

Basidiomycetes Mushroom Biotechnology for the Development of Functional Products: The Effect of Drying Processes on Biological Activity

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Abstract: *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* mushrooms were cultured in a 14 L bioreactor using low-cost, non-conventional culture media. To produce useful raw material for the development of functional products, the biomass produced in the bioreactors was subjected to the following drying processes: convection, infrared radiation, spray drying and freeze-drying. The highest antioxidant activity (ORAC values) and HMGCoA reductase inhibitory capacity were obtained with *G. lucidum*, (ORAC value of 10,594±128.1 and HMGCoA reductase inhibitory capacity of 78.22±0.01), followed by *G. frondosa* (ORAC value of 6,919±231.6 and HMGCoA reductase inhibitory capacity of 71.45±1.48) when the biomass was processed via freeze-drying.

Keywords: Antioxidant capacity, biomass, forced convection, freeze-drying, HMGCoA Reductase, infrared radiation, spray drying.

INTRODUCTION

Several basidiomycete mushrooms have been used for decades in traditional oriental medicine, and their consumption is widespread in many Asian countries, Europe and the United States [1, 2]. Many of these mushrooms are considered to be medicinal because they contain a rich content of bioactive compounds [3-5]. *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* are mushroom species that are widely recognized throughout the world due to their medicinal properties, which stem from the presence of compounds including polysaccharide-protein complexes, intra and exopolysaccharides (IPS and EPS), phenolic compounds, proteins (fungal immunomodulating proteins (FIPs), lectins, glycoproteins, non-glycosylated proteins and peptides), lipid components (ergosterol), fatty acids and terpenoids. These compounds promote a wide range of biological activities such as antitumor/anticancer, antimicrobial and antifungal, immunomodulatory, antiatherogenic, antioxidant capacity and hypocholesterolemic effects [3, 4, 6].

The possible mechanisms underlying the antioxidant activity of edible mushroom extracts include the scavenging of free radicals generated during lipid peroxidation (e.g., by peroxy radicals through their hydrogen-donating capacity, which is generally correlated with higher amounts of total phenolics) [7]. Ergothioneine, a specific antioxidant, has

been identified and quantified in various species, including *Lentinus edodes*, *Pleurotus ostreatus*, *G. frondosa* and *A. blazei*, and this metabolite is a cellular protector against oxidative damage [8]. The ORAC (Oxygen Radical Absorbance Capacity) unit, also known as the ORAC value, is a method of measuring the antioxidant capacity of different foods and supplements. The ORAC value was developed by scientists at the National Institutes of Health. Despite the fact that the exact relationship between the ORAC value of a food and its health benefit has not been established, it is believed that foods with higher ORAC values are more effective at neutralizing free radicals. According to the free-radical theory of aging, neutralizing free radicals will slow the oxidative processes and inhibit free radical damage that may contribute to age-related degeneration and disease (USDA National Nutrient Database for Standard Reference) [9-11].

In addition, mushrooms have several compounds that are capable of inhibiting the key enzymes involved in cholesterol metabolism. One such example is lovastatin, a compound that lowers cholesterol levels by inhibiting 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCoAR) [8-10]. Additionally, research on *P. ostreatus* and *L. edodes* has suggested that their inhibitory activity might be due to proteoglycans and demonstrated that HMGCoAR inhibitory capacity was dependent on the commercial variety, the cultivation parameters and the developmental stage of the mushrooms [8-10].

Traditionally, mushroom culture has emphasized fruiting body production. The basidiocarps are dried by conventional processes [forced convection] and processed by grinding and

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pulverizing the dry biomass to obtain final the product [11]. However, in recent years, special attention has been directed toward finding the optimal conditions for submerged culture to enable the efficient production of mycelial biomass and bioactive compounds using both flasks and bioreactors [12-16].

