (e.g. rye, wheat, sorghum), straw spawn (e.g. paddy rice straw, wheat straw) or other plant waste materials, and sterilized before use. Alternatively the mushrooms can be grown by using cut wood logs. Different strains will be suitable depending on the climate and incubation conditions. The most commonly cultivated *Pleurotus* species is *P. ostreatus*. Other frequently cultivated species include grey oyster

mushroom or phoenix-tail mushroom (*P. sajor-caju* (Fr.) Sing.), abalone mushroom (*P. cystidiosus* O.O. Miller), golden oyster mushroom (*P. citrinopileatus* Sing.), pink oyster mushroom (*P. flabellatus* (Berk. and Br.) Sacc.), black oyster mushroom (*P. sapidus* (Schulzer) Kalchbremer), *P. eryngii* (DC.: Fr.) Quel., *P. djamor* (Fr.) Boedjin *sensu* Lato and *P. tuberregium* (Fr.) Sing.

C. Production of oyster mushrooms

9. The cultivation of oyster mushrooms world-wide has increased more than 25-fold during the last thirty years. The worldwide production of oyster mushrooms was 35,000 tons in 1981 and around 875,000 tons in 1997. In 1997 the *Pleurotus* spp. accounted for 14.2% of the world production of mushrooms (Chang and Miles, 2004). However, Chang and Miles (2004) also refer to a publication in Chinese, *The Market of Edible Fungi*, which states that *Pleurotus* spp. are the second most important cultivated mushroom in China (26% of the market), and that in 2000 the production was more than 1,722,000 tons. No data on world production from the last fifteen years have been found.

D. Consumption of *Pleurotus ostreatus*

10. The oyster mushroom is generally consumed cooked or preserved. Older data on world-wide production of oyster mushrooms is available, along with some data on the extent of export and import. It should be noted that all mushrooms produced are not used as food. For example, these mushrooms are used for the production of enzymes and products for medicinal purposes. Data on the actual consumption of *P. ostreatus* has not been found.

E. Processing of *Pleurotus ostreatus*

11. The harvested fresh mushroom has a relatively short shelf life. The oldest method of preserving *P. ostreatus* and other *Pleurotus* species is by air-drying cleaned samples. Mushrooms can also be preserved in brine or canned, but their texture is best fresh. If heat treatments are used during processing, it is acknowledged that flavour may be lost, particularly if the mushrooms are cooled too slowly. Water has been shown to remove less flavour than steam (Chang and Miles, 2004).

F. Appropriate comparators for testing new varieties

12. This document suggests parameters that oyster mushroom breeders should measure when developing new modified varieties of *P. ostreatus*. The data obtained in the analysis of a new *P. ostreatus* variety should ideally be compared to those obtained from an appropriate near isogenic non-modified variety, grown and harvested under the same conditions.[1] The comparison can also be made between values obtained from new varieties and data available in the literature, or chemical analytical data generated from other commercial *P. ostreatus* varieties.

13. Components to be analysed include key nutrients, anti-nutrients, toxicants, allergens, and other metabolites. Key nutrients are those which have a substantial impact in the overall diet of humans (food) and animals (feed). These may be major constituents (fats, proteins, and structural and non-structural carbohydrates) or minor compounds (vitamins and minerals). Similarly, the levels of known anti-nutrients and allergens should be considered. Key toxicants are those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Standardized analytical methods and appropriate types of material should be used, adequately

[1] For additional discussion of appropriate comparators, see the Guideline for the Conduct of Food Safety Assessment Foods Derived from Recombinant DNA Plants CAC/GL 45/2003 of the Codex Alimentarius Commission (paragraphs 44 and 45).

adapted to the use of each product and by-product. The key components analysed are used as indicators of whether unintended effects of the genetic modification influencing plant metabolism have occurred or not.

G. Genetic modification of *Pleurotus* mushrooms

14. Several methods to introduce DNA into *Pleurotus* mushrooms have been studied, including those based on polyethylene glycol (Peng et al., 1993; Yanai et al., 1996; Jia et al., 1998; Kim et al., 1999; Honda et al., 2000; Amore et al., 2012), electroporation (Peng et al., 1992), restriction enzyme mediated integration (Irie et al., 2001; Joh et al., 2003; Fan et al., 2006), particle bombardment (Sunagawa and Magae, 2001), and *Agrobacterium tumefaciens* mediated transformation (Ding et al., 2011). A drawback, limiting the use of some of these methods, are the low transformation efficiency, heterogeneous integration into genomic loci and the need for using protoplasts, although improved procedures with enhanced efficiencies have been published (Li et al., 2006; Ding et al., 2011).

15. Marker or reporter genes successfully employed in identification and selection of mushroom transformants include those conferring antibiotic (hygromycin B) resistance (Peng et al., 1992; Irie et al., 2001), antimetabolite (5-fluoroindole, 5'-fluoro-orotic acid) resistance (Jia et al., 1998; Kim et al., 1999), metabolite (uracil) auxotrophy (Joh et al., 2003), fungicide (carboxin) resistance (Honda et al., 2000), herbicide (bialaphos) resistance (Yanai et al., 1996), as well as reporting successful transformation by expressing the green fluorescent protein (Li et al., 2006; Amore et al., 2012).

H. Traditional characteristics screened by developers of *Pleurotus* strains

16. The development of breeding programs for edible mushrooms such as the oyster mushroom relies on efficient methods to perform directed crosses between fungal strains. This requires in depth understanding of the biology of the mushroom, including knowledge about the mating genes, genome structure and genetic breeding of higher mushrooms. In *P. ostreatus*, the genes of two independent loci on one of the eleven chromosome pairs orchestrate the mating control system (Ramírez et al., 2000). The molecular map of the *P. ostreatus* genome is starting to become available. In 2000, Ramírez and co-workers had developed a map based on 196 RAPD (random amplified polymorphic DNA) and RFLP (restriction fragment length polymorphism) markers, as well as functional characters. Traits to be explored in the breeding programs are being mapped into the species genome in order to facilitate future breeding. The first review on gene sequences of *Pleurotus* intracellular and secreted proteins were published by Whiteford and Thurston (2000).

