Nutritional characterization of *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. produced using paper scraps as substrate

Ângela Fernandes [a,b], Lillian Barros [a], Anabela Martins [a], Paulo Herbert [c], Isabel C.F.R. Ferreira [a,*]

[a]Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

[b]REQUIMTE/ Depto. de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal.

[c]*Lidergraf - Artes Gráficas, S.A., Travessa N 1 do Galhano 15, 4480-089 Vila do Conde, Portugal*

*Author to whom correspondence should be addressed (I.C.F.R. Ferreira: iferreira@ipb.pt, tel. +351-273303219, fax +351-273325405).

ABSTRACT

Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm. is the third most produced edible mushroom worldwide due to its ability to colonize and degrade a large variety of lignocellulosic substrates. Therefore, the objective of this work was to evaluate the chemical composition of fruiting bodies of *P. ostreatus* grown in blank and printed paper substrates, in comparison with samples grown in oat straw (control). The nutritional properties of the control sample were similar to the ones reported in literature. Nevertheless, the chemical composition of the samples obtained using paper scraps, either blank or printed, was highly satisfactory. The results obtained validated the nutritional characteristics of the samples, highlighting a profit alternative to re-use paper.

Keywords*:* Mushrooms production; *Pleurotus ostreatus*; Blank and printed paper; Oat straw; Chemical/Nutritional composition

1. Introduction

Research about the production of edible mushrooms has been focused on the development of technologies able to reduce production costs, leading to lower prices to the consumer, and thus stimulating mushrooms consumption (Cardoso, Demenjour & Paz, 2013).

The mushrooms of the genus *Pleurotus* occupy the third position in the production of edible mushrooms, behind the species of the genus *Agaricus* and *Lentinula* (Cardoso et al., 2013). *Pleurotus* spp. are found in tropical and subtropical rainforests around the world, and can be artificially cultivated (Bonatti, 2004) due to their ability to colonize and degrade a wide variety of substrates containing cellulose, hemicellulose and lignin, using them in their own development (Pokhrel, Kalyan, Budathoki, & Yadav, 2013). Furthermore, these species have a quick mycelium growth and fruiting and a low cost of culture, being slightly affected by diseases, and requiring minimal monitoring of the cultivation environment due to an easy adaptation and maintenance (Bonatti, 2004; Ramos, Sapata, Ferreira, Andrada, & Candeias, 2011; Pokhrel et al., 2013). Therefore, and also due to nutritional and functional characteristics, *Pleurotus* spp. are considered increasingly popular in a commercial point of view.

In general, the production of mushrooms may be divided into several stages: composting and filling, sterilization, inoculation, incubation, fruiting and harvest (Loss, 2009). Contrary to other mushrooms (*e.g.*, *Agaricus bisporus* (J.E.Lange) Emil J. Imbach and *Lentinula edodes* (Berk.) Pegler), *Pleurotus* genus does not require a composting substrate (Fan, Soccol, & Pandey, 2000), due to the presence of a powerful enzyme complex (with cellulases, hemicelulases, ligninases, peroxidases, laccases, proteases, among other enzymes) (Rajarathnam, Shashireka, & Bano, 1992). *Pleurotus* spp. only require crushed materials in order to acquire the desired texture for a good mycelial colonization.

The production of *Pleurotus* spp. has been tested using different substrates, *e.g.*, cotton waste textile (Chang, Lau, & Cho, 1981), rice straw (Mehta, Gupta, & Kaushal, 1990) by-products of corn chain (Loss, 2009), bark of coffee (Dias, Koshikumo, Schwan, & Silva, 2003), wheat straw (Ramos et al., 2011) and sugarcane (Cardoso et al., 2013).

The adaptation of this genus to new wastes represents one of the main methods for bioconversion of agro-industrial waste into edible products with high nutritional value (Cohen, Persky, & Hadar, 2002; Mandeel, Al-Laith, & Mohamed, 2005).

The recycle of different materials is one of the most important contributions of fungi in nature (Sanchez, Ysunza, Beltran-Gracia, & Esqueda, 2002). Therefore, the objective of this study was to produce *P. ostreatus* using paper scraps, either blank or printed, and validate its nutritional and chemical composition, by comparison with a common substrate (oat straw- control).

2. Material and Methods

2.1. Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, tocopherol, organic acid and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Samples

The mycelium of the fungus (*Pleurotus ostreatus*) was a commercial strain (Biowin) purchased from BioInvitro (Portugal) and further grown on oat seeds. The substrates used were: oat straw (control), blank paper scraps and printed paper scraps. All substrates were used without any addition (pure).

Each one of the substrates (500 g) was poured in bags of PEHD (High density polyethylene; Deltalab - Spain) and autoclaved in a pressure cooker at about 120 °C for 30 minutes with a little of water in the pan. The bags were kept open during the process. Afterwards, the bags were transferred to an improvised flow chamber at 25 °C.