Technological advances in the food and biotechnology fields, specifically in the area of submerged culture conditions, have facilitated new developments aimed at designing products and transforming existing products, minimizing production costs and giving the final product a greater added value with regard to both nutrition and health [3, 11, 17, 18]. However, it is necessary to evaluate the entire production process, especially for edible and medicinal mushrooms, and the cultivation process must not only ensure the uniform production of biomass and its active metabolites under standardized processes but also guarantee the stability and quality of the obtained biomass. The latter consideration is indispensable to evaluate changes that occur in the biomass after drying by different systems, and it is critical to determine how the drying process directly affects biological activity. This consideration is important because it is common to observe changes in the organoleptic characteristics of biomass, such as odor, taste and texture that are completely different under different processing methods. Color can also vary depending on the drying method.

The purpose of this work was to evaluate the biological activity, in terms of antioxidant capability [ORAC values] and HMGC_oAR inhibitory capacity, of the biomass of *Ganoderma lucidum*, *Grifola frondosa* and *Agaricus blazei* produced in a 14 L bioreactor after different drying processes: forced convection, infrared radiation, spray drying and freeze-drying. The goal of this study was to determine the most appropriate system for the maintenance of functional properties of the biomass and one that could be used for different products in the near future. Thus, this report addresses the issue of how the different drying processes affect the bioactivity of the functional raw material obtained from mushroom species.

MATERIALS AND METHODS

Strain Maintenance and Preinoculum Preparation

G. lucidum (GL), *G. frondosa* (GF) and *A. blazei* (AB) strains were provided by the Plant Biotechnology Research Group of the Universidad de Antioquia. The strains were maintained in a solid medium containing MGL1 with the following components (g L⁻¹): Barley flour, 30; yeast extract, 3; saccharose, 5.3; and agar, 8; with a pH of 5.5±0.1. The strains were incubated at 24°C in darkness and stored at 4°C [19]. Mycelial agar disks 1 cm in diameter were removed from the margin of an actively growing colony, and six pieces were inoculated in 1000 mL flasks containing 450 mL of MGL1 liquid media consisting of the following components [g l⁻¹]: Barley flour - 50, NaNO₃ - 0.08, MgSO₄·7H₂O - 0.02, KCl - 0.01 and KH₂PO₄ - 0.03; pH 5.5±0.1. Cultures were maintained at 100 rpm, 24±1°C and 40 micromoles m⁻²s⁻¹ for nine days [19].

14 L Bioreactor Culture

The fermentation process for the three species was carried out in a 14 L bioreactor with 9 L of working volume

in MGL1 liquid modified culture medium (with 20 g L⁻¹ of barley flour and 0.1% (v/v) olive oil as anti-foam agents). The culture medium was inoculated with 900 mL of preinoculum through a peristaltic bomb. The culture conditions were as follows: 150 rpm of agitation, aeration rate of 6 vvm, temperature of 30°C and a pH of 5.5±0.1. These conditions were maintained for a period of 3, 4 and 15 days for *G. lucidum*, *G. frondosa* and *A. blazei*, respectively.

Drying Methods

After cultivation, the biomass of each fungus was homogenized in an ULTRA-TURRAX disperser (IKA® T18 Basic) at 25000 rpm for 10 min and subjected to four different drying treatments.

Infrared (ID) and Convection Drying (CD)

One liter of homogenized biomass was separated by centrifugation at 9000 rpm for 10 min, and the different samples were dried at 70°C in an infrared or convection oven until a constant weight was obtained. The dry biomass from both processes was grounded using a friction grinder, packed in plastic bags, sealed and stored at 25±2°C for subsequent trials.

Spray Drying (SD)

One liter of homogenized product was diluted in distilled water to a final concentration of 50% (p/v) to decrease the amount of suspended solids and to avoid injector blockage. The drying agent used was maltodextrin (6.25%), and the biomass feeding solution was carried to a spray dryer (Buchi® B-290) at an inlet temperature of 150°C, outlet temperature of 70-90°C, 100% aspiration rate, 35-45% pump, two-nozzle cleaner and 50 L/h air flow rate.

Freeze-drying (FD)

One liter of homogenized product was frozen in a cooling bath at -20°C. Then, the biomass was carried to the freeze-dryer (Eyela® 1100) with a temperature of -50°C and 10 Pa of pressure.

