Characterization of *Agaricus Blazei* by TG/DTA and Solid State NMR

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Abstract: *The Agaricus mushroom species has been well known for its medicinal properties. Research conducted in Japan revealed the presence of a certain amount of beta-glucan, a kind of monosaccharide that has a beneficial effect on the human organism by increasing the number of macrophages. This study evaluated a lineage of Agaricus species, Agaricus Blazei Jun-17 by using Thermal Analysis for the assessement of their chemical and physical properties. Also, Fourier Transform Infrared Spectroscopy was used to detect functional groups and Nuclear Magnetic Resonance to evaluate chemical structure. These three techniques made itpossible to confirm the presence of proteins and polysaccharides.*

Introduction

Many protective substances have been found in specific diets, particularly in mushrooms. *Agaricus Blazei is* a mushroom native to Brazil, and *Agaricus Blazei Jun-17* is a variant of this species. *Jun-17* is a component of the basidiomycete group, which grows very well in hot and wet weather. In Brazil, almost 90% of the production of this mushroom is directed to the international market - mostly to Japan, Canada, the USA, and others. It has been confirmed that *Agaricus* has specific effects on humans, as it develops the immune response through an increase of the number of macrophages. It also has already been shown to act as an anti mutagenic agent on microorganisms due to unsaturated compounds, such as linolenic acid. The advantages of this foodstuff include the improvement of digestive, circulatory, breathing, and reproductive systems.[1] Polysaccharides, proteins, fat, fiber, iron, calcium, and phosphorus may be present as part of its composition. Although this mushroom is native to Brazil, research on the qualitative and quantitative composition of *Agaricus Blazei* Jun-17 is scant both in the national and international This lack of data may be explained by the fact that *Agaricus blazei* Jun-17 is rather sensitive to the purification protocol usually followed in the study of functional foods, in contrast with other mushroom species[2,3]. In this study, our aim is to

characterize the functional groups present in a raw sample of *Agaricus Blazei* Jun-17. The techniques used are the following: Thermogravimetry (TG), Derivative Thermogravimetry (DTG), Differential Thermal Analysis (DTA), ^{13}C Solid State NMR and High Resolution ^{1}H NMR (HRMAS). We also aim to evaluate the possibility of using these techniques in the quality control of raw samples of *Agaricus.*

Experimental

Agaricus Blazei Jun-17 was purchased from Mitsuko Abe – SP/ Brazil and analyzed in the raw form. The sample was characterized by thermal analysis (TG/DTG/DTA) using a TA Instruments, 2960, at a heating rate of 10ºC/ min in a temperature range of 25 to 800ºC in nitrogen atmosphere.

Solid state NMR data were acquired on a Bruker Avance 400 NMR spectrometer, using a 4mm Bruker probe; Bloch decay with high power proton decoupling (HPDEC, with a pulse delay of 0.3s) and cross polarization with ramp (CP; contact time 1 ms); pulse sequences were used to acquire spectra at the spinning rate of 10 kHz. ^{1}H HRMAS spectra were obtained on a Bruker DRX-400 spectrometer, by using 4mm rotors with 50µL cavity and $CDCl_3$ as solvent.

Results and discussion

Figure 1 shows TG/DTG and DTA curves in nitrogen atmosphere of an *Agaricus Blazei Jun-17* sample, where three stages of degradation can be observed. The first stage is in the temperature range of 70ºC to 80ºC (weight loss of 4.3%); the second is at around 200ºC, probably due to protein denaturation (8% of weight loss); and the third degradation is at 300ºC, which represents the main decomposition, suggesting polysaccharides degradation (40% of mass loss).

An amount of 18% of dry residue was found. The DTG curve exhibits four decomposition stages in the temperature range of 60ºC to 380ºC, showing the fastest decomposition at 290ºC. DTA analysis show three endothermic events at 120ºC, 290ºC (both smooth), and at 700ºC, suggesting the presence of inorganic compounds, corroborating the TG results. All peaks are related with decomposition. The FTIR spectrum (not shown) presented a strong band at 3274 cm^{-1} (O-H stretching), referring to a polysaccharide, with bands at around 2950 cm^{-1} (C-H stretching) due to lipid hydrocarbon chains, and at 1630 cm^{-1} (C=O stretching) corresponding to amide carbonyl groups of proteins. These findings agree with the decomposition pattern observed by TG/DTG/DTA.