17. Although commercial transgenic mushroom strains are not yet available, molecular breeding studies of mushrooms have been carried out world-wide. *Pleurotus ostreatus* is not only one of the most important cultivated mushrooms but also a good model for understanding biochemical and physiological processes in mushrooms, including the production of enzymes and other biologically active compounds. Possible target genes to be introduced by genetic transformation include: genes producing sporeless strains with reduced ability to cause respiratory disease in mushroom cultivation workers (Baars et al., 2004), senescence genes to improve mushroom quality, substrate utilisation genes (especially with increased lignin degradation capability) to enhance yields (Ha et al., 2001), and developmental genes to control mushroom fruiting. There are numerous potential pest and disease resistance targets in strain development, including genes involved in response to fungal pathogens, toxicity to insects and natural pest resistance. In addition, transformations with mating type genes that regulate inter-strain compatibility can alter breeding behavior, whereas genes that influence the contents of essential nutrients may result in functional foods or foods with medicinal effects (Sunagawa and Magae, 2001; Aida et al., 2009). Other genes contributing to efficient agro-industrial waste bioconversion (Cohen et al., 2002), toxic heavy metal biosorption activities (Pan et al., 2005), hydrophobicity (Ma et al., 2008), and production of live vaccines for animal feeds and human health might also be targets for strain development.

SECTION II – NUTRIENTS

A. Composition of the oyster mushroom (*Pleurotus ostreatus* L.)

18. There is a considerable variation in the data published on nutritional parameters of the oyster mushrooms (*Pleurotus* spp). Most of this variation reflect *de facto* differences in chemical composition due to different strains having been investigated (e.g. Bautista Justo et al., 1998; Manzi et al., 1999; Rai et al., 1988), and different conditions having been present during mushroom cultivation. Factors during and after cultivation that would influence the level of individual constituents include chemical composition of the substrate (e.g. Bonatti et al., 2004; Papaspyridi et al., 2010; Shashirekha et al., 2005; Wang et al., 2001; Yildiz et al., 1998), temperature during cultivation (Pedneault et al., 2007), flushing cycle sampled (Mendez et al., 2005), as well as the conditions after harvest, including storage (Hammond, 1980; Mäkinen et al., 1978) and processing (Jaworska et al., 2011; Manzi et al., 2001). As constituent levels may also vary between different parts of the mushroom (e.g. Synytsya et al., 2008; Yilmaz et al., 2006), it is important to know which parts of the mushroom have been analysed. In part, differences in data may also reflect discrepancies in the analytical methods used. However, most studies referred to in this document have utilised standardized AOAC[2] methods, or similarly validated procedures.

19. This document only considers data on the chemical composition of fruiting bodies of various strains of *Pleurotus ostreatus,* including synonymous strain as defined in Section IA, 2nd paragraph. The mushrooms analysed were either cultivated under controlled conditions or wild mushrooms.

1. Proximates

20. Representative data on proximate analysis of the *P. ostreatus* are presented in Table 1. All data originally reported on a fresh weight basis were recalculated and expressed on a dry weight basis in order to facilitate comparisons.

2. Proteins

21. The protein content is generally calculated from analytical measurements of total nitrogen content. As proteins contain about 16% nitrogen, a conversion factor of 6.25 (1/0.16) is commonly used in nutrition research to convert total nitrogen to protein content (FAO/WHO, 1991; Merrill and Watt, 1973). For mushrooms, this conversion factor is commonly adjusted to account for significant amounts of non-protein nitrogen present. Thus, a conversion factor of 4.38 (Bano and Rajarathnam, 1988; Crisan and Sands, 1978; USDA, 2010) or a factor close to this value (Fujihara et al., 1995; Mattila et al., 2002b) has been suggested by several authors, while the traditional conversion factor of 6.25 has been used in other publications. To facilitate comparisons of results, protein values obtained from total nitrogen using conversion factors other than 4.38, have been recalculated using the conversion factor 4.38. These cases are indicated by a footnote in Table 1.

[2] "The Association Of Analytical Communities" AOAC INTERNATIONAL

Table 1. Proximate composition of *Pleurotus ostreatus*

	Bautista Justo et al 1998[b]	Beluhan & Ranogajec 2011[c]	Bonatti et al 2004[d]	Chirinang & Intarapichet 2009[ef]	Coli et al 1988[e]	Jaworska et al 2011[gd]	Khanna & Garcha 1984[eh]	Mattila et al 2002b[gi]
	Range[a]	*Mean*	*Range*[a]	*Mean*	*Range*[a]	*Mean*	*Range*[a]	*Mean*
Dry matter	9.9-10.2	11.7	11.9-14.4	..	10.7-12.0	8.8	8.5-10.8	8.0
Carbohydrates	70.4-73.2	61.9	71.2-74.5	78.0	54.0-67.4	70.9	59.2-65.1	62.5
Protein	17.3-20.0	24.9	13.1-16.9	15.3	13.6-23.7	16.7	19.2-26.1	24.6
Total dietary fibre	32.1-36.8	..	..	47.6	..	..	..	30.0
Fat	1.1-1.9	2.1	6.0-6.3	0.6	3.4-3.9	5.5	2.3-3.7	4.4
Ash	7.7-8.8	7.6	5.6-6.1	6.1	5.0-6.4	6.7	11.0-13.4	8.0

	Manzi et al 2001[ij]	Rai et al 1988[dhi]	Shah et al 1997[bf]	Sturion & Oetterer 1995[k]	USDA 2010[i]	Wang et al 2001[e]	Yang et al 2001[b]	Obodai & Apertorgbor 2008[k]
	Mean	*Range*[a]	*Mean*	*Range*[a]	*Mean*	*Range*[a]	*Mean*	*Mean*
	(% fresh weight)							
Dry matter	8.7	6.0-7.1	9.6	5.6-7.0	10.8	..	11.4	9.1
	(g/100g dry weight)							
Carbohydrates	67.0	63.6-64.6	75.7	66.3-73.5	56.3	51.7-57.9	66.4	70.4
Protein	18.6	25.8-26.2	15.9	17.4-24.1	30.6	29.1-37.4	23.9	20.0
Total dietary fibre	47.3	..	..	..	21.3	..	..	..
Fat	4.2	1.5-1.7	1.9	1.5-1.9	3.8	4.3-4.7	2.2	2.0
Ash	10.3	7.9-8.7	6.5	7.5-8.1	9.3	6.7-8.4	7.6	7.6

[a] Range of means due to different strains and/or substrates.
[b] Original carbohydrate value not including fibre have been recalculated.
[c] Wild mushrooms.
[d] Carbohydrate value here presented as carbohydrates by difference. Value originally presented as analysed.
[e] Protein value recalculated using the conversion factor 4.38. Carbohydrates by difference recalculated accordingly.
[f] Data recalculated based on true dry matter content.
[g] Protein value calculated by summing the amino acid residues.
[h] Both *Pleurotus ostreatus* and *Pleurotus ostreatus* var. *florida*.
[i] Original data given on fresh weight basis have been recalculated on dry weight basis.
[j] Original carbohydrate value including ash. Value recalculated excluding ash.
[k] Original data not including carbohydrates have been complemented with this data if sufficient information was available for such calculation.

