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Part II

NUTRITIONAL AND MEDICINAL ASPECTS OF MUSHROOMS

Champignon do Brasil (*Agaricus brasiliensis*): Nutrition, Health, Market Demands and Regulatory Concerns

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Abstract: *Agaricus brasiliensis* Wasser et al., until recently thought to be *Agaricus blazei* Murrill is one of the most valued mushrooms in the world market. Although the vast majority of the literature deals with its virtues as a medicinal mushroom, due to its nutritional quality and peculiar characteristics of taste, almond flavour and excellent texture, it has also a large potential as a health and gourmet food, being therefore highly prized. The common name Champignon do Brasil was recently proposed as a reference to the Champignon de Paris (*Agaricus bisporus*), the most consumed commercial mushroom in the world. Some aspects on its nutritional qualities and gastronomical potential, flavour and taste compounds, potential toxic constituents, medicinal properties claims, market demands and regulatory concerns will be discussed.

Key words: *Agaricus brasiliensis, Agaricus blazei*, Agaricaceae, Basidiomycetes, edible and medicinal mushrooms, nutrition and health

1 Introduction

Mushrooms have become more and more attractive as gourmet and functional foods, as well as a source for the development of new drugs. *Agaricus brasiliensis* Wasser et al., until recently thought to be *Agaricus blazei* Murrill, is one of the most promising species. The following common names have been used for this mushroom in Brazil: Cogumelo Piedade, Cogumelo do Sol and Cogumelo de Deus. The most popular one is Cogumelo do Sol. This denomination, however, is a trade mark of the company Cogumelo do Sol Agaricus do Brasil Com. Imp. & Exp. Ltda. Thus, the indiscriminate use of this commercial name comprises an infraction of the laws of trademarks and patents, and punishments are foreseen for such irregularities, as announced by the company and widely known. As an alternative to the mushroom growers, especially those wishing to explore it as a gourmet food, we recently proposed the name Champignon do Brasil, alluding to the well-known and the most consumed commercial mushroom in the world, Champignon de Paris (*A. bisporus*), the button mushroom.[1] In Japan, *A. brasiliensis* was initially called Kawariharatake and then Himematsutake, while the Chinese call it the Brazilian Mushroom. In the USA, it is known as Royal Agaricus, Royal Sun Agaricus, King Agaricus or Almond Portobello.[2] A detailed discussion of its taxonomic identity is given by Wasser et al.[3]
Originally from Brazil, the species was taken to Japan in 1965. There, its medicinal activities have been extensively studied. It is said that the Japanese immigrant Takatoshi Furumoto, who worked at growing the Champignon de Paris and the Shiitake (*Lentinula edodes*), one day found a different mushroom in his small farm, in the region of Piedade, São Paulo State, which attracted his attention. Unable to identify it, he sent it to the Instituto de Botânica in São Paulo and to the Iwade Mushroom Institute in Japan. As attempts to identify the material failed at both institutions, samples were sent to the leading fungal taxonomists David Pegler (Great Britain) and Paul Heinemann (Belgium). Pegler reported no identification in any publication, although the president of the company Cogumelo do Sol Agaricus do Brasil Com. Imp. & Exp. Ltda. affirms that he has been told that the mushroom belongs to the species *Agaricus silvaticus*, a cosmopolitan fungus from forest humus. Some results