Each bag was inoculated with approximately 2% of the fungus and not hermetically closed in order to allow gas exchange, being further incubated at 23 °C for 20 days. After the incubation period, holes (4 per bag) of about 3 cm diameter were performed in the bag sides, which were then placed in a room with indirect lighting and a relative humidity of about 70 %. The average temperature during the fruiting process was about 22 °C. After the first fruiting, the bags were kept open until the appearance of new *Primordia*. Only the first two flushes were considered in the characterization of the production of each substrate (for each sample, a mixture of the two flushes was used in the chemical analyses).

Prior to analysis, all the samples (~150 g each one) were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas City, MO, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenate samples.

2.3. Chemical composition

2.3.1. Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a

known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × ($g_{protein}$ + $g_{carbohydrate}$) + 9 × (g_{fat}).

2.3.2. Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) following the extraction procedure described by the authors (Reis et al., 2011; Barreira et al., 2012), using raffinose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

2.3.3. Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by the authors (Reis et al., 2011; Barreira et al., 2012). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID at 260 ºC and a

Macherey-Nagel (Duren, Germany) column (50% cyanopropyl-metyl-50% phenylmethylpolysiloxane, 30 m × 0.32 mm ID × 0.25 µm d*f*). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.3.4. Tocopherols

Tocopherols were determined after an extraction procedure previously described by the authors (Fernandes et al., 2013; Sarmento, Barros, Fernandes, Carvalho, & Ferreira, 2014), using tocol as IS. The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 ºC. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols content was further expressed in µg per 100 g of dry weight (dw).

2.3.5. Organic acids

Organic acids were determined following a procedure previously described by the authors (Barros, Pereira, & Ferreira, 2013; Fernandes et al., 2013). Analysis was performed by ultrafast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Cooperation, Koyoto, Japan). Detection was carried out in a PDA, using 215 nm as preferred wavelength. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

2.4. Statistical analysis

For all the experiments, three samples were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values ± standard deviation (SD). The differences between the different samples were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic. This treatment was carried out using SPSS v. 22.0 program.

3. Results and Discussion

Nutritional values and energetic contributions of *P. ostreatus* cultivated in different substrates are shown in **Table 1**. The samples obtained using paper scraps (blank or printed paper) revealed similar moisture contents, but higher than the control sample. The obtained values are in accordance with literature; Bonatti (2004) reported a moisture value of 88.1 g/100 g for *P. ostreatus* cultivated in banana straw, while Reis, Barros, Martins, & Ferreira (2012) described 89.2 g/100 g in a commercial sample. The sample obtained in printed

paper gave similar contents in fat and carbohydrates in comparison with the control. Nevertheless, the latter showed the highest energetic contribution, due to its highest protein levels. No significant differences were found in protein levels of the samples obtained using printed or blank paper scraps as growth substrates.

Total fat of the studied samples is lower than the values reported in literature: 5.97 and 6.32 g/100 g for *P. ostreatus* cultivated in banana and rice straw, respectively (Bonatti, 2004). Regarding protein contents, the values described in literature are highly variable: 19.9 to 34.7 g/100 g for samples cultivated in wheat straw supplemented with sugar beet (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999); 21 g/100 g in *P. ostreatus* cultivated in wheat straw (Patil, Ahmed, Telang, & Baig, 2010) and 9.62 g/100 g in a sample cultivated in coffee husk (Fan et al., 2000).

The samples obtained using paper scraps, mostly blank paper, revealed higher ash content than the control. The content observed in samples obtained with blank paper was similar to the one reported by Mehta et al. (1990) in a sample cultivated in rice straw (15 g/100 g), while the value of the control sample was similar to the content described by Patil et al. (2010) in a sample obtained using wheat straw (6.35 g/100 g). Manzi et al. (1999) found values ranging from 6.89 to 9.70 g/100 g in *P. ostreatus* cultivated in wheat straw supplemented with sugar beet. The dissimilarity observed in the reported results can be partially explained by the use of different cultivation substrates. According to Mendez, Castro, Casso, & Leal (2005), the nutritional value of mushrooms can be affected by the cultivation in different substrates.

The results obtained for sugars composition are presented in **Table 2**. The control sample and the sample produced in printed paper presented mannitol and trehalose (**Figure 1A**), but the highest levels were found in the first one. The sample obtained using blank paper

presented only trehalose (**Figure 1A**). Reis et al. (2012) also described the same sugars in a commercial sample of *P. ostreatus*.