22. The protein content in *P. ostreatus* has been reported to be in the range 13.1-37.4 g/100 g dry weight (Table 1). The difference in protein content reported by various investigators has partly been linked to the problem of estimating the true total nitrogen content. It has also been linked to several other factors influencing protein quantities, including mushroom strain studied (Coli et al., 1988; Bautista Justo et al., 1998; Manzi et al., 1999), substrate used for cultivation (Wang et al., 2001), time of harvest (Mendez et al., 2005), storage and processing *(*Jaworska et al., 2011; Manzi et al., 2001).

23. Table 2 presents data on the total content of the various amino acids in *P. ostreatus*. The total amino acid composition includes free amino acids and those in proteins. Essential amino acids comprise 29-41% of the total amino acid content. The most abundant amino acids are glutamine/glutamic acid and asparagine/aspartic acid, whereas the least abundant are cysteine, methionine, and tryptophan.

24. Several studies have investigated the content of free amino acids in *P. ostreatus* (Abe et al., 1980; Beluhan and Ranogajec, 2011; Ginterova and Maxianová, 1975; Kazuno and Miura, 1985; Kim et al., 2009; Oka et al., 1984; Rai et al., 1988; Sato et al., 1985; Tsai et al., 2009; Yang et al., 2001). The reports differ in methodology used for free amino acids analysis and in the various amino acids analysed. Consequently, reported levels of total free amino acids differ considerably (0.4-16.1 g/100 g dry weight (dry matter)). The most common free amino acids in *P. ostreatus* are glutamine/glutamic acid and alanine. Several non-protein amino acids have been detected, with ornithine as the major constituent (Kazuno and Miura, 1985; Kim et al., 2009; Manzi et al., 1999; Oka et al., 1984; Sato et al., 1985). The occurrence of non-protein amino acids in *P. ostreatus* does not raise a safety concern.

3. Carbohydrates

25. This document uses a common understanding of total carbohydrates in proximate analysis, that is, carbohydrate is calculated as the remaining component when crude protein, crude fat, ash, and moisture have been determined and summed up and the total subtracted from 100%. By this definition, dietary fibre is included in total carbohydrates. Publications with no presented value for carbohydrates by difference, but with sufficient data to calculate it, have been complemented with a calculated value. Changes in presentation of original data have been highlighted by a footnote in Table 1.

26. The nature of determining the total carbohydrate content is reflected by the broad range 51.7 to 78.0 g/100 g dry weight reported in Table 1. As for other nutrients, the influence of external factors on the carbohydrate content has been studied, *inter alia* composition of the substrate for cultivation (Shashirekha et al., 2005; Wang et al., 2001), strain of *P. ostreatus* used (Kim et al., 2009; Rai et al., 1988), post-harvest storage (Hammond, 1980) and processing (Manzi et al., 2001) of the mushrooms.

27. Total dietary fibre comprises the carbohydrate elements (remnants of plant cells, polysaccharides, lignin and associated substances) resistant to hydrolysis (digestion) by human digestive enzymes. Standard analyses for total dietary fibre are based on enzymatic-gravimetric or enzymatic-chemical methods in accordance with AOAC recommendations. Both methods permit separation of an insoluble fraction and a fraction that is soluble in 80% alcohol. Reported amounts of total dietary fibre in *P. ostreatus* are in the range 21.3-47.6 g/100 g dry weight (Chirinang and Intarapichet, 2009; Bautista Justo et al., 1998; Manzi et al., 2001; Mattila et al., 2002b; USDA, 2010). Of this quantity, soluble fibre are reported to constitute 5.1-21.4%, and insoluble fibre constitutes 78.6-94.9% (Lee et al., 2008; Manzi et al., 2001; Synytsya et al., 2008). The insoluble fibre fraction of carbohydrates is primarily made up of chitin from the cell walls, at 3.6-5.5 g/100 g dry weight (Manzi et al., 2001; Vetter, 2007).

Table 2. Amino acid composition of *P. ostreatus* (g/100 g total amino acids)

	Bautista Justo et al. 1999	Chirinang & Intarapichet 2009[c]	Mattila et al. 2002b[d]	Manzi et al 1999	Shah et al. 1997	USDA 2010	Wang et al. 2001	Range of mean values (g/100 g total a.a.)	Range of mean values (g/100 g d.w.[e])
Alanine	6.1-6.2	9.0	5.4	6.0-8.3	6.2	8.7	8.2	**5.4-9.0**	**0.95-2.86**
Arginine	6.6-8.5	15.5	7.8	7.0-11.5	6.2	6.6	7.9	**6.2-15.5**	**0.95-2.76**
Aspartic acid[a]	11.0-12.2	9.7	12.8	9.2-12.1	9.3	10.7	9.0	**9.0-12.8**	**1.42-3.66**
Cysteine[b]	1.5-1.7	n.d.	1.2	1.2-1.7	0.8	1.0	1.1	**0.8-1.7**	**0.12-0.38**
Glutamic acid[a]	18.4-22.6	23.7	15.9	13.1-16.6	17.7	22.9	15.3	**13.1-23.7**	**2.71-5.84**
Glycine	4.2-4.5	3.9	4.2	4.4-4.8	4.6	4.5	4.9	**3.9-4.9**	**0.70-1.71**
Proline	2.9-3.1	1.4	4.1	3.6-4.8	6.2	1.5	4.3	**1.4-6.2**	**0.39-1.52**
Serine	4.6-4.9	5.2	4.8	3.5-6.0	4.7	4.5	5.2	**3.5-6.0**	**0.72-1.81**
Tyrosine	3.3-3.5	2.7	9.6	3.6-4.6	3.5	3.0	3.8	**2.7-9.6**	**0.54-2.74**
Histidine	2.6-2.7	2.6	2.8	3.6-4.3	2.0	2.5	3.6	**2.0-4.3**	**0.31-1.24**
Isoleucine	3.7-4.1	2.9	3.6	3.9-4.7	5.8	4.1	4.7	**2.9-5.8**	**0.71-1.62**
Leucine	5.9-6.9	5.4	6.1	6.3-7.3	8.2	6.1	7.4	**5.4-8.2**	**1.18-2.57**
Lysine	6.7-7.1	3.4	5.5	5.4-6.4	7.2	4.5	6.6	**3.4-7.2**	**1.10-2.29**
Methionine	1.9-2.0	1.3	1.5	1.5-2.3	1.7	1.5	1.1	**1.1-2.3**	**0.26-0.44**
Threonine	4.9-5.1	4.8	4.7	4.7-5.3	4.8	5.1	4.9	**4.7-5.3**	**0.73-1.71**
Tryptophan	1.8-2.2	0.7	n.a.	1.1-1.6	1.5	1.5	1.4	**0.7-2.2**	**0.23-0.48**
Phenylalanine	3.5-4.8	3.5	4.9	3.8-4.7	4.3	4.1	4.4	**3.5-4.9**	**0.66-1.52**
Valine	4.5-4.9	4.3	4.9	4.3-5.2	5.0	7.1	6.0	**4.3-7.1**	**0.77-2.10**
Non-essential	59%	71%	66%	66-70%	59%	63%	60%	**59-71%**	
Essential	41%	29%	34%	30-34%	41%	37%	40%	**29-41%**	

