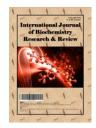
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Evaluation of Biochemical and Microbiological Properties of *Pleurotus ostreatus* Mushrooms Cultivated and Sold in M'Badon Village (Abidjan, Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: The present study aims to constitute a database necessary for the efficient valorisation of the local cultivated edible mushrooms in the Ivorian diet. This work consisted in evaluating the biochemical characteristics and microbiology analysis of cultivated *Pleurotus ostreatus* species sold and used in rural and urban people food.

Methodology: Standard methods proposed by AOAC made this study possible to determine the biochemical parameters such as dry matter, ash, pH, moisture, protein and lipid content. The microbiological analyses enabled the enumerations of yeasts and molds, fecal coliforms, aerobic mesophilic germs, detection and enumeration of *Escherichia coli* and Salmonella were performed. **Results:** The results showed that the cultivated *Pleurotus ostreatus* is a food, rich in protein (16.37)

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 \pm 0.6 % dw), crude fibre (24.85 \pm 0.08 dw) and in ash (11.00 \pm 1.33 % dw). On the other hand, this mushroom is relatively low in lipids (4.16 \pm 0.13% dw) and reducing sugars (1.04 \pm 0.07 % dw) with a low moisture content (6.40 \pm 1.13 % dw). These results also revelated the absence of Salmonella and *Escherichia coli* in the flour.

Conclusion: Local cultivated *Pleurotus ostreatus* mushroom is an undeniable source of protein and crude fibre. Thus it would be used as a substitute for meat diet, allow a good functioning of the immune system and the good development of bones. It also show satisfactory microbiological criteria. Thus Pleurotus ostreatus mushroom is a safety food for ivorian.

Keywords: Cultivated mushrooms; Pleurotus ostreatus; biochemical parameters; microbiological analyses.

1. INTRODUCTION

Mushrooms have been regarded as gourmet cuisine across the globe since antiquity for their unique taste and subtle flavor. They are considered as sources of important nutrients including dietary fibre, minerals, and vitamins, in particular, vitamin D [1]. More than 2,000 species of mushrooms exist in nature, but only around 25 are widely accepted as food and few are commercially cultivated [2]. Recently, they have become increasingly attractive as functional foods due to their potential beneficial effects on human health. Hence, food industry is especially interested in both wild and cultivated edible mushrooms.

In Côte d'Ivoire, as mushroom cultivation is not yet developed, most of the mushrooms consumed are of wild origin [3]. However, it has been shown that wild mushrooms disappear or stop growing as soon as their tree partners are cut down [4]. Moreover, they are not widely available or even sufficiently available, as their appearance is often random and concentrated in a few weeks per year, mainly during the rainy season. Hence the rise and interest in cultivated mushrooms are initiated to compensate this deficit [5].

Therefore, Pleurotus mushrooms are artificially and easily cultivated in tropical and subtropical regions [6]. Their nutritional value includes carbohydrates and proteins, which are the main components, accounting for 70 to 90 % of dry matter. Besides, they have about 7.0 - 8.0 % ash but, fat content is low (1-2 %) [7]. Proteins of Pleurotus mushrooms are present with essential amino acids [8]. And among the genus Pleurotes, we can mention the species *Pleurotus ostreatus*.

It is reported that *Pleurotus ostreatus* (*P. ostreatus*) like other oyster mushroom species, is a basidiomycete mushroom cultivated for consumption. Its fruiting body is highly valued for

its sensory qualities [9]. In terms of nutritionally, *P. ostreatus* is known to be an excellent provider of protein of high biological value, dietary fibre and trace elements, but it is also known for its low fat content [10]. This mushroom could help to enrich the diets of vulnerable populations from rural areas in malnutrition [11]. In addition to its nutritional potential, *P. ostreatus* also contains certain bioactive compounds such as non-starch polysaccharides and flavonoids, identified for their anticancer and antioxidant activities [10]. In this regard, a recent study reveals that these mushrooms have been considered as functional foods [12].