The results of fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are shown in **Table 3**. The control sample revealed the highest levels of SFA (20.2%) with the main contribution of palmitic acid (C16:0; 11.2%), followed by pentadecanoic acid (C15:0; 2.55%) and stearic acid (C18:0; 2.53%). This sample also showed prevalence of PUFA (69.1%), with linoleic acid (C18:2n6c; 68.1%) as the major fatty acid. The samples obtained using paper scraps showed the highest MUFA levels, mainly due to the contribution of oleic acid (C18:1n9). A similar fatty acids profile was observed among the samples obtained with paper scraps and the control (see for example **Figure 1B**).

Despite some differences in the percentage of individual fatty acids, the global percentages obtained for SFA, MUFA and PUFA are similar to those described in other studies (Reis et al., 2012; Papaspyridi, Sinanoglou, Strati, Katapodis, & Christakopoulos, 2013).

The four vitamers of tocopherols detected in the studied samples are presented in **Table 4**. δ-Tocopherol was only found in control, while β-tocopherol was detected in the samples obtained using blank and printed paper; in the sample produced using blank paper, this isoform was the major compound, and the concentration of total tocopherols was the highest one. The sample cultivated in printed paper gave the highest levels of α- and γ-tocopherols. Reis et al. (2012) described the presence of α-, γ- and δ-tocopherols in a commercial sample of *P. ostreatus*, which is more similar to the profile found for the control sample.

The composition in organic acids is presented in **Table 5**. Citric acid was only detected in the control, which also gave the highest total content of organic acids. The samples cultivated in paper scraps, both blank and printed, revealed a similar profile (**Figure 1C**)

with quinic, oxalic and fumaric acids. The profile of organic acids described by Barros et al. (2013) for commercial *P. ostreatus* was slightly different since the authors detected malic instead of quinic acid.

Regarding future commercial production, it is important to consider the conversion ratio of paper into fresh mushroom. A parameter commonly used to evaluate mushroom yield is biological efficiency (BE), defined as the ratio of weight (g) of fresh mushroom harvested to initial dry weight (kg) of the substrate (Norouzi, Peyvast, & Olfati, 2008) or as the ratio of weight of fresh mushroom harvested (g) to initial dry weight (g) of substrate expressed as a percentage (Pathmashini, Arulnandhy, & Wijeratnam, 2008).

Biological efficiency mainly depends on the characteristics of the substrate and the environmental conditions of the growth process (Aguilar-Rivera, Moran, Lagunes, & Gonzalez, 2012), as well on the number of flushes achieved. In the present study BE was determined for each substrate used (BE = weight fresh mushroom (g)/initial dry weight substrate). The values of total BE (%) obtained for the first two flushes (mean ± SD) for blank paper (10.3±2.5), printed paper (14.9±1.9) and oat straw (16.7±2.8) substrates, although low, are in accordance with those reported by Pathmashini et al. (2008). These authors reported maximum BE of 30.8% and minimum 12.0 % for 5 harvests in sawdust as substrate. Nevertheless, Patil et al. (2010) reported maximum BE of 85.2% and minimum 71.8 % for 3 harvests in different straws as substrates. In the present work, statistically (Tukey test) significant differences ($p<0.05$) between BE values were found between control and blank paper (lower BE). Biological efficiency can be further improved by substrate and culture conditions optimization. Moreover, the production of mushrooms using paper could be faced as a viable alternative (despite being low profiting) for people that do not have other substrates to produce/obtain food.

In conclusion, the control was the sample that showed nutritional properties closer to the ones reported in literature. Nonetheless, the chemical characteristics of the samples obtained using paper scraps were highly satisfactory. In fact, *P. ostreatus* is known for its ability to grow and fructify in different substrates. One of the ways to re-use paper, printed or not printed, is the production of fruiting bodies of the genus *Pleurotus*, taking advantage of the facility that this genus has to degrade lignocellulosic material. Moreover, paper can be used in the commercial production of *P. ostreatus* with significant income since the nutritional and chemical composition of the obtained samples was characterized, showing to be similar to control samples. The present study was focused in the analysis of nutritional molecules, however, further studies should be carried out in order to guarantee that heavy metals or toxic molecules present in printed paper do not appear in the produced mushrooms.

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Table 1. Nutritional value and energetic contribution of *Pleurotus ostreatus* (mean ± SD).

	Control	Blank paper	Printed paper
Moisture (g/100 g)	84.3±0.2[b]	90.3±0.5[a]	91.0±1.5[a]
Fat (g/100 g)	1.53±0.25[a]	1.18±0.01[a]	1.68±0.49[a]
Protein (g/100 g)	14.7±0.4[a]	9.71±0.02[b]	9.29±0.08[b]
Ash (g/100 g)	5.69±0.64[c]	15.9±1.2[a]	10.5±0.8[b]
Carbohydrates	78.1±0.8[a]	73.2±1.2[b]	78.6±0.3[a]
Energetic value (Kcal/100 g)	385±4[a]	342±5[c]	367±6[b]

Results are expressed in a dry weight basis, except moisture that is expressed in fresh weigh. In each row different letters mean significant differences ($p<0.05$).