The ^{13}C solid state HPDEC/MAS spectrum (Figure 2, top) obtained by employing short recycle delay times allows us to detect specially high-mobility phases.[4] Following this approach, the signal at 30 ppm is assigned to $(CH_2)_n$ in the lipid chain, and the signals at 130 and 128 ppm to HC=CH groups present in the unsaturated linear chain.

In the ^{13}C CPMAS spectrum (Figure 2, bottom) it is possible to confirm the presence of a protein moiety through signals at 175-173 ppm (N-C=O) and at 20-50 ppm (C-N, C-H).

The presence of polysaccharides is evidenced by the signals in the range of 61-64 ppm (CH_2-O), 68-73 ppm (CH-O) and 103.5 ppm (O-C-O) carbons.[5,6]

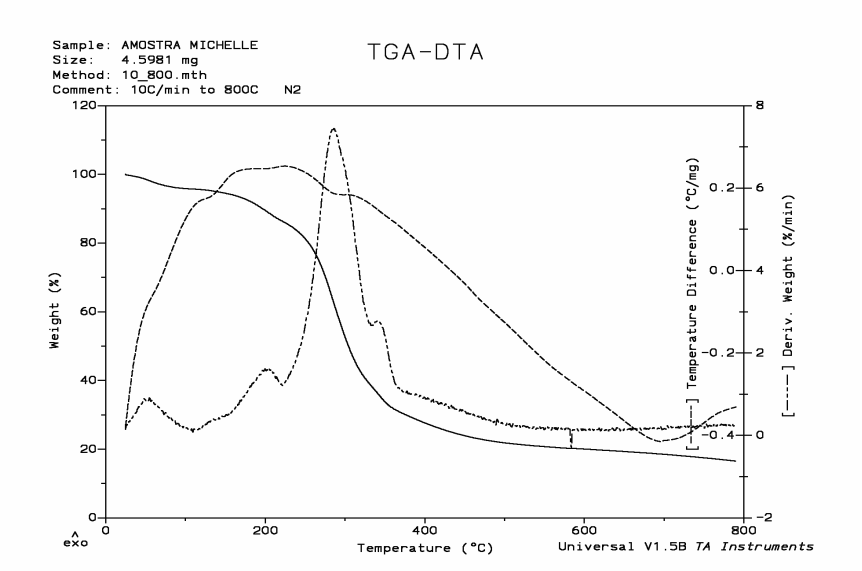

Figure 1. TG/DTG/DTA curves obtained for *Agaricus blazei Jun-17* (nitrogen flux)

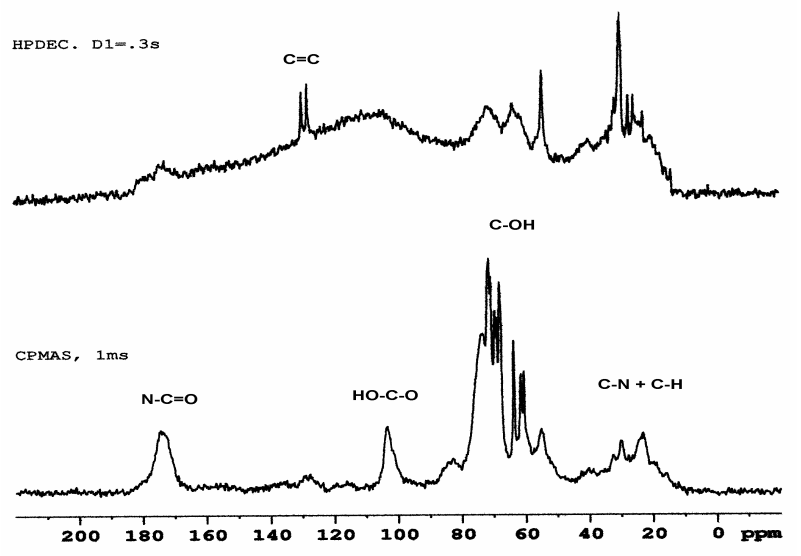

Figure 2. ^{13}C BD/MAS AND ^{13}C CP/MAS spectra of *Agaricus blazei Jun-17*

Low intensity signals in the aromatic region can be also detected. Pizzoferrato et al.[7] have compared the signals at 170/105 ppm, 20/90 ppm and 20/105 ppm in order to estimate the ratios between protein and polysaccharide levels of intact food samples. Following this approach, it can be qualitatively stated that, in the studied sample, the amount of polysaccharides is higher than that of the protein.

