Photoacoustic spectroscopy applied to the direct detection of bioactive compounds in *Agaricus brasiliensis* mycelium

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Abstract This paper describes the application of the photoacoustic spectroscopic (PAS) for detection of bioactive compounds in *Agaricus brasiliensis* mycelium. The mycelium was cultivated by solid-state fermentation and by submerged fermentation. Vegetal residues from food industry were used as substrates for fermentation: apple pomace (*Malus domestica*), wheat (*Triticum aestivum*), peel and pomace of pineapple (*Ananas comosus*), malt (*Hordeum vulgare*) and grape pomace (*Vitis vinifera*). Dry and ground samples of biomass were directly put into the PA cell. The optical absorption spectra indicated the existence of three main absorption bands: one around 280 nm related to phytosterols (ergosterol), phenolic acids, flavonoids and aromatic amino acids, another at 340 nm, due to phenolic and flavonoid compounds, and the third one at around 550 nm associated with anthocyanins and anthocyanidins. A correlation between the PA signal and the total phenolic content was satisfactory, as well as for the analyzed spectrum region (270 nm up to 1000 nm), using multivariate methods. Our results indicated that PA technique may be considered as an analytical tool to quickly detect bioactive compounds in mushrooms without the need of sample pretreatment.

Keywords *Agaricus brasiliensis* · Total phenolic content · Photoacoustic spectroscopy · Multivariate analysis

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1 Introduction

In the last decades, several researches have been made in the attempt to obtain biocompatible antioxidant agents, in order to assist in the treatment of chronic diseases [1–3]. In this perspective, mushrooms are widely used due to its high levels of phytosterols, proteins, carbohydrates, phenolic compounds, vitamins and minerals [4]. Among the species of edible mushrooms *Agaricus brasiliensis* is a suitable alternative for human and animal consumption and it is considered as a nutraceutical food due to its nutritional and pharmacological properties. Immunostimulant properties, strong antitumor activity, as well as effects against diabetes and heart diseases have been described for this mushroom [5]. However, *A. brasiliensis* employment as an antioxidant agent, for example, as a food preservative or in nutraceutical products, requires the assessment of the presence of phenolic compounds and also their quantification. In this context, photoacoustic spectroscopy (PAS), as a non-conventional technique for analysis of foods, is of advantage because it is a direct, easy and rapid analytical technique which does not need any step of sample extraction or pretreatment. Additionally, PAS does not employ organic solvents and only few grams of in nature samples are directly analyzed, so PAS can be considered as a green analytical technique. It is nondestructive and allows a fast characterization of the thermal and optical properties of the matter. The intensity of the PA signal, in the absence of saturation, is directly proportional to the concentration of absorbing analytes. The application of simpler and direct analytical techniques and methodologies, such as PAS, to determine unknown analytes in complex mixtures is an alternative to existing methods that require several steps of sample pretreatment or the use of chemical reagents [6–8].

Previous researches indicate photoacoustic spectroscopy technique as a tool to provide the total phenolic contents in red sorghum flours, in a fast and efficient way [9]. This technique gives information of the optical absorption processes that occur in the material, being also possible to characterize samples regarding to its atomic or molecular composition. In addition, PA spectra can be analyzed through mathematical techniques such as normalizations, derivatives, deconvolutions in Gaussian kernels, and linear regression models [10, 11]. Among these techniques, the construction of a linear correlation model employing multivariate analysis methods, such as principal components analysis and partial least squares regression, presents the advantage of building a rigid model, to test it with calibration and validation data, and to obtain information in respect of the sample set dissimilarities. Furthermore, it is also possible to predict new samples properties by means of the correlation model [11].

The purpose of this paper was to apply the PA technique to characterize the optical absorption processes of samples of *A. brasiliensis* mycelium grown in different agroindustrial residues. Additionally, the study was aimed at associating the PA absorption spectra with the known phenolic content and finally a linear correlation model was developed by means of multivariate analysis methods.

2 Materials and methods

2.1 Samples

A. brasiliensis was cultivated by solid-state (SSF) and submerged (SF) fermentation, as described in Ref. [12], using agroindustrial vegetal residues as substrates (S): apple pomace

(AP) (*Malus domestica*), wheat (W) (*Triticum aestivum*), pineapple peel (PL), pineapple pomace (PM) (*Ananas comosus*), malt (M) (*Hordeum vulgare*), and grape pomace (G) (*Vitis vinifera*). Afterward, the mycelium biomass and the unfermented substrates were dried and ground and directly analyzed by PAS without any further sample treatment. SSF and SF produced a mycelium biomass which can not be separate from the substrate, and the resulting biomass is composed by mushroom mycelia and substrate particles.