of clinical observations in patients with cancer, using its products either associated to surgical treatment, chemotherapy and radiotherapy or alone, were even published in Brazil under this denomination.[4-8] Heinemann,[9] on the other hand, identified the fungus as *A. blazei*, a naturally occurring species from North America described from Florida by Murrill[10] in 1945, and communicated his identification to the scientific community in a paper published in 1993. This late denomination has been the most universally used in the literature on biotechnological and medicinal aspects of the mushroom, as well as in most of its commercialised products.
The majority of the strains spread over the world most probably came from the culture originally sent from Piedade to Japan, as no further discovery in nature was reported until January 2001, when the species was found growing naturally on a heap of mowed grass at Embrapa Florestas, Colombo, State of Paraná, Brazil. It was then suspected that the name *A. blazei* was not correct for this fungus, which motivated the mycologist André de Meijer to write a circular letter to several specialists, suggesting re-identification. The proposal was recalled in an article published in Belgium, in December 2001, where the rebaptism of the fungus as *Agaricus brasiliensis* was informally proposed.[11] One year later (December 2002), Solomon Wasser and collaborators, in a detailed comparative morphological study, demonstrated that the North American endemic species *A. blazei* ss. Murrill and the widely cultivated medicinal *A. blazei* ss. Heinem. are two different species. *A. blazei* ss. Heinem. was then proclaimed as a new species and nominated *A. brasiliensis*, based on the collection made at Embrapa Florestas, and so this area has become the type locality.
Therefore, in the light of present knowledge, the use of the name *A. brasiliensis*, replacing previous denominations, should be encouraged. In practice, however, this is not an easy task. There are commercial implications due to previous legal registration of products either under the name *A. blazei* Murrill or *A. sylvaticus* Shaeffer (wrong spelling; the correct form would be *A. silvaticus* Schaeff.: Fr.). This was most evident by the passionate reaction to my recent lecture during the Second International Symposium on Mushrooms in Brazil (II SICOG), held in Brasília, 6-9th December, 2004.[12] Although the Cogumelo do Sol Agaricus do Brasil Com. Imp. & Exp. Ltda. has sent us their mushrooms for taxonomic identification and we have certified them as *A. brasiliensis* Wasser, Didukh, Amazonas et Stamets (C.P&D Florestas n° 59/2003, March 18, 2003), pre-clinical and clinical studies supported by the company presented in the II SICOG still referred to *A. sylvaticus*.[13, 14] Nevertheless, the denomination *A. brasiliensis* was used in the 16th International Congress on the Science and Cultivation of Edible and Medicinal Fungi, held in Miami, USA, 14-17th March, 2004.[15]

2 Nutritional and Gastronomical Aspects

2.1 Nutritional Qualities and Gastronomic Potential

The vast majority of the international literature on *A. brasiliensis* deals with its virtues as a medicinal mushroom and, as a result, it is presently one of the most valued cultivated mushrooms on the world market. However, due to its nutritional quality and peculiar characteristics of taste, almond flavour and excellent texture, it also has a large potential as a health and gourmet food, and is therefore highly prized. The dried mushrooms retain about 7% of their weight as moisture. The dry matter has the following average composition: 38% protein, 40% carbohydrates, 3% fat, and about 7% of mineral compounds including 2.5% potassium, 1% phosphorus and 0.1% magnesium. Moreover, *A. brasiliensis* contains nutritionally important amounts of B vitamins, niacin, and even vitamin D, plus the essential elements iron, manganese, zinc and copper.[11, 16] Evaluation of the protein quality in animal model systems is under investigation by our research group and some preliminary results will be presented at this conference.
Stamets[2] recommends cooking the sliced mushrooms simply in olive oil at high temperature, and to season with salt, soy sauce and tamari. The gastronomist Paulo Siqueira included the mushroom in traditional Brazilian recipes for, among others, soups, crackers, pastry, fruit juices, ice tea and pudding. To appeal to the German taste, he has even introduced the mushroom in sausages, which were successfully presented at the agricultural

trade fair BioFach 2003, held at Nuremberg, Germany. The Bratwurst was prepared, as the other recipes, with the support of GAPI - Grupo Agaricus de Pilar Ltda., a company managed by five rural high quality organic *A. brasiliensis* producers in the State of São Paulo, Brazil. Because of the reasons discussed before, they are still using the name *A. blazei* commercially.

The development of alcoholic beverages by mushroom fermentation is being considered in Japan. Potent alcohol dehydrogenase (ADH) was found in cell-free extracts of *A. brasiliensis*, which motivated an investigation of its use in the fermentation of grape juice.[17] The authors state that the fermented beverage produced with *A. brasiliensis* contained 0.68% ß-D-glucan and thus can be expected to be effective in preventing cancer. Moreover, fibrinolytic activity was shown and so it may also have a preventive effect on thrombosis. These results can stimulate the development of new fermented foods with attractive functional properties.

In an effort to promote *A. brasiliensis* as a healthy gourmet food in Brazil, we have recently published a booklet containing information on relevant aspects of this new promising business, including many mouth-watering recipes.[1] This booklet was presented to support the cooperative work between Embrapa Florestas, Gapi and Paulo Siqueira, during the fair Sabores do Paraná, held at Curitiba, December 2003, where canned *A. brasiliensis* (Champignon do Brasil) was submitted to and successfully approved by a large public for the first time. At the occasion, some biscuits produced in collaboration with the food company Tip Top Alimentos Ltda. were also most appreciated.