d.w. = dry weight; n.a. = not analysed; n.d. = not detected; a.a. = amino acids

a The analytical method converts asparagine and glutamine to aspartic acid and glutamic acid, respectively. Values represent a total amount of both forms.
b The analytical method converts cysteine to the dimer cystine, and is analysed as cysteic acid. Values represent a total amount of both forms.
c Cysteine not included in total amino acids.
d Tryptophan not included in total amino acids.
e Data presented on a fresh weight basis have been recalculated on a dry weight basis.

28. The most important constituents in the soluble fibre fraction of oyster mushrooms are the β-glucans. β-glucans have been studied for their potential medical uses (Bobek et al., 2001; Dey et al., 2010; Gutiérrez et al., 1996; Karácsonyi and Kuniak, 1994; Lavi et al., 2006; Nosálóvá et al., 2001; Palacios et al., 2012; Patra et al., 2013; Rop et al., 2009; Rovenský et al., 2009; Sarangi et al., 2006; Sun and Liu, 2009; Tong et al, 2009; Yoshioka et al., 1975 and 1985; Zhang et al., 2007). Most studies have concentrated on isolation and characterization of specific β-glucans, and only a few have tried to quantify these polysaccharides. The chemical method used for analysis of these constituents in various types of mushroom has been shown to influence the quantity of β-glucans detected (Park et al., 2009). Using an enzymatic method, Manzi and co-workers determined the β-glucan content in *P. ostreatus* to be 0.14-0.38 g/100 g dry weight (Manzi and Pizzoferrato, 2000; Manzi et al., 2001), which is about 5% of the total dietary fibre. Two other studies, using a commercial kit for analysis, found levels in the range of 27.4-50.0 g/100 g dry matter *(*Papaspyridi et al., 2010; Synytsya et al., 2008).

29. To date, no investigators have been able to give a complete picture of the distribution of individual carbohydrate components in oyster mushrooms. Due to solubility and stability issues different studies for a given carbohydrate fraction are often contradictory. The soluble sugar portion of carbohydrates is usually extracted with 80% ethanol, and analysed after chromatographic separation of the mono- and oligo-saccharide components. Glucose (0.1-1.8 g/100g d.w.) and mannose (0.1-1.3 g/100g d.w.) are the most abundant monosaccharides found in oyster mushrooms (Kazuno and Miura, 1985; Yoshida et al., 1986; Yang et al., 2001; Kim et al*.,* 2009; Tsai et al., 2009; Beluhan and Ranogajec, 2011), while low quantities of fructose (Yoshida et al*.,* 1986); Reis et al., 2012a) and ribose (Kim et al*.,* 2009) have also been reported. Most investigators have identified the disaccharide trehalose (two molecules of glucose) in *P. ostreatus* but the reported levels vary between 0.2 and 40.8 g/100g dry weight (Yoshida et al., 1986; Yang et al*.,* 2001; Kim et al., 2009; Tsai et al*.,* 2009; Beluhan and Ranogajec, 2011; Reis et al*.,* 2012a). The most common sugar alcohol in the mushroom is mannitol, at quantities between 0.3 and 5.0 g/100 g dry weight (Kazuno and Miura, 1985; Yoshida et al., 1986; Yang et al., 2001; Tsai et al., 2009; Beluhan and Ranogajec, 2011; Reis et al*.,* 2012a). Other sugar alcohols occurring at lower amount include arabitol, sorbitol and myo-inositol (Kazuno and Miura, 1985; Yoshida et al., 1986; Yang et al., 2001; Tsai et al*.,* 2009), although the latter is not a classical sugar alcohol.

4. Lipids

30. The crude fat content of *P. ostreatus* ranges from 0.6 to 6.3% of mushroom dry weight (Table 1). Individual fatty acids are generally analysed as methyl esters by gas-liquid chromatography or gas chromatography coupled to mass spectrometry. They are usually presented in relative terms, as percentage of total fatty acids. This means that an accurate presentation requires approximately equal efficiency to identify and quantify the different fatty acids.

31. Table 3 presents literature data on profiles of major fatty acid constituents in *P. ostreatus*. Linoleic acid (C18:2) is the most common fatty acid, at 50 - 78% of the total fatty acids. Oleic acid (C18:1) and palmitic acid (C16:0) are the next most prominent fatty acids with ranges of 6-20% and 11-26% of total fatty acids, respectively. Also studies that only analysed for a few fatty acids found these fatty acids be the major ones (Bautista Justo et al., 1998; Hadar and Cohen-Arazi, 1986; Khanna and Garcha, 1981; Rashad et al., 2009; Shashirekha et al., 2005). Stearic acid (C18:0), palmitoleic acid (C16:1), and myristic acid (C14:0) occur in lesser quantities. Occasional data is available for other individual fatty acids. The most complete picture of the fatty acid profile has been reported by Pedneault et al (2007). They noted that all saturated fatty acids not mentioned above with a chain length between 12 and 24 carbons (except fatty acids with a chain length of 19 and 21 carbon atoms), as well as the unsaturated fatty acids linolenic acid (18:3), gadoleic acid (C20:1), erucic acid (C22:1), and nervonic acid (C24:1) were minor fatty acids in the range 0.01-0.82% of total fatty acids. Stancher et al. (1992a) made similar observations. Small quantities of arachidic acid (C20:0) have been reported by Rashad et al., (2009) and Yilmaz et al.