Extraction and Quantification of IPS

For IPS extraction, 1 g of dried mycelial biomass from each drying method was collected. The polysaccharides were extracted in boiling water for 20 min, and the hot solution was filtered with Ø 125 mm filter paper (Whatman®). Then, the IPS precipitate was made by the addition of four volumes of 96% ethanol at 4°C for 1 h followed by centrifugation at 4000 rpm for 20 min. The supernatant was discarded, and the pellet was diluted in 5 mL of distilled water [20]. For quantification, we used the colorimetric method of phenol-sulfuric acid, as described by Dubois *et al.* (1956) and modified by Masuko *et al.* (2005) using a glucose standard curve. The samples were measured in a microplate format [21-25].

BIOLOGICAL ACTIVITY

Oxygen-radical Absorbance Capacity Assay (ORAC_{FL})

The antioxidant activity of the biomass from each mushroom was measured using the ORAC assay [9-11]. Dry biomass obtained from each of the different drying methods was diluted in a 10 mM phosphate buffer at pH 7.4 to obtain

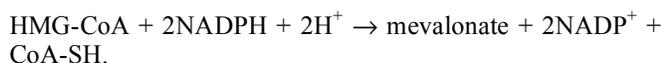
Table 1. Biomass and IPS Under 14 L Bioreactor Conditions after Freeze-Drying

Species	Biomass (g/L)	Productivity (g biomass /day)	IPS (mg/mL)	Productivity (g IPS/day)
<i>G. lucidum</i>	19.72±1.06	6.57	13.29±2.29	4.43
<i>G. frondosa</i>	24.49±0.44	8.16	12.28±1.87	4.09
<i>A. blazei</i>	23.48±1.31	1.56	42.56±3.46	2.84

a final concentration of 1 µg/mL. Trolox (0–200 µM) was used as the standard. A mixture of the fluorescent probe, FL (150 µL of a 1 µM solution), and sample solution (25 µL of a 1/10 dilution) was preincubated for 30 min at 37°C. Then, 25 µL of 250 mM AAPH solution in phosphate buffer was added. Fluorescence intensity was measured every 2 min for 120 min with excitation and emission wavelengths of 485 and 520 nm, respectively. The results are reported as micromoles of Trolox equivalents per 100 g of biomass (µmol TE/100 g of biomass) [9-11].

Determination of HMGC_oA Inhibitor Activity

Mushroom powders from each drying method (3 g) were mixed with 20 mL of 96% ethanol. Samples were subjected to ultrasound for 2 h at 30% amplitude and 20 kHz of operating frequency (Misonix® S4000-010), and the cycle was repeated. Subsequently, the samples were shaken in a vortex for 30 s and filtered with Ø 125 mm filter paper (Whatman®). Extracts were subsequently concentrated by evaporation under partial vacuum, and the residue was resuspended in PBS buffer (pH 7.4) to obtain a final concentration of 3 µg/µL. These solutions were used as the source of HMGC_oAR inhibitors. HMGC_oAR activity was measured using the commercial HMG-CoA Reductase Assay (Sigma® CS1090) according to the user's manual. This assay is based on the spectrophotometric measurement of the decrease in absorbance at 340 nm at 37°C, which represents the oxidation of NADPH by the catalytic subunit of HMGC_oAR in the presence of HMG-CoA according to the following reaction:



Mushroom solutions (1 µL) were applied into a 96-well plate, and the absorbance change was read every 20 s for up to 10 min using a microplate reader (BioTek Powerwave 340®). Pravastatin was used as a positive control for inhibition. This control was considered as exhibiting 100% inhibitory activity, and tested samples were measured for their percentage of activity inhibition. All assays were performed in triplicate.

RESULTS

14 L Bioreactor Culture and IPS Content

This work aimed to study the performance of each of the mushroom when cultured in a stirred tank reactor. To obtain optimal cell growth, the concentration of the carbon source was lowered from 50 g/L in the flask culture [23-27] to 20 g/L in the bioreactor because we found that an important remnant of the cereal flour was not used by the fungus at the

end of the culture time, indicating that the carbon source was added in excessive amounts. Additionally, when the cultures were maintained in flasks and/or the bioreactor, they required between 5 and 20 days for cultivation, depending on the species [14, 26-28]. However, in our bioreactor operating conditions and the culture medium established, *G. lucidum* and *G. frondosa* reached their maximum biomass and IPS production at 3 days, whereas *A. blazei* reached its maximum value at 15 days. The biomass and IPS were obtained by freeze-drying because there was no loss during the drying process, as discussed below (Table 1).