However, some studies indicate that substrates used for mushroom cultivation may alter the biochemical, functional and microbiological characteristics of fruiting bodies, as well as their sensory properties [9; 13]. It should also be noted that rare species census studied by Marshall and Nair [14] and Kara and Khendriche [15] do not address the biochemical and microbiological aspect in any way. And to our knowledge. investigations on biochemical properties and microbiological characteristics of local cultivated Pleurotus ostreatus are very limited. Therefore, this is a limiting factor in its vulgarisation in Côte d'Ivoire. Thus, this study aims to assess the proximate composition, in vitro protein, reducing sugars, lipids as well as the most relevant microorganisms of the local cultivated and sold P. ostreatus mushroom in the village of M'Badon.

2. MATERIALS AND METHODS

2.1 Biological Material

In the present study, the biological material used for analysis was the edible mushroom *Pleurotus ostreatus* grown and purchased at the village market of M'Badon (Abidjan, Lagoon region, Côte d'Ivoire) (Fig. 1). The production of *Pleurotus ostreatus* is done in soilless culture. This technique required the use of a nutrient substrate whose constituents are sawdust, dry cassava peel and sorghum.

2.2 Preparation of *Pleurotus ostreatus* Flour

The mushroom samples (400 g) were oven-dried for 72 hours at 45 °C. They were then ground in a MOULINEX blender to obtain a flour. These flours were kept in pre-dried jars for possible analysis (Fig. 2).

2.3 Proximate Analysis

The standard analytical procedures for food analysis were adopted for the determination of

the moisture content, crude protein, crude fibre, percentage lipids, carbohydrate, ash and calorific value.

2.3.1 Determination of moisture content

Two grammes of the sample were put into the crucibles, dried in an oven at 105 °C overnight. The dried sample was cooled in a desiccator for 30 min and weighed to a constant weight. The percentage loss in weight was expressed as percentage moisture content on dry weight basis [16]. This was repeated three times to obtain triplicate values.



Fig. 1. Cultivated Pleurotus ostreatus mushrooms from M'Badon

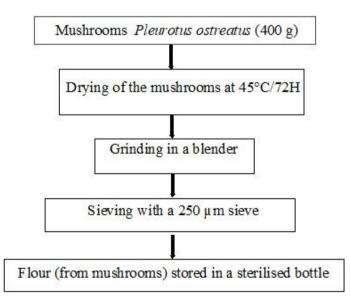


Fig. 2. Flow diagram of the process for producing mushrooms Pleurotus ostreatus flour

2.3.2 Determination of ash content

From the dried and ground sample, 3.00 g was taken in triplicates and placed in pre-weighed crucibles and ashed in a muffle furnace at 550 ± 15 °C for 24h. The hot crucibles were removed and cooled in a desiccator and weighed. The percentage residual weight was expressed as ash content [16].

2.3.3 Crude lipid content determination

From the pulverized sample, 3.00 g was used for determining the crude lipid by extracting the lipid from it for 5 h with (60 to 80°C) petroleum ether in a soxhlet extractor [16]. Triplicate samples were extracted to obtain triplicate values that were later averaged.

2.3.4 Protein determination

Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed in triplicate into a filter paper and put into a Kjedahl flask, 8 to 10 cm³ of concentrated H₂SO₄ were added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with about 10 cm³ of 40 % NaOH solution. The condenser tip was dipped into a conical flask containing 5 cm³ of 4 % boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flaskwith 0.01 M HCl until the solution turned red [16]. The crude protein was calculated by multiplying the estimated nitrogen by 6.25.

2.3.5 Determination of crude fibre

From the pounded sample, 3.00 g were used in triplicates for estimating the crude fibre by acid and alkaline digestion methods using 20 % H_2SO_4 and 20 % NaOH solutions [16].

2.3.6 Carbohydrate determination

The carbohydrate content was calculated using the following formula: Available carbohydrate (%), = 100 - [protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Crude Fat (%)] [17].