(2006). Only a few studies have presented data on absolute quantities of fatty acids; linoleic acid levels were in the range between 7.0 and 11.9 mg/g of dry weight oleic acid between 1.6 and 2.9 mg/g of dry weight, palmitic acid between 1.8 and 5.7 mg/g of dry weight, and stearic acid between 0.3 and 0.5 mg/g of dry weight (Hadar and Cohen-Arazi, 1986; Bautista Justo et al., 1998; USDA, 2010).

Table 3. Fatty acid composition of *P. ostreatus* (g/100 g total fatty acids)

	Pedneault et al. 2007[a,b]	**Coli et al. 1988[a]**	**Stancher et al. 1992a[b]**	**Reis et al. 2012a**
C14:0	0.1-0.2	n.d.-0.3	1.2	..
C16:0	11.6-12.8	19.0-25.8	21.5	11.2.
C16:1[c]	0.4-0.5	n.d.-0.2	0.9	..
C18:0	1.8-2.8	1.7-2.2	2.8	1.6
C18:1[c]	6.2-12.0	13.5-20.0	9.7	12.3
C18:2	69.6-78.0	50.5-51.6	59.4	68.9
C20:0	..	1.3-1.6	0.14	..
Others	1.9	5.5-6.1	1.3	5.9

n.d. = not detected

a Range is due to data from different strains and cultivation conditions.
b Original data separated in polar and non-polar fatty acids. Data recalculated into total fatty acids.
c Data presented as undifferentiated by double-bond position and configuration.

32. A few investigators have reported data on free fatty acids in *P. ostreatus* (Kazuno and Miura, 1985; Stancher et al., 1992b). Linoleic acid (C18:2) is not only the most common fatty acid in lipids but also the most common free fatty acid, at 61-64% of the total free fatty acids. The next most common free fatty acid is palmitic acid (C16:0) at around 21% of total fatty acids.

5. Minerals

33. Mushrooms are usually good at taking up minerals and heavy metals from soil. Several reports demonstrate that the content of minerals and heavy metals in the fruiting bodies of *P. ostreatus* mirrors the content in the substrate (Bressa et al., 1988; Favero et al., 1990a; Favero *et al.*, 1990b; Sales-Campos et al., 2009). Consequently, there are considerable differences in mineral and heavy metal levels presented in studies on cultivated and wild oyster mushrooms dependent on substrate and environmental factors. This document therefore separates mineral data on wild grown mushrooms and cultivated mushrooms. Still, substantial differences due to production areas are likely, and there is a great variability within the presented data. Data from studies on mushrooms collected from pronounced contaminated areas have been omitted. Table 4 summarizes data on the content of the most important minerals and heavy metals in the cultivated *P. ostreatus*.

34. Occasional data on other minerals or trace elements have been reported, i.e. Al, As, B, Ba, Li, Mo, Ni, Se, Sr, Ti, and V (Costa-Silva et al., 2011; Haldimann et al., 1995; Mattila et al., 2001; Petrovska, 1999; Procida and Pertoldi Marletta, 1995; Santoprete and Innocenti, 1984; USDA, 2010; Vetter, 1989 and 2005; Vetter et al., 2005).

35. Numerous studies have determined the content of toxic heavy metals in the cultivated *P. ostreatus*. These data are presented in Table 5. Table 6 summarize a selection of studies on the content of the most important minerals and heavy metals in collected wild *P. ostreatus*.

Table 4. Mineral content of the cultivated *P. ostreatus* *[continues on next page]*

	Çaglarirmak 2007[a]	Çoli et al. 1988[b]	Kawai et al. 1994[c]	Manzi et al. 1999[b]	Mattila et al. 2001	Obodai & Apertorgbor 2008	Petrovska 1999[b]	Procida & Pertoldi Marletta 1995[b]
(mg/100g dry weight)								
Calcium	110.1	40.2-42.0	4	23.5-48.6	1.0	43.1	36.0-48.0	..
Iron	20.1	11.4-23.1	8	..	5.4	42.6	3.4-3.8	7.2-21.6
Magnesium	301	129-151	156	161-203	200	..	380-722	..
Phosphorus	1355	697-1027	1061	..	1390	939	310-400	..
Potassium	3019	967-2503	2720	2185-3444	3730	3334	..	..
Sodium	1049.8	1400-1485	74	25.2-136.0	13.0	56.2	..	..
Zinc	15.2	6.2-10.4	10.8	..	8.3	..	1.7-2.6	11.3-14.2
Copper	..	8.8-11.4	1.6	..	0.8	..	1.0-2.4	0.5-4.6
Manganese	..	..	1.5	..	1.1	..	0.3-1.1	0.8-1.0
(µg/100g dry weight)								
Cobalt	..	..	..	..	..	..	n.d.-51	..
Chromium	..	..	..	..	..	..	n.d.-22	35-47

n.d. = not detected

a Original data given on fresh weight basis have been recalculated on dry weight basis.
b Range of means due to different strains and/or substrates.
c Average of 25 samples of different oyster mushroom cultivations on sawdust substrate
d *Pleurotus ostreatus* var *florida*
e *Pleurotus ostreatu*s var *columbinus*
f Analysed part of the fruit body was the pileus
g *Pleurotus ostreatus* var *salignus*
h The value 30 mg/100 g is a suspected outlier. The other values are in the range 3-5 mg/100 g dry weight

Table 4. *[Continued]* **Mineral content of the cultivated *P. ostreatus***

	Rashad & Abdou 2002[b]	Sales-Campos et al. 2009[b]	Sesli & Tüzen 1999	Strmisková et al. 1992[b]	Tshinyangu 1996[b,e]	USDA 2010[a]	Vetter 1989	Vetter et al. 2005[f]	Wang et al. 2001	Yildiz et al 1998[b,g]
(mg/100g dry weight)										
Calcium	10.9-19.0	34.0-60.0	..	12.8-17.5	101.7-108.3	28.0	89.0	82.0	n.d.	1.0-20.0
Iron	42.9-209.9	11.6-15.1	5.8	5.7-14.2	6.7-8.2	12.3	..	15.6	7.1	1.0-19.0
Magnesium	136-166	157-250	..	134-208	178-193	166	190	137	182	..
Phosphorus	-	695-1060	..	942-1755	790-880	1109	1198	698	1648	..
Potassium	632-2020	3683-4218	..	2240-4734	2615-2860	3882	3988	3 074	2171	3440-4500
Sodium	433-654	15.4-19.4	..	13.2-27.5	72.3-87.7	166.0	..	26.9	21.9	..
Zinc	7.0-8.9	8.2-12.4	4.3	4.7-7.7	10.8-11.7	7.1	8.0	7.66	13.7	10.0-13.0
Copper	6.0-11.9	0.9-1.2	0.7	0.8-2.7	1.5-2.0	2.3	2.2	1.87	2.5	3-30[h]
Manganese	1.6-3.5	1.6-2.3	1.3	0.5-1.0	1.0-1.4	1.0	1.1	0.96	1.6	2.0-4.0
(µg/100g dry weight)										
Cobalt	..	..	20	..	..	..	n.d.-19	4	..	..
Chromium	..	..	..	16-101	..	..	n.d.-131	89	..	..