2.2 Flavour and Taste Compounds

The flavour and taste compounds of *A. brasiliensis* were recently investigated at the Nestlé Research Centre, Lausanne, Switzerland.[18] Dried mycelium and corresponding mushrooms were obtained from Paul Stamets, Olympia, USA. Several dried fruit body samples were also supplied by commercial growers from the States of Paraná and Minas Gerais in Brazil. A wild-growing dried sample from the type locality (*Embrapa Florestas*) was also included in the study. Other agarics analysed were obtained in Switzerland and in France.

An investigation of the mushroom's pleasant almond flavour revealed that benzaldehyde and its precursor benzoic acid were the major components of the volatile fraction. Other benzilic compounds contributing to the flavour were benzyl alcohol, methyl benzoate and 4-hydroxybenzaldehyde. When soaking the dried mushrooms in water, the almond flavour develops, presumably by enzymatic conversion of benzoic acid to benzaldehyde and benzyl alcohol. The authors argue that benzoic acid is a classic food preservative and, since it is naturally present in *A. brasiliensis* at concentrations of 0.13% - 0.30% on dry weight, it may well contribute to its excellent shelf life. Interestingly, benzoic acid also occurs in several close relatives of *A. brasiliensis*, suggesting that this compound could be a taxonomic marker.

Among the non-volatile taste compounds, mannitol predominated to the extent of 22% on a dry weight basis. The content of taste-enhancing free glutamic and aspartic acid was comparable to those reported in the white button mushroom. The mycelium of *A. brasiliensis* was found to be poor in nearly all compounds investigated. No almond flavour was observed and its crude protein content was only 13% compared to an average value of 30% in the dried mushrooms. Moreover, it had less than 1% mannitol and only very low levels of free amino acids.

2.3 Potential Toxic Constituents

There is misleading information about the content of agaritine, a hydroxymethylphenylhydrazine derivative suspected to be a potential carcinogen, in *A. brasiliensis*. Stamets[19] and Wasser et al.,[3] both based on Stijve (2001, pers. comm.), state that agaritines (in the plural!) account for 1% of the dried mass of fruit bodies, while products from the mycelium vary substantially from <0.02% to 0.2%[19] and <0.2% to 2%[3] depending upon the manufacturer and source. Stamets[20] and Didukh et al.[21] once again emphasized the content of agaritines in *A.*

brasiliensis, this time based in a general article first published in Belgium,[11] then in Switzerland, Germany and Australia. However, there was no mention of agaritine in this article, which has also appeared in France under a different title.[22] In fact, in a study on potential toxic constituents in *A. brasiliensis*, as compared to other cultivated and wild-growing edible mushrooms,[23] agaritine, agaritinal, urea and free tryptophan were analysed in samples from Brazil (fruit body samples: eight from commercial growers, mainly from the State of Paraná, and two collections of the wild-growing mushroom, made at the type locality of the latter area), China (one fruit body sample purchased in Singapore) and USA (some samples, including both mycelium and mushrooms, supplied by Paul Stamets). Tree *A. brasiliensis*-derived medicinal products from Japan were also included in this study. The analysis was done at the Nestlé Research Centre, Lausanne, Switzerland. The following values were found for agaritine: <0.02% in the mycelium, 0.1% to 0.65% in fruit bodies and <0.005% to ~0.03% in *A. brasiliensis*-derived medicinal products. The mushroom is in all probability exempt of other phenylhydrazine compounds, as not even agaritinal, the formylphenylhydrazine analogue, could be detected. The fluctuating agaritine levels are in the same order of magnitude as those reported in other members of the genus, including the button mushroom.

The important point in this question is the evaluation of a possible human health risk from consumption of the mushroom, and for that we can learn from the studies with *A. bisporus*. So far, conclusive data are not available. The Nordic project group on inherent natural toxicants in food plants and mushrooms has followed the scientific information on the toxicity of *A. bisporus* and the phenylhydrazine derivatives occurring in the mushroom since the end of the 1980s. A detailed update review prepared by the group is given by Andersson & Gry.[24] This report, initiated in 1996, has revealed that more studies are required - particularly in relation to carcinogenicity, other toxicity and chemical studies - to prepare a better founded risk assessment of the phenylhydrazines in *A. bisporus*. The full report can be accessed as a pdf file (http://www.norden.org/pub/velfaerd/livsmedel/sk/TN2004558.pdf).