Biological Activities

Oxygen-radical Absorbance Capacity Assay (ORAC_{FL})

The effect of the drying method on biomass antioxidant activity was evaluated using ORAC_{FL}. ANOVA revealed that each of the factors evaluated and their interactions significantly affected antioxidant activity (p<0.05).

G. lucidum (10,594±128.1) had the highest ORAC values among the mushrooms tested, followed by *G. frondosa* (6,919±231.6) and *A. blazei* (4,101±150.5) (Table 2). These results were compared with reference values from the USDA National Nutrient Database for Standard Reference, which is a comprehensive database of foods and their antioxidant levels expressed as ORAC values.

Determination of HMGC_oA Inhibition

Next, we tested the influence of each drying process on HMGC_oAR inhibitory activity. ANOVA revealed that each of the factors evaluated and their interactions significantly affected the inhibitory activity of the extracts (p<0.05) (Table 3). All of the mushroom extracts displayed the highest HMGC_oAR inhibitory activity under the freeze-drying process, with percentages of inhibition of 78.22%±0.01% and 71.45%±1.48% for *G. lucidum* and *G. frondosa*, respectively.

DISCUSSION

Currently, there are several established techniques for drying raw materials in the fields of cosmeceuticals and pharmaceutical products, especially in the food sectors. The drying and dewatering processes are of great interest due to increasing quality standards, especially in the production of natural ingredients and additives for different formulations [29].

It is important to note that the culture medium and operating conditions of the bioreactor used in this study are suitable for obtaining biomass and IPS for the three fungi studied. Comparing these values with previous reports, we

Table 2. Antioxidant Activity ORAC of Biomass Obtained by Different Dry Methods Compared with USDA National Nutrient Database for Standard Reference (<http://www.ars.usda.gov/Services/docs.htm?docid=8964>, <http://www.oracvalues.com/>)

Sample	ORAC ($\mu\text{mol ET}/100\text{ g sample}$)	ORAC Values Reference
GLSD	2,279 \pm 38.2	2,294 Cereals ready-to-eat, granola, low-fat, with raisins 2,252 Asparagus, raw
GLFD	10,594\pm128.1	13,541 Nuts, walnuts, English 10,460 Juice, black raspberry
GLCD	6,224 \pm 128.6	6,257 Baby food, fruit, peaches 6,100 Plums, raw
GLID	7,718 \pm 20.58	7,957 Currants, European black, raw 7,675 Nuts, pistachio nuts, raw
GFSD	480.4 \pm 15.13	481 Spices, poppy seed 438 Lettuce, iceberg [includes crisphead types], raw
GFFD	6,919\pm231.6	7,282 Lentils, raw 6,681 Apples, dried to 40% moisture [purchased in Italy]
GFCd	5,413 \pm 27.2	5,708 Garlic, raw 5,409 Soybeans, mature seeds, raw
GFID	5,497 \pm 135.4	5,708 Garlic, raw 5,409 Soybeans, mature seeds, raw
ABSD	1,328 \pm 80.5	1,346 Lemons, raw, without peel 1,326 Potatoes, red, flesh and skin, baked
ABFD	4,101\pm150.5	4,123 Baby food, fruit, applesauce, strained 3,898 Apples, Granny Smith, raw, with skin
ABCD	2,496 \pm 19.25	2,496 Cabbage, red, raw
ABID	946.8 \pm 4.43	951 Mushrooms, brown, Italian or Crimini, raw 943 Pineapple, raw, extra-sweet variety

Table 3. HMGC_oAR Inhibitory Activity of Mushroom Extracts Obtained with Different Drying Processes