n.d. = not detected

a Original data given on fresh weight basis have been recalculated on dry weight basis.
b Range of means due to different strains and/or substrates.
c Average of 25 samples of different oyster mushroom cultivations on sawdust substrate
d *Pleurotus ostreatus* var *florida*
e *Pleurotus ostreatu*s var *columbinus*
f Analysed part of the fruit body was the pileus
g *Pleurotus ostreatus* var *salignus*
h The value 30 mg/100 g is a suspected outlier. The other values are in the range 3-5 mg/100 g dry weight.

Table 5. Content of toxic heavy metals in cultivated *P. ostreatus* (µg/100 g dry weight)

Heavy metal	Range	References
Cadmium	20-294	García et al., 2009; Haldimann et al., 1995; Kawai et al., 1994; Maihara et al., 2008; Mattila et al., 2001; Petrovska, 1999; Procida & Pertoldi Marletta, 1995; Reguła & Siwulski, 2007; Santoprete & Innocenti, 1984; Sesli & Tüzen, 1999; Strmisková et al., 1992; Wahid et al., 1988; Vetter, 1989; Vetter et al., 2005; Zurera-Cosano et al., 1987
Lead	n.d.-440	García et al., 2009; Haldimann et al., 1995; Mattila et al., 2001; Petrovska, 1999; Procida & Pertoldi Marletta, 1995; Reguła & Siwulski, 2007; Santoprete & Innocenti, 1984; Sesli & Tüzen, 1999; Strmisková et al., 1992; Wahid et al., 1988; Zurera-Cosano et al., 1987
Mercury	n.d.-110	Haldimann et al., 1995; Kawai et al., 1994; Melgar et al., 2009; Reguła & Siwulski, 2007; Santoprete & Innocenti, 1984; Sesli & Tûzen, 1999; Strmisková et al., 1992; Zurera-Cosano et al., 1988

n.d. = not detected

Table 6. Mineral and toxic heavy metal content in wild *P. ostreatus* (mg/100 g dry weight)

Mineral/ Heavy metal	Range	References
Calcium	82-317	Gençcelep et al., 2009; Vetter, 1989
Iron	9.9-68.2	Gençcelep et al., 2009; Isildak et al., 2004; Tüzen et al., 1998; Vetter, 1989; Zhu et al., 2011
Magnesium	120-190	Gençcelep et al., 2009; Vetter, 1989
Phosphorus	326-1198	Gençcelep et al., 2009; Vetter, 1989
Potassium	1993-3988	Gençcelep et al., 2009; Vetter, 1989
Sodium	19-153	Gençcelep et al., 2009; Vetter, 2003
Zinc	1.9-14.2	Alonso et al., 2003; Gençcelep et al., 2009; Isildak et al., 2004; Tüzen et al., 1998; Vetter, 1989; Zhu et al., 2011
Copper	0.5-4.7	Alonso et al., 2003; Dogan et al., 2006; Gençcelep et al., 2009; Isildak et al., 2004; Tüzen et al., 1998; Vetter, 1989; Zhu et al., 2011
Manganese	0.7-3.7	Dogan et al., 2006; Gençcelep et al., 2009; Isildak et al., 2004; Tüzen et al., 1998; Vetter, 1989; Zhu et al., 2011
Cadmium	0.023-0.30	Dogan et al., 2006; Isildak et al., 2004; Tüzen et al., 1998; Vetter, 1989; Zhu et al., 2011; Zurera-Cosano et al., 1987
Lead	0.012-0.297	Dogan et al., 2006; Tüzen et al., 1998; Zhu et al., 2011; Zurera-Cosano et al., 1987
Chromium	n.d.-4.1	Dogan et al., 2006; Isildak et al., 2004; Vetter, 1989; Zhu et al., 2011
Mercury	0.002-0.142	Nnorom et al., 2012; Tüzen & Soylak, 2005; Tüzen et al., 1998; Vetter & Berta, 1997

n.d. = not detected

6. Vitamins

36. Table 7 summarize data on the vitamin content of the oyster mushroom. Levels of β-carotene, the precursor of vitamin A, is reported to be very low (<3.1 mg/100g dry weight) and frequently below the limit of quantification. Several studies have not been able to detect any vitamin C in oyster mushrooms (Okamura, 1998; USDA, 2010; Yang et al., 2002), while others have presented quantities in the range 20.0-45.9 mg/100 g dry weight (Çaglarırmak, 2007; Bautista Justo et al., 1998; Li and Chang, 1985;

Mattila et al., 2001; Rai et al., 1988), and one as high value as 113 mg/100 g dry weight (Bano and Rajarathnam, 1986). The latter observation was in a sample of *P. ostreatus* var. *florida*. These observations can partly be explained by to the analytical method used, as Okamura (1998) showed that ascorbic acid occurs in the form of analogues (6-deoxyascorbic acid, erythroascorbic acid, 6-deoxy-5-O-(α-D-xylopyranosyl)-ascorbic acid, 6-deoxy-5-O-(α-D-glucopyranosyl)-ascorbic acid, 5-O-(α-D-glucopyranosyl)-erythroascorbic acid and 5-O-(α-D-xylopyranosyl)-erythroascorbic acid) rather than ascorbic acid itself in *P. ostreatus* and other mushrooms. The total level of the reduced and oxidised forms of these analogues, converted to ascorbic acid, was around 5 mg/100 g. Most of the analogues occurred in the reduced form (Okamura, 1998). Also reported vitamin E levels differ between investigators. Whereas one research team found α-tocopherol to be more common than γ-tocopherol and δ-tocopherol (Tsai et al., 2009), another research team made the opposite observation (Reis et al., 2012a).