Table 2. Composition of *Pleurotus ostreatus* in free sugars (mean ± SD).

	Control	Blank paper	Printed paper
Mannitol	2.73±0.01[a]	nd	0.36±0.01[b]
Trealose	23.5±0.5[a]	17.6±0.6[b]	9.09±0.01[c]
Total (g/100 g)	26.2±0.5[a]	17.6±0.6[b]	9.45±0.01[c]

Results are expressed in a dry weight basis. nd - not detected. In each row different letters mean significant differences (p<0.05).

Table 3. Composition of *Pleurotus ostreatus* in fatty acids (mean ± SD).

	Control	Blank paper	Printed paper
C6:0	0.06±0.01	0.05±0.00	0.04±0.00
C8:0	0.12±0.03	0.17±0.01	0.04±0.00
C10:0	0.10±0.02	0.16±0.01	0.03±0.00
C12:0	0.28±0.06	0.27±0.01	0.11±0.01
C13:0	0.04±0.01	0.03±0.00	0.02±0.00
C14:0	0.96±0.10	0.75±0.01	0.46±0.01
C14:1	0.06±0.00	0.04±0.00	0.02±0.00
C15:0	2.55±0.11	1.38±0.02	1.49±0.01
C16:0	11.2±0.0	10.5±0.0	10.3±0.0
C16:1	0.44±0.01	0.99±0.02	0.77±0.01
C17:0	0.53±0.03	0.38±0.01	0.26±0.00
C18:0	2.53±0.01	1.95±0.01	1.34±0.01
C18:1n9	9.50±0.04	18.00±0.01	20.78±0.01
C18:2n6	68.1±0.2	61.5±0.2	61.3±0.1
C18:3n3	0.20±0.02	0.16±0.01	0.13±0.01
C20:0	0.14±0.02	0.15±0.01	0.08±0.00
C20:1	0.12±0.00	0.26±0.01	0.11±0.00
C20:2	0.28±0.04	0.21±0.02	0.17±0.01
C20:3n3+C21:0	0.25±0.01	0.26±0.01	0.15±0.02
C20:5n3	0.26±0.01	0.13±0.04	0.16±0.02
C22:0	0.47±0.01	0.52±0.02	0.30±0.02
C22:1n9	0.11±0.01	0.14±0.01	0.09±0.01
C23:0	0.22±0.01	0.14±0.02	0.10±0.01
C24:0	1.00±0.01	0.91±0.05	0.66±0.03
C24:1	0.51±0.01	0.94±0.01	1.06±0.03
SFA (percent)	20.2 ± 0.2^{a}	17.3 ± 0.1^{b}	15.3 ± 0.1^{c}
MUFA (percent)	10.8 ± 0.1^{b}	20.4 ± 0.1^{a}	22.8 ± 0.1^{a}
PUFA (percent)	69.1 ± 0.2^{a}	62.3 ± 0.1^{b}	61.9 ± 0.1^{b}

C- carbon atoms; SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids.

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Tridecanoic acid (C13:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1); cis-11,14-Eicosadienoic acid (C20:2); cis-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); Eicosapentaenoic acid (C20:5n3); Erucic acid (C22:1n9). Behenic acid (C22:0); Tricosanoic acid (C23:0); Lignoceric acid (C24:0); Nervonic acid (C24:1). In each row different letters mean significant differences ($p<0.05$).

Table 4. Composition of *Pleurotus ostreatus* in tocopherols (mean ± SD)

	Control	Blank paper	Printed paper
α-tocopherol	2.44±0.49^{c}	4.85±0.64^{b}	6.19±0.29^{a}
β-tocopherol	nd	81.0±2.0^{a}	36.2±1.0^{b}
γ-tocopherol	14.9±0.1^{c}	28.5±2.1^{b}	37.7±1.2^{a}
δ-tocopherol	25.0±2.7	nd	nd
Total (μg/100g)	42.4±3.3^{c}	114±3^{a}	80.1±0.5^{b}

nd - not detected. In each row different letters mean significant differences (p<0.05).

Table 5. Composition of *Pleurotus ostreatus* in organic acids (mean ± SD).

	Control	Blank paper	Printed paper
Oxalic acid	0.30±0.04[b]	0.17±0.03[c]	0.48±0.06[a]
Quinic acid	0.10±0.01[c]	1.38±0.01[a]	0.94±0.04[b]
Citric acid	2.25±0.02	nd	nd
Fumaric acid	0.25±0.01[b]	0.32±0.02[a]	0.30±0.01[a]
Total (g/100 g)	2.89±0.08[a]	1.86±0.00[b]	1.71±0.10[b]

Results are expressed in a dry weight basis. nd - not detected. In each row different letters mean significant differences (p<0.05).