2.2 Total phenolic content

The total phenolic content of the samples was previously assessed by the general Folin–Ciocalteu method [13] and was reported in Ref. [14]. Briefly, the analytical curve was obtained by reacting 300 μL of each standard solution of gallic acid at different concentrations with 500.0 μL of the Folin–Ciocalteu reagent and 500.0 μL of an aqueous saturated solution containing carbonate and sodium tartrate ($\text{pH} = 11.0$). Ultrapure water was added to a final volume of 5.0 ml. After 1 h, the absorbance was read at $\lambda = 760$ nm in a UV–vis spectrophotometer. To determine the total phenolic content in extracts the same procedure was employed, simply replacing the gallic acid solutions by methanolic extracts of nonfermented substrates or the mycelium of *A. brasiliensis* obtained from SF and SSF.

2.3 Photoacoustic spectroscopy analysis

The samples were analyzed by conventional photoacoustic spectroscopy (PAS) technique. The experimental PAS set-up comprised a 1000 W Xenon arc lamp (Oriel-66921), whose emission covers the range from ultraviolet up to near infrared. The wavelength was selected by a monochromator (Oriel-74004) and filtered to eliminate the light diffraction of superior orders. Afterward, the light beam was modulated by a variable frequency mechanical modulator (Stanford-SR540), set at 16 Hz, crossing then a set of lens. The resultant modulated light beam hits the sample placed in the PA cell, closed upward by a quartz window. The incidence of radiation on the sample generates pressure variations in the boundary air layer, being detected by a sensitive acoustic microphone (Brüel & Kjær-2690-0S2). This procedure generates an electrical signal which is sent to a lock-in amplifier (Stanford-SR830), to be cleaned of electrical noises, and amplified. Finally, the lock-in sends the data to a computer to be analyzed and interpreted. In this study, the absorption spectra in the wavelength range of 270 nm up to 1000 nm was investigated. Data acquisition was performed with a step of 1 nm per second at a nominal applied potency of 700 W. A volume of approximately 80 mm³ of each sample was used for the PAS analyses. The PA signal acquisition was performed in triplicate for each sample. The PAS spectra were always normalized to the signal obtained from a charcoal powder.

2.4 Statistical and multivariate analysis

In this study, principal components analysis (PCA) and partial least squares regression (PLS) were applied to the experimental data using Matlab software with PLS Tool Box version 5.8. A (18 $\times$ 731) matrix was used to evaluate the PAS spectra. This matrix was composed by the normalized and mean center PA data, obtained in the spectral region of 270 nm up to 1000 nm. PLS analysis was performed for the best PCA model, applying the cross-validation method of leave-one-out.

3 Results and discussion

3.1 PA absorption spectra

Figure 1 shows the PA absorption spectra of the sample set, in the range of 270 nm up to 1000 nm, displayed in groups of food agroindustrial residues (S) before and after the fermentation process (SSF and SF).

According to Fig. 1, PA signal of all fermented samples increased in relation to the PA signal of unfermented substrate for almost the entire spectral range, regardless of the fermentation process (solid state or submerged). In the case of the fermented samples of pineapple peel (C) and grape (F), this increase was for the whole spectral range, while the other samples exhibited a crossing of the PA signal of fermented sample and substrate around 300 nm. This result is in agreement with our previous report [14] for these samples where the highest content of antioxidant compounds was obtained after fermentation, in extracts of mycelium regardless of the fermentation type or the vegetal substrate. Additionally, in the current study, the fermentation of grape resulted in the highest increase in PA signal intensity of fermented substrates in comparison with the unfermented ones. On the other hand, the most intensive PA signal was produced by fermentation of pineapple peel (Fig. 1c) also in agreement with our previous report [14] where this biomass showed the highest phenolic and flavonoid levels.

In order to verify the behavior of optical absorption of all samples with the same analytical weight, the PA data were normalized by zero up to one according to the expression: $Y'_i = (Y_i - Y_{min}) / (Y_{max} - Y_{min})$. Figure 2 shows the normalized PA absorption spectra in the range of 270 nm up to 1000 nm of all the sample set, also plotting the signal of pure *A. brasiliensis* mycelium for comparison.