Table 7. Vitamin content of the *P. ostreatus*

Compound	Unit	Range	References
Vitamin C	mg/100 g d.w.	n.d.-113.0	Bano and Rajarathnam, 1986; Çaglarırmak, 2007; Bautista Justo et al., 1998; Li and Chang, 1985; Mattila et al., 2001; Okamura, 1998; Rai et al., 1988; USDA, 2010; Wang et al., 2001; Yang et al., 2002
Vitamin B_1	mg/100 g d.w.	0.1-2.0	Bano and Rajarathnam, 1986; Çaglarırmak, 2007; Bautista Justo et al., 1998; Mattila et al., 2001; USDA, 2010; Wang et al., 2001
Vitamin B_2	mg/100 g d.w.	2.3-7.9	Bano and Rajarathnam, 1986; Çaglarırmak, 2007; Bautista Justo et al., 1998; Mattila et al., 2001; USDA, 2010; Wang et al., 2001
Vitamin D_2	µg/100 g d.w.	0.3-6.5	Mattila et al., 2001; Teichmann et al., 2007; USDA, 2010; Phillips et al., 2011
Vitamin D_4	µg/100 g d.w.	18.3	Phillips et al., 2012
Folates	mg/100 g d.w.	0.1-1.4	Bano and Rajarathnam, 1986; Çaglarırmak, 2007; Lasota et al., 1983; Mattila et al., 2001; USDA, 2010
Niacin	mg/100 g d.w.	36.0-90.0	Bano and Rajarathnam, 1986; Çaglarırmak, 2007; Bautista Justo et al., 1998; Lasota et al., 1983; Mattila et al., 2001; USDA, 2010; Wang et al., 2001
Vitamin E	mg/100 g d.w.	n.d.-70	Reis et al., 2012a; Tsai et al., 2009; USDA, 2010; Yang et al., 2002
β-carotene	mg/100 g d.w.	n.d.-3.1	Tsai et al., 2009; USDA, 2010; Yang et al., 2002

d.w. = dry weight, n.d. = not detected

37. The biosynthesis of vitamin D2 from ergosterol is ultraviolet light dependent, and its formation is influenced both by the amount of the precursor available, the moisture content of the mushroom, the supply of daylight, and the temperature during exposure. Ergosterol has been reported to occur at quantities between 0.68 and 6.7 mg/g dry weight (Jasinghe and Perera, 2005; Koyama et al., 1984; Mattila et al., 2002a; Teichmann et al., 2007; Phillips et al., 2011). Recently, Phillips et al. (2012) demonstrated that several mushrooms, including *P. ostreatus*, also contain vitamin D4, being produced in an ultraviolet light dependent process from the precursor ergosta-5,7-dienol (22,23-dihydroergosterol). The level of vitamin D4 in P. ostreatus was 18.3 µg/100 g dry weight, and the level of the precursor ergosta-5,7-dienol around 0.87 mg/g dry weight. As no information on the cultivation conditions were available in the studies in Table 7 reporting vitamin D levels, it is not known whether the reported amounts fully describe the range in levels of these vitamins in *P. ostreatus*.

7. Other metabolites

38. A few investigators have studied the composition of the flavour compounds of the oyster mushroom, volatile as well as soluble compounds. Tsai et al. (2009) and Zhang et al. (2008) studied

these compounds in the fresh mushroom, and Misharina et al. (2009) studied them in cooked mushrooms. The volatile flavour compounds identified by Tsai et al. (2009) and Zhang et al. (2008) comprised six compounds with eight carbon atoms (1-octen-3-one, 1-octen-3-ol, 3-octanol, 3-octanone, 1-octanol and 2-octen-1-ol) and two aromatic compounds (benzaldehyde, benzyl alcohol), with 1-octene-3-ol, 3-octanone and 1-octen-3-one predominating. The aromatic compounds made up only about 1% of the volatile flavour compounds. Soluble flavours included several soluble sugars and polyols, free amino acids, and 5'-nucleotides (Tsai et al., 2009).

39. Only limited data are available on the occurrence of other constituents in the oyster mushroom. Compounds that have been identified or quantified in P. ostreatus include organic acids (Yoshida et al., 1986), phenolic compounds (Del Signore et al., 1997; Rajarathnam et al., 2003; Reis et al., 2012b; Kim et al., 2008), indoles (Muszyńska et al., 2011), steroidal compounds (Chobot et al., 1997; Plemenitaš et al., 1999), glycoinositolphosphosphingolipids (Jennemann et al., 2001), and lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Gunde-Cimerman and Cimerman, 1995).

SECTION III – OTHER CONSTITUENTS

A. Anti-nutrients

40. Lectins are carbohydrate-binding proteins found in most vegetables and a broad range of mushrooms (Goldstein and Winter, 2007; Guillot and Konska, 1997). They are biologically active in higher animals by binding to cell-wall components in the gastro-intestinal tract, but their principal function in fungi has not been established (Goldstein and Winter, 2007; Guillot and Konska, 1997). One lectin protein, a glycoprotein containing 14% neutral carbohydrate, has been isolated (Conrad and Rüdiger, 1994; Kawagishi et al., 2000; Wang et al., 2000) and crystallized (Chattopadhyay et al., 1999) from P. ostreatus. Its molecular weight has been established to approximately 80-87 kDa using gel filtration and sodium dodecyl sulfate polyacrylamide gel electrophoresis (Conrad and Rüdiger, 1994; Kawagishi et al., 2000; Wang et al., 2000). Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry confirmed the molecular weight to 81.6 kDa (Kawagishi et al., 2000).

41. Quantification of lectins in the mushroom is difficult as it is influenced by the efficacy of the purification. The lectin in the oyster mushroom belongs to the group of lectins causing erythrocyte agglutination and is frequently quantified by its haemagglutinating activity. Kawagishi et al. (2000) and Conrad and Rüdiger (1994) report a lectin content of 20 mg/100 g of fruiting bodies, whereas Wang et al. (2000) report a content of 7.9 mg/100 g fruiting bodies.

42. The toxicological data on the *P. ostreatus* lectin is limited and there are no incidents of human intoxication specifically related to the lectin in the oyster mushroom. However, decreased food intake has been reported in laboratory animals fed a diet including powder of dried *P. ostreatus* (Kawagishi et al., 2000; Nieminen et al., 2009) whereas another study did not observe a similar effect (Bobek et al., 1991). Kawagishi et al (2000) linked the reduced feed intake to the lectin fraction of the feed; the lower the lectin content of the oyster mushroom powder, the lower the influence on feed intake. Extrapolation of these experiments of repeated intake of relatively high doses of mushrooms pelleted into the animal's diet to the human situation has not yet been undertaken. Kawagishi et al (2000) used a diet containing 5% mushroom powder and Nieminen et al (2009) utilised a diet that resulted in a mushroom powder intake ranging from 1.8%-5.4% of total feed. According to Nieminen et al (2009), the latter range corresponds to an intake of 1.2-2.7 kg mushroom per day for a human weighing 70 kg.