Drying Process	<i>G. lucidum</i> activity inhibition of HMGC _o AR (%)	<i>G. frondosa</i> activity inhibition of HMGC _o AR (%)	<i>A. blazei</i> activity inhibition of HMGC _o AR (%)
Spray drying	60.23 \pm 0.03	55.31 \pm 0.01	40.59 \pm 0.55
Freeze-drying	78.22 \pm 0.01	71.45 \pm 1.48	60.90 \pm 0.23
Forced Convection	43.32 \pm 0.01	53.27 \pm 0.06	43.61 \pm 0.13
Infrared Radiation	38.64 \pm 0.32	59.89 \pm 0.25	40.74 \pm 0.03

Table 4. Biomass and IPS Productivity (P) Under 14 L Bioreactor Conditions Using the Freeze-Drying Method Compared with the Maximum P Values Reported by other Authors

Species	P for Biomass	P Reported	Reference	P for IPS	P Reported	Reference
<i>G. lucidum</i>	6.57	1.37	[28]	6.57	0.395	[28]
<i>G. frondosa</i>	8.16	6.20	[33]	8.16	0.325	[32]
<i>A. blazei</i>	1.56	1.54	[19]	1.56	0.12	[34]

achieved higher productivity biomass and IPS for *G. lucidum* and *G. frondosa* in fewer days (three days) [26, 30, 31]. The biomass of *A. blazei* was similar to previous reports, but its IPS value was higher [15, 32] (Table 4). Additionally, the culture media used in our work significantly reduced the cost compared with those reported by other authors [19, 28, 32-35].

Regarding antioxidant activity, our results were consistent with previous reports demonstrating that freeze-drying is generally the best drying method to preserve the nutrient quality and antioxidant activity. This characteristic is likely due to the constant conditions of this process and the fact that the low temperature of the process prevents damage related to the chemical degradation of substances; the damage reported with the other methods is likely caused when the sample is submitted to high temperatures with different exposure times. The samples are subjected to spray drying for just a few seconds, and the process takes 48 h for convection and infrared radiation. These findings suggest that the substances to which antioxidant activity can be attributed are thermo-sensitive. However, the reduction observed with convection and infrared processes was not as marked compared to spray drying, in which the lowest ORAC values were obtained for *G. lucidum* and *G. frondosa* and the second lowest value for *A. blazei*. During the spray-drying process, it is necessary to add a drying agent that increases the final yield value by increasing the weight and volume of the final product. Therefore, it is possible that the amount of the metabolite of interest is at a lower concentration and is masked by the presence of maltodextrin [29].

With regard to HMGCAR activity inhibition, the best results for the three mushrooms were achieved with the freeze-dried biomass, and lower values were obtained using both convection and infrared radiation. Thus, prolonged exposure times have a greater effect on bioactivity, which decreased the yield by 39.58% for *G. lucidum*, 18.18% for *G. frondosa* and 20% for *A. blazei*.

Taken together, these results suggest that freeze-drying is the best drying method because it protects flavor and aroma compounds as well as biologically active compounds. These results were confirmed by Shofian *et al.* [36] and Yang *et al.* [37], who showed that the amounts of phenolic compounds, β -carotene and ascorbic acid were better preserved after freeze-drying compared to other drying processes. The drying process caused dramatic changes in both activities, the antioxidant activity and HMGCAR Reductase inhibitory capacity, which is in agreement with the results obtained by Yang *et al.*, who demonstrated that the lowest activity was observed in hot-air-dried samples. During freeze-drying, ice sublimation causes significant changes in the shape and volume, but the active compounds are preserved [33].

The most popular method of mushroom dehydration is convective drying. However, this method takes a long time at a high temperature, which decreases the quality of the dried product and directly affects its bioactivity. Therefore, it is necessary to optimize the drying process of the raw material and verify its biological activities prior to the development of products that promise health benefits [29, 34].

CONCLUSIONS

The drying of mushroom products can substantially change their biological activities. The highest antioxidant activity (ORAC values) and HMGCAR inhibition values were obtained when applying the freeze-drying method; however, for different industrial purposes, further studies should be conducted to minimize alterations in the chemical composition and/or bioactivity of the samples.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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