2.3.7 Caloric value

The caloric value was calculated in kilocalories per 100 g (kcal/100 g) by multiplying the crude fat, protein and carbohydrate values by Atwater factors of 9, 4 and 4 respectively.

2.3.8 pH measurement

The pH values of the samples were determined by suspending 10 % W/V of the sample in distilled water in each case. It was then thoroughly mixed in a 100 cm³ beaker, stirred and the pH was taken. This was repeated three times and the average calculated [18].

2.4 Microbiological Analyses (Enumeration of Microorganisms)

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were carried out according to Coulin et al. [19]. For all determinations, 10 ml of the sample were homogenized in a stomacher with 90 ml of sterile diluent containing 0.85% NaCl and 0.1% peptone (Difco, Becton Dickinson, Sparks, MD, USA). Tenfold serial dilutions of stomacher fluid, ranging from 10¹ to 107, were prepared and spread-plated for the determination of microbial counts. So. enumeration of coliforms was carried out using VRBL (Violet crystal and neutral Red Bile Lactose) plates containing agar (VRBL agar. Oxoid Ltd., Basingstore, UK), incubated for 24 h at 30 °C for total coliforms and 44 °C for fecal coliforms. Yeasts and moulds were enumerated on Sabouraud chloramphenicol agar (Fluka, Biochemica 89579, Sigma-Aldrich Chimie GmbH, India) incubated at 30 °C for 4 days. Highlighting Salmonella sp is done in three (Pre-enrichment, enrichment stages and isolation) according to the reference standard NF/ISO 6579:2002 Amd 1: 2007. Preenrichment is performed in media Buffered Peptone Water (BPW) by incubating the stock solution at 37°C for 24 h. Samonella was tested on Hektoen agar after the pre-enrichment and enrichment phases. The results of the counts are given by the following formula (ISO n°7218 of May 1996):

$$N = \frac{\Sigma a}{V(n1 + 0, 1n2) x d}$$

N: Numbers of colonies ∑a: The sum of the Ufc in two dilutions V: Volume of seeded inoculum n1: number of first dilution boxes d: dilution factor corresponds to the 1st dilution n2: number of second dilution boxes n: number of box

2.5 Statistical Analysis

All measurements were performed in triplicate. Statistical analyzes of the data were performed

using STATISTICA 7 software (Statsoft Inc, Tulsa-USA Headquarters). This software was also used to calculate mean values and standard deviations of the trials.

3. RESULTS AND DISCUSSION

3.1 Biochemical Properties

Table 1 shows the results of proximate composition of Pleurotus ostreatus mushroom. The result revealed that the moisture content in the sample was 6.40 ± 1.13 %. The low moisture content as saw in the sample is an evidence that this specimen may not be more inclined to decay, since nourishments with high dampness substance are more inclined to perishability [20]. According to Soro et al. [21] a low moisture content of less than 12 % would allow a better conservation of flours. Therefore, it might be profitable in perspective of the specimen timeframe of realistic usability. Indeed, according to Siddiqui and Chowdhury [22] moisture is an important parameter that significantly affects the shelf life and and development of microbial contaminants in flour. This outcome is not shocking in perspective of the way that the advertisers of the item assert to the truth, that it can be put away for a long period.

The ash content of the sample was 11.00 ± 1.33 %. This result suggest that the sample have high ash content when compare to 7.0 - 8.0 \pm 0.0 % from Pleurotus geesteranus reported by Oka et al. [23]. This result is approximately close to 12.36 ± 0.14 from Termitomyces letestui, a wild edible mushroom reported by Kouamé et al. [3]. The result of the ash content in the sample is a suggestion of a high deposit of mineral elements in the samples compare to the recommended values by the FAO. This may indicate that Pleurotus ostreatus mushroom would likely contain very high qualities essential minerals. These results are aggreed with those reported by Chapon et al. [24]. Indeed, according to these authors, mushrooms are highly rich in mineral elements (phosphorus, potassium, magnesium, iron, copper, zinc, iodine, fluorine, cobalt, chromium, chlorine, sulphur and selenium). This would explain the high ash content of the P. ostreatus species and their contribution to health.