Dried *A. brasiliensis* was also investigated for toxic trace elements, such as heavy metals, radioactive isotopes, and pesticide residues in the aforementioned samples.[23] The results were rather reassuring: pesticides and radioactive isotopes tested negative in A. brasiliensis from the USA and Brazil. A sample from China, on the other hand, contained a little benzene hexachloride (BHC, one of the notorious organochlorine pesticides) and deltamethrin, an insecticide belonging to the class of synthetic pyrethroids. Among the trace elements, excessive cadmium may be a potential problem: concentrations of this heavy metal in the mushrooms from Brazil and USA complied with European legislation, but the sample from China did not. However, cadmium levels in oyster and shiitake, two widely consumed mushrooms, also occasionally exceed the EEC limit, which may have been set too low. This also applies to the horse mushroom (*A. arvensis*) cultivated in Holland, which recently appeared on the markets in Geneva and Lausanne, where, apart from the time-honoured button mushroom, there are few, if any, cultivated *Agaricus* species on the market. The first flush of its fruitvbodies often had slightly too much cadmium, but the following flushes are approved by the EEC legislation.[16]

3 Claims of Medicinal Properties

Among the medicinal properties claimed for *A. brasiliensis*, the immunomodulating and anti-tumour effects are the most prominent, as for other mushrooms like *Ganoderma lucidum*, *L. edodes*, *Schizophyllum commune*, *Trametes versicolor* and *Grifola frondosa*. Glucose and cholesterol reduction activity have also been claimed. Furthermore, the high content of ergosterol, the vitamin D precursor, gives the mushroom important attributes in the fight against bone-related diseases such as rachitis and osteoporosis. It is worthwhile to remember also that vitamin D is an antioxidant.

Many bioactive compounds have been isolated and characterized from *A. brasiliensis*, such as cytotoxic steroids,[25] lectin,[26] polysaccharides,[27-35] peptide-glucan,[36] proteoglucan,[37-39] ergosterol,[40] des-A-ergostane-type compounds,[41, 42] and recently sodium pyroglutamate, an anti-angiogenic substance with antitumour and

antimetastatic actions.[43] A few papers on polysaccharide production in liquid culture have also been published.[44-47] Although the detailed mechanisms of their physiological effects are still not thoroughly understood, *in vitro* and animal model trials demonstrating bioactivity for several extract fractions and purified compounds have been reported. Most of these studies have originated from Japan.[25-32, 35-43, 46-50] In the last four years, studies from Brazil,[46, 47, 53-62] China,[33, 34, 63] Korea[52] and Australia[44] have also been reported.

At the University of California, USA, Dr. Mamdooh Ghoneum concluded that the Royal Agaricus mushroom could be considered as a potent biological response modifier for treatment of cancer. His studies in mice, presented at the 9th International Congress of Immunology, California, July 23-29, 1995,[64] encouraged the Brazilian oncologist Dr. Jorge Laerte Gennari to use the mushroom as a complement to traditional therapies in the treatment of human cancer patients. Other physicians joined him to establish the Ricardo Veronesi Research Institute - InFito (www.infito.com.br). The eminent Brazilian infectologist, Prof. Veronesi, who died on May 8, 2004, was an enthusiastic defender of the use of this mushroom in the immunotherapeutic approach to fight diseases like cancer. Just one year before his death, we were privileged to organize a seminar in Curitiba, the capital of the State of Paraná, Brazil, where he and Dr. Gennari discussed their experiences on mushroom and immunotherapy. Regrettably, Prof. Veronesi was himself victim of a malign tumour!

Although some impressive results based on a decade-long clinical trial following 70 cancer patients have been shown by Dr. Gennari and colleagues,[4-8, 14, 15] his protocol does not include placebo controls and, therefore, is subject to criticism. In a double-blind clinical trial recently reported by scientists from Korea,[65] 100 gynaecological cancer patients undergoing chemotherapy were randomised for treatments with a mushroom extract from a Japanese company (*A. blazei* Murrill Kyowa-ABMK) and placebo. The patients were recruited from among those visiting the Department of Obstetrics and Gynaecology, Kangnam St. Mary's Hospital (Seoul, South Korea) in the 3-year period from 1999 to 2001, and were monitored for six weeks. It was observed that natural killer (NK) cell activity was significantly higher in ABMK-treated group (n=39) as compared to non-treated placebo group (n=61). However, no significant difference was observed in the activities of lymphokine-activated killer (LAK) and monocyte (H_2O_2 assay), as well as the count of white blood cells, lymphocytes, monocytes, and percent of specific immune cell populations (CD3, CD4, CD8, CD56 and CD48) between ABMK-treated and non-treated groups. At the time of the treatment completion, the patients were asked to fill out questionnaires to evaluate their own physical and mental conditions. It was observed that chemotherapy-associated side effects, such as loss of appetite, alopecia, nausea/vomiting, emotional instability, and general weakness, were all improved by ABMK treatment, as compared to placebo, suggesting a positive effect of the mushroom extract consumption on the patients' overall condition. The authors concluded the following: "oral delivery of ABMK provides an additional alternative therapeutic modality to maintain innate NK cell activity and, in particular, reduces many severe side effects caused by chemotherapy in gynaecological cancer patients".