The ^{1}H HRMAS spectrum (Figure 3) is dominated by the signals due to an unsaturated linear chain; the presence of a signal at 2.8 ppm with high intensity confirms bis-allyl carboxylic acid moieties as the principal component.

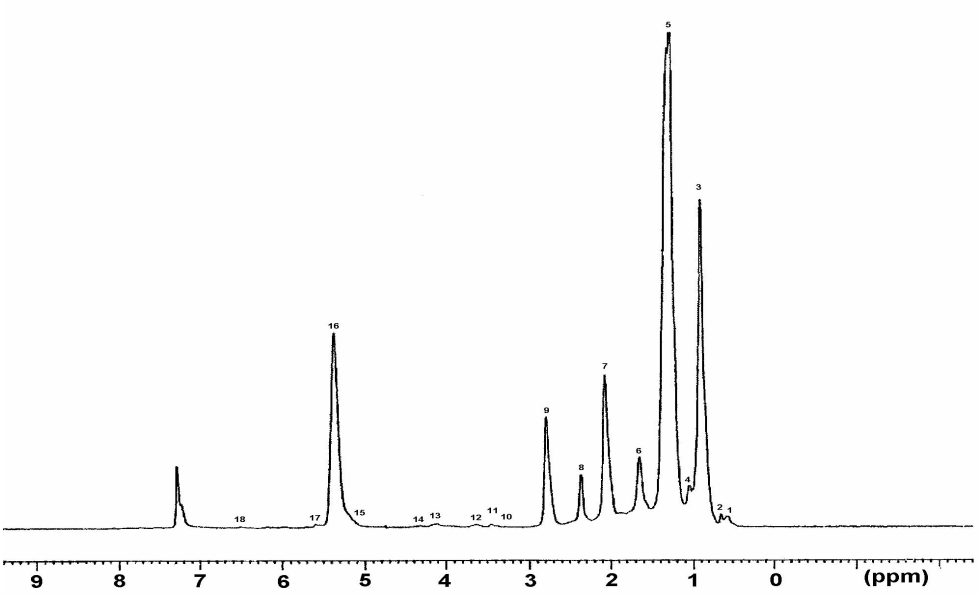

Figure 3. ^{1}H Solid state NMR (HRMAS) of a raw sample of *Agaricus blazei Jun-17*

Table 1 summarizes the chemical shifts and assignments proposed for the signals observed. The presence of ergosterol was confirmed through the signals at 0.65, 1.05 and 5.6 ppm. Low intensity signals could be attributed to triacylglycerides, diglycerides and/or etanolamine diacylglycerophospholipid derivatives. Aromatic protons could be also detected in a very low concentration, which can possibly be related to aromatic amino-acids and/or polyphenols.

Table 1. ^{1}H Chemical shifts and assignments proposed from ^{1}H HRMAS NMR

Signal	δ (ppm)	type of H	Signal	δ (ppm)	type of H
1	0.57	CH_3 sterols	10	3.3	HC-N
2	0.65	CH_3 ergosterol(C18)	11	3.4	HC-N
3	0,91	CH_3 linear chain	12	3.6	HCN-CH-O
4	1.05	CH_3 ergosterol(C21)	13	4.2	$C\underline{H_2}$-O lipid
5	1.28/1.31	$(CH_2)_n$	14	4.4	CH-Oβ linkage
6	1.65	$C\underline{H_2}$-CH_2-COO	15	5.2	CH-O lipid
7	2.1	$C\underline{H_2}$-CH=CH	16	5.4	HC=CH
8	2.4	CH_2-COO	17	5.6	HC=CH ergosterol
9	2.8	CH=CH-$C\underline{H_2}$-CH=CH	18	6.6	HC=C arom.(phenol deriv.)

Conclusion

It was possible to demonstrate that thermal analysis is a potential technique to evaluate the thermal behavior of *Agaricus Blazei Jun-17*. Also, solid state NMR can be used to characterize the raw sample of this mushroom species.

Overall, the results suggest that both techniques can be used to characterize raw samples of that functional food, without extraction steps. Thermal and NMR analyses of samples from different commercial sources are in progress to validate this claim. Particularly, 2D NMR experiments will be required for a more precise determination of the molecules present in the sample.

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