Investigations into total carbohydrates $(37.22 \pm 0.61 \% \text{ DW})$ and dietary crude fibre $(24.85 \pm 0.08 \% \text{ DW})$ show that the mushroom *P. ostreatus* is an excellent source of these (Table 1). Similar levels of carbohydrate were observed in

mushrooms usually consumed in the Gbêkê, Bélier and N'zi regions, where they are considered to be excellent energy providers [25]. The total carbohydrate content is evidence that mushrooms in general are also a source of energy. Fibre plays a crucial role in the proper functioning of the intestinal tract. Its consumption is an asset in a healthy and balanced diet.. Thus, Kouamé et al. [26] reported that a high-fibre diet stabilises glycaemic profile of diabetic patients. Thus the fibre contained in *P. ostreatus* could be advised to diabetic patients by decreasing the rate of glucose absorption and/or delaying gastric emptying. It could also contribute to the prevention of colon cancer by binding to the cancer-causing chemicals, driving them away from the cells lining the colon [27]. In addition, dietary fiber serves as a useful tool in the control of oxidative processes in food products and as functional food ingredient [28]. Fibre content of foods helps in digestion process and prevention of cancer [29; 30] Crude fiber also decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes [31]. The values of crude fibre in this study, remain high compared to Psathyrella atroumbonata (12.6 g/100 g), Pleurotus tuber-regium (15.6 g/100 g) from Nigeria [32] and to some mushrooms of the genus Pleurotus cultivated in Bangladesh [33] such as P. sajor-caju (22.87 g/100 g) and P. florida (23.29 g/100 g).

The species Pleurotus ostreatus has a low reducing sugar content (1.04 \pm 0.07 % DW). This result is much lower than that reported by Oka et [23] on different crops of Pleurotus al. geesteranus mushroom (114.4 - 199.9 mg/100g DW). But, it is slightly higher than those reported by Kouamé et al. [3] on wild edible mushrooms Psathyrella tuberculata (0.48 ± 0.04 % DW) and Termitomyces letestui (0.85 ± 0.13 % DW). This difference may be due to the phenomenon of diffusion during drying and the loss of reducing sugars. It may also be due to the different substrates on which the mushrooms were harvested. Indeed, the nature of the soil and the cultivation conditions can influence the physicochemical composition of a given food.

The results of the analyses showed that *Pleurotus ostreatus* flour has a low fat content (4.16 \pm 0.13 %). This content is similar to those reported by Kouamé et al. [3] on the wild edible mushrooms *Volvariella volvacea* (3.90 \pm 0.21 % DW) and *Termitomyces letestui* (4.91 \pm 0.11 %

DW). But, it is higher than those reported by Oka et al. [23] on different crops of *P. geesteranus* which values varied from 1.2 to 2.1 % DW. Indeed, its low fat content can rebalance or complement the overly rich menus used in rich menus used in the diet of people who are advised against eating fatty foods, or can be included in low-calorie diets [24]. The oyster mushrooms would therefore reduce the risks associated with cardiovascular disease. The introduction of mushroom into the consumption habits of consumption habits of the population should thus contribute to weight control and prevent the risk of obesity.

Table 1. Biochemical characteristics of Pleurotus ostreatus mushroom

| Composition | Values (% DW) |
|---------------------|------------------|
| Dry matter | 93.60 ± 3.71 |
| Moisture | 6.40 ± 1.13 |
| Ash | 11.00 ± 1.33 |
| Total sugar | 6.50 ± 0.40 |
| Carbohydrates | 37.22 ± 0.61 |
| Crude Fat | 4.16 ± 0.13 |
| Crude fibre | 24.85 ± 0.08 |
| Reducing sugar | 1.04 ± 0.07 |
| Crude Protein | 16.37 ± 1.60 |
| Energy Value (Kcal) | 251.80 ± 2.75 |
| pH | 6.72 ± 0.35 |

The values are means of triplicate determinations ± standard deviations (SD).