4 Market Demands and Regulatory Concerns

A. brasiliensis is a highly valued and prized edible and medicinal mushroom, with sales having reached 25 billion Japanese yen in 2000.[66] At present, the mushroom is cultivated commercially in Brazil, Japan, China, Korea, and the USA. In Europe, small-scale growing has just started in some countries.[16] Brazil is the world leading producer and 90% of its production is exported to Japan as raw material for further processing. Great efforts are being made to popularise the mushroom as a health food for consumption in the country. Despite the fact that this foodstuff is not part of the normal diet, it has been shown to be easily incorporated in traditional Brazilian recipes.

On the other hand, from the global point of view, there is a growing demand for fungal products and derivatives by the nutraceutical industry, for which a shift from the focus based on a marketing paradigm to a science-driven paradigm is in motion.[67] This will demand considerable scientific documentation relating to the quality, safety and efficacy substantiation for these products worldwide. The testing procedures, studies, and research

required to satisfy this new paradigm and recent regulatory concerns represent a big challenge. A variety of regulations and safety concerns in the United States, the European Union, Canada, Australia, New Zealand, Japan and Israel have been discussed.[67-70] In addition, it is worthwhile mentioning here the position of Brazil. While the country's legislation is being reviewed, the latest documentation from the National Agency for Sanitary Vigilance (ANVISA), dated Jan 31, 2003 (http://www.anvisa.gov.br/alimentos/informes/06_310103.htm), stated the following decisions:

1. The dried forms, either whole or fragmented, and the canned forms of the species below mentioned are considered as foods (Resolution CNNPA 12/78 and Resolution CNNPA 13/77) and are dispensed from the registry obligation:
   Agaricus campestris
   Agaricus blazei Murrill (popularly known as himematsutake or cogumelo do sol)
   Agaricus sylvaticus Shaeffer and
   Lentinula edodes or Lentinus edodes (popularly known as shiitake)
2. The species of mushrooms different from the mentioned, in the dried whole or fragmented and in the canned forms, must prove safety of use and absence of risk to health based on the Resolution ANVS/MS n°17/99.
3. The mushrooms, either isolated or associated to other substances, in the forms not previewed in the specific technical regulation (Resolution CNNPA n°12/78 and Resolution CNNPA 13/77), as powders, capsules, tablets, pills and liquids, among others, independently from the species of mushroom, are not considered as foods.
4. Medicinal and or therapeutic claims (prevention, treatment and cure of diseases) in the labels and advertisement material of the mushrooms, previewed in the items 1 and 2 (when evaluated and approved by Anvisa) are not allowed.

5 Concluding Remarks

Although many biological properties have been claimed for *A. brasiliensis* and its derived products, it is clear there is a need for many more studies (toxicokinetics, short-term tests, effects on experimental animals and clinical trials) to support safety and efficacy claims of products aimed for therapeutic use.

Brazil has a great opportunity to become established as a leader in the world market relating to this particularly genuine Brazilian mushroom. However, one must be aware that this will happen only if a proper research and development policy is set, joining scientists, mushroom growers, gastronomists, and entrepreneurs. Although the country is not a traditional mushroom consumer, the interest for those organisms is rapidly increasing, especially due to claims relating to their functional properties. At present, however, the inclusion of the Champignon do Brasil in the diet habit seems to be a much more plausible approach to satisfy regulatory concerns. Moreover, so far, the country is mainly providing raw material (dried mushrooms) for processing derived products by companies outside, often sold as miracle medicines. This system is not fair to the local mushroom growers who derive little benefit from the business. Diversification with focus in the food market could well be a more appropriate way of popularising mushroom consumption inside the country. This, in turn, would demand a much greater mushroom production and so a fairer social and health benefit to the local population would be expected.

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