The normalized PA signal exhibits clearly the presence of a significant absorption band centered around 550 nm for the unfermented substrate of grape, which is not observed or defined for the other analyzed samples. This band is due to the presence of high levels of anthocyanins and anthocyanidins, which show an absorption band between 465 nm and 550 nm as well as weak absorption between 270 nm and 280 nm, observed in grapes and

Fig. 1 Optical absorption spectra made by PAS of all samples under study

Fig. 2 Normalized PA absorption spectra of the sample set and pure mycelium of *Agaricus brasiliensis* (A)

many species of fruits and flowers [15, 16]. The absorption band around 280 nm, visible for all the studied samples, could be associated mainly with the presence of phytosterols, such as ergosterol [12], phenolic acids (gallic acid), and hydrolyzable tannins (ellagitannins and gallotannins) [17], flavonoids and aromatic amino acids [15, 16]. Additionally, absorption around 340 nm, observed in some samples, may be attributed to flavonoids and phenolic compounds, such as cinnamic acid and related derivatives. For example, p-coumaric acid showed maximum UV-absorption at 230 nm and 310 nm whereas caffeic and caffeoylquinic acids exhibit strong absorption at 230 and 330 nm. Most flavonoids show at least two absorption bands, one ranging from 240 nm to 280 nm and another from 300 nm to 400 nm [18].

The correlation between the PA absorption spectra of the samples and their total phenolic content was investigated following the procedure described in Ref. [9]. The phenolic content of the samples was linearly associated with its normalized PA signal at 475 nm, as depicted in Fig. 3. This wavelength was chosen in order to compare our results with those reported in Ref. [9]. For that, the samples with total phenolic content up to approximately 40 mg/g were chosen, since for higher concentrations there is not a linear correlation, showing an erratic behavior. In Ref. [9] this correlation has shown to be valid up to around 30 mg/g. According to the linear correlation between the total phenolic content, denoted by (P), and the normalized PA signal amplitude at 475 nm, denoted by (I), displayed in Fig. 3, these variables can be associated by the expression $P[\text{mg/g}] = (-6.3 \pm 2.9) + (52.0 \pm 4.8) * I[\text{a.u.}]$. This linear correlation model presents a correlation coefficient (R) of 0.974, and is valid for the analyzed range of phenolic content from 10 mg/g up to 40 mg/g. The obtained R value is higher than the 0.91 reported in Ref. [9].

3.2 Statistical analysis

Figure 4 shows the principal component analysis (PCA) performed with the normalized PA signal data. This multivariate analysis was made assuming a confidence interval of 95%, employing the cross-validation method of leave-one-out.

Fig. 3 Correlation between the normalized PA amplitude at 475 nm and its total phenolic content (mg/g)

According to the PCA plot, excepting the group of samples derived from grape, there is a clustering tendency in relation to the fermentation type. The distinction of the grape samples from the others is probably because of their high levels of anthocyanins and anthocyanidins, presenting a remarkable absorption band centered at around 550 nm, as shown for the unfermented and fermented substrates of grape in Fig. 1.

The PA signal of the samples whose total phenolic content did not exceed 40 mg/g was also associated with its phenolic content by means of partial least squares (PLS) regression, using the cross-validation model with leave-one-out method, displayed in Fig. 5. The correlation between PA signal data and phenolic content, performed with PLS, was satisfactory

Fig. 4 Principal component analysis scores plot PC1 x PC2 of the normalized PA absorption spectra data of the sample set

Fig. 5 Partial least squares regression of the correlation between the normalized PA absorption spectra data of the sample set with its total phenolic content (mg/g). The dotted green line represents the actual linear fit, and the continuous red line represents the model linear fit

for the analyzed content range of up to approximately 40 mg/g, as well as the investigated PA spectral range, from 270 nm up to 1000 nm. This regression model was built with three latent variables, exhibiting a prediction correlation coefficient (R) of 0.979.

4 Conclusion

The results indicate the PA intensity is increased after both processes of fermentation (SF and SSF), in comparison with unfermented substrates. For all samples, two notable absorption bands were observed, the first one related to ergosterol and aromatic amino acids, around 280 nm, and the second one associated to phenolic and flavonoids content, around 340 nm. The findings express a directly proportional linear correlation between the PA signal at 475 nm and total phenolic content, in the range of 10 mg/g up to 40 mg/g. In addition, the applied multivariate tools demonstrate PAS technique can be employed to evaluate efficiently the total phenolic content of *A. brasiliensis* mycelium growth in peels and pomaces of different foods, as a fast, low cost and efficient method.

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Compliance with Ethical Standards

Conflict of interest Fernando Maia de Oliveira declares that he has no conflict of interest. João Benhur Mokochinski declares that he has no conflict of interest. Yohandra Reyes Torres declares that she has no conflict of interest. Herta Stutz Dalla Santa declares that she has no conflict of interest. Pedro Pablo González Borrero declares that he has no conflict of interest.

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