Concerning proteins content, this study indicated that the analysed flour is rich in protein (16.37 \pm 1.60 %). The high protein content of *Pleurotus ostreatus* is therefore comparable to that of milk, soybean and beans [34]. However, its protein content is lower than that of poultry co-product meal (61.54 %) according to the work of Ouédraogo et al. [35]. It is therefore an important source of protein for human consumption on the one hand and for animal feed. Therefore *P. ostreatus* mushroom remains a good source of protein. This fungus could thus play a major role in the supply of protein, especially since it can be available through the promotion of a mass [36]. By comparing the protein contents to those of the

mushroom species in the Haut-Sassandra region in Côte d'Ivoire such as *Volvariella volvacea* (15.73 g/100 g) and *Psathyrella tuberculata* (15.95 g/100 g), higher values can be observed [3].

3.2 Microbiological Analyses

The results of the microbiological analyses (Table 2) showed a high level of contamination of non-pathogenic spoilage germs. The microbial loads of spoilage germs are respectively (137.10⁶ cfu/g) of aerobic mesophilic germs, (16.10² cfu/g) of yeasts and moulds and (75.10² cfu/g) of faecal coliforms. These microbial loads do not comply with the microbiological criteria for edible fungi. These microbiological criteria for mushrooms are 5.10⁵ cfu/g for mesophilic aerobic germs, 10 cfu/g for faecal coliforms and 10³ cfu/g for yeasts and moulds. This high contamination of these microorganisms in the flour could be due to a bad post-harvest treatment, to a bad storage condition of the mushrooms. In fact, in the harvesting areas, mainly rural areas, the mushroom is sold at the roadside and packed in jute bags that have been used several times before. This method of storage is thought to increase the rapid growth of these microorganisms [22]. However, there is a complete absence of Salmonella and Escherichia coli in Pleurotus ostreatus mushroom flour, which are two bacteria that are dangerous by their mere presence in the food because they are pathogenic. This result could reflect the respect practices good of hvaienic durina flour production. It is in good agreement with the standards of the Official Journal, which indicates the total absence of these germs [37]. The flour obtained in this study is of acceptable microbiological quality. In summary, in the presence of micro-organisms indicative of general hygiene and in the absence of identified potentially pathogenic germs, it can be concluded that the consumption of this P. ostreatus mushroom flour does not present a danger to health. These results are consistent with some studies [38; 39; 40].

Table 2. Assessment of bacterial, mould and yeast load of Pleurotus ostreatus mushroom

| Germs wanted | | | | | | |
|----------------------------|---------------------------|--------------------------|--------------|---------|---------|--|
| | AMG | YM | FC | SAL | E. coli | |
| Charges average | 137.10 ⁶ cfu/g | 16.10 ² cfu/g | 75.102 cfu/g | Absence | 0 | |
| Compliance to the criteria | *NC | NC | NC | С | С | |

*NC: Non Conforming, C: Conforming, AMG: Aerobic Mesophilic Germs, YM: Yeasts and Moulds, FC: Faecal Coliforms, SAL: Salmonella, E. coli: Escherichia coli

4. CONCLUSION

The fruiting body of the *P. ostreatus* mushroom is a good source of protein. This body is also rich in carbohydrates (dietary fibre), with a low fat content. The fruiting bodies of this oyster mushroom can be eaten regularly as a protein supplement. The high fibre content with a high fraction of physiological and functional properties could be a potentially beneficial health nutrient, especially beneficial against certain metabolic metabolic diseases such as diabetes. This fungus would allow a good functioning of the immune system and the good development of bones It also presents satisfactory microbiological criteria, given the absence of Salmonella and Escherichia coli in the flour.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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