B. Toxicants

46. Two proteins with hemolytic and cytolytic properties (hemolysins) have been isolated from oyster mushroom, pleurotolysin (Bernheimer and Avigad, 1979) and ostreolysin (Berne et al., 2002). Pleurotolysin consists of two non-associated protein components with molecular weights of 17 kDa and 59 kDa, respectively (Sakurai et al., 2004; Tomita et al., 2004). These components co-operatively, in a larger assembled complex of 700 kDa, generate a pore structure in the cell membrane producing lysis. Ostreolysin is a membrane binding protein of 15-17 kDa interacting in particular with lipid membranes highly enriched in cholesterol and sphigomyelin, hypothetically involved in the development of fruit bodies (Berne et al., 2002; Skočaj et al., 2013). Besides showing hemolytic activity and increasing permeability of endothelial cell membranes in vitro (Berne et al., 2002; Maličev et al., 2007; Sepčić et al., 2003), ostreolysin contracts coronary blood vessels in laboratory animals supplied the protein intravenously (Juntes et al., 2009; Rebolj et al., 2007). An intravenous LD_{50}-value of 1170 µg ostreolysin

per kg body weight has been determined in the mouse (Zuzek et al., 2006). No data on quantities of pleurotolysin or ostreolysin in the fruit body of oyster mushroom is available in the literature.

47. There is no data on the toxicity of *P. ostreatus* hemolysins in oyster mushroom consumers. Oyster mushroom is considered a non-toxic mushroom and the presence of hemolysins does not influence this conclusion. Hemolysins are thermo-labile, and potential toxicity would be considered only for raw mushrooms. Furthermore, proteins are usually degraded in the gastrointestinal tract when ingested. Administration of aqueous extracts of *P. ostreatus* to mice demonstrated no acute effects (Al-Deen et al., 1987; Bedry et al., 2001) and repeated oral feeding of the mushroom to rodents revealed no histopathological changes of cardiac or hepatic tissue (Bobek et al., 1998; Nieminen et al., 2009).

C. Allergens

43. Two types of allergy can be distinguished – food allergy manifested after consumption of allergenic mushrooms, and respiratory allergy after inhalation of allergenic mycelia or basidiospores. The latter type of allergic disease may be due to the compost/cultivation conditions and is then frequently independent of the mushroom species cultivated. If it is due to mushroom tissues, usually spores, then it is frequently species dependent.

44. No case reports on individuals being allergic to oyster mushroom as food have been found in the literature.

45. Like many other cultivated mushrooms, *Pleurotus* species have been shown to give rise to mushroom grower's disease. Most likely mushroom grower's disease 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 and where basidiospores are common. Characteristic symptoms include allergic rhinoconjunctivitis, asthma, and hypersensitivity pneumonitis (Lehrer et al., 1994; Saikai et al., 2002; Helbling et al., 1999; Mori et al., 1998). All these symptoms of allergy have been described in worker's cultivating *P. ostreatus* and its subspecies (Senti et al., 2000; Vereda et al., 2007), but hypersensitivity pneumonitis being particularly frequent (Zadrazil, 1973 and 1974; Noster et al., 1976 and 1978; Cox et al., 1988; Mori et al., 1998; Kamm et al., 1991). In addition, allergic contact dermatitis after exposure to *Pleurotus* mushrooms has been described. Symptoms appeared around harvest and included red scaly vesicular lesions on the hands, sometimes spreading to the upper and lower limbs, face and trunk (Rosina et al., 1995). The agent responsible for the contact dermatitis has not been identified.

SECTION IV – SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FOOD USE

A. Identification of *Pleurotus ostreatus* food products

48. Oyster mushrooms stand for around 15% of the world production of cultivated mushrooms, and *P. ostreatus* is the most commonly cultivated oyster mushroom species. A significant part of the harvest is destined for human consumption. The mushrooms are either sold fresh or processed by industry for easy storage (dried, frozen, canned, and 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. Recommendation of key components to be analysed related to food use

49. The key constituents suggested to be analysed in new varieties of *P. ostreatus* using appropriate methodology are shown in Table 8. In case minerals are also analysed, iron, phosphorus, potassium, zinc, copper, manganese and chromium is suggested. As all food products of the oyster mushroom used by consumers and the 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 oyster mushroom.

Table 8. Suggested constituents to be analysed in fresh fruit bodies of cultivated oyster mushroom, *P. ostreatus*, for food use

Constituents	Fruit bodies
Proximates	X
Amino acids	X
Fatty acids	X
Vitamins	X[a]

a: The B-vitamins thiamine (B1), riboflavin (B2), niacin (B3) and folic acid (B9) are suggested

SECTION V – SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FEED USE

50. Mushrooms are not typically included as animal feed ingredients, and it is unlikely that mushrooms would become a large significant nutrient contributor in animal feed. In the rare cases when by-products of oyster mushroom cultivation and mushroom processing (mainly stipes) may be used as animal feed, it is probably locally in the neighbourhood of the mushroom farms.

51. Although it is unlikely that fresh or processed fruit bodies of *P. ostreatus* will be used as animal feed, fragments of the vegetative mycelium might be consumed. It has been observed that several agro-industrial byproducts locally available in many nations, for example, cocoa pod husks, which when untreated have a limited value as animal feed due to high contents of lignin and non-starch polysaccharides such as cellulose, hemicelluloses and pectin, have improved nutritional utility after they have been chemically modified by being substrates during mushroom cultivation. However, in this case it is recognized that the main proportion of the animal feed ingredient will be the bio-converted agro-industrial byproduct, with mushroom mycelia being only a minor part. When included in this form, oyster mushroom would still be considered a very minor animal feed. No specific studies on the chemical composition of by-products of oyster mushroom cultivation and processing are needed for considering these as animal feeds. Therefore, no specific requirements for constituents to be analysed for animal feed are recommended in this document. Any required compositional information can be obtained from the analysis of proximates performed as part of an assessment for food uses of new varieties of oyster mushroom.

SECTION VI – REFERENCES

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