Chemical composition and nutritional value analysis of AbCi hybrid mushrooms by GC-MS and HPLC

Characterization of selected nutrients of AbCi hybrid mushrooms

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***Abstract*- The aim of the present study was to characterize and compare the chemical composition and nutritional value of the four fruit body developing *AbCi* somatic hybrid mushrooms and their parent strains *Agaricus bisporus* and *Calocybe indica*. *AbCi* hybrids were developed using PEG-mediated protoplast fusion technique. The protein, fat, carbohydrate, moisture and ash content of mushroom samples were analyzed. The fatty acid and sugar profile was studied using Gas chromatography-mass spectrometry coupled with Electron Ionization Detector (GC-MS/EID) and high performance liquid chromatography coupled to a refraction index detector (HPLC/RID), respectively. The macronutrient content revealed that the hybrids were rich source of protein (18-20g/100g dry weight) and carbohydrates (20-35 g/100g) and were low in fat (2-3 g/100g) content. Fatty acids profile depicted high percentage of unsaturated fatty acids (Oleic acid, Linoleic acid and Palmitoleic acid) compared to saturated fatty acids in hybrids compared to parent strains. Among the free sugars mannitol and trehalose were the dominating sugars in the hybrid strains. The moisture content was quite high and it ranged between 82-91%. So, based on the present proximate analysis, it can be concluded that *AbCi* hybrid mushrooms are good source of proteins and have very low-fat content. Thus, they may become part of healthy balance diet especially for people with diabetics and malnutrition.**

***Key words*: chemical composition, *Agaricus bisporus*, *Calocybe indica*, hybrid mushroom, edible mushroom, GC-MS, HPLC**

I. INTRODUCTION

Edible mushrooms have an excellent nutritional value as they are good source of protein containing essential amino acids, vitamins [1] and are poor in fat content [2, 3]. They serve as source of different unsaturated fatty acids, tocopherols, trace minerals, fibre and carbohydrates. The nutritional value of mushroom makes them a gradual replacement of animal protein. About 140,000 species of mushrooms are known of which only 25 species are widely accepted as safe food [5]. The pharmacological potential of mushrooms such as antimicrobial [4], antiviral, antitumor, antiallergic, immunomodulating, anti-inflammatory, antiatherogenic, hypoglycemic, and hepatoprotective properties [5], makes them more attractive as a source for the development of drugs and nutraceuticals.

In our present research work we were interested in the nutritional characterization of *AbCi* hybrids and parent mushrooms (*Agaricus bisporus* and *Calocybe indica*). Four *AbCi* hybrids were developed previously by intergeneric polyethylene glycol mediated protoplast fusion between *Agaricus bisporus* and *Calocybe indica* var. APK2 [6]. *A. bisporus* is the most cultivated mushroom all over the world because of its very high protein content, vitamin and high food value [7]. The aim of this protoplast fusion experiment was to produce hybrids with better nutritional and chemical properties than their parent strains. So this proximate analysis will give an idea of nutritional properties of hybrids. The *AbCi* hybrids nutritional properties were not examined before so we documented it, as it will help in better management and conservation of natural resources.

Herein a comparison study was done for nutritional value and chemical composition of the hybrids and to study whether the hybrids were better than the parent species or not. The protein, fat, carbohydrate, moisture and ash content of mushroom samples were analyzed. Along with these the sugar profile and fatty acid profile of the *AbCi* mushrooms was studied in details. The fatty acid and sugar profile was studied using Gas chromatography-mass spectrometry coupled with Electron Ionization Detector (GC-MS/EID) and high performance liquid chromatography coupled to a refraction index detector (HPLC/RID), respectively, the latter methodology being completely validated.

II. MATERIALS AND METHODS

Mushroom sample

Parents *Agaricus bisporus* and *Calocybe indica* var. APK2 were collected from 'National Research Centre for Mushroom', Solan, Himachal Pradeshand Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India respectively. Through PEG-mediated protoplast fusion four somatic hybrids (*AbCi* 6, *AbCi* 7, *AbCi* 8 and *AbCi* 9) were successfully developed and cultivated in the laboratory conditions [6]. Fresh fruit bodies were collected from all the mushroom strains and were dried and grounded into fine powder (1 mm particle size). The powdered samples were stored in the refrigerator for further analysis.

Nutritional value analysis

Chemical composition (moisture, protein, fat, carbohydrates and ash) of the parent and hybrid mushroom samples was analysed [8]. The macro-Kjeldahl method was followed for estimation of the crude protein content (Nx4.38) [9]; the determination of crude fat was done with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at (600 ± 15) ºC for 5 to 6 hr. Total carbohydrates were calculated by difference: **Total carbohydrates =100−(moisture+protein+ fat + ash).** Total energy was calculated according to the following equations: **Energy (Kcal) = 4 × (protein + carbohydrate) + 9×(lipid).**

Sugar profile analysis by HPLC

Sugar extraction procedure: HPLC-RID (High-Performance Liquid Chromatography) analysis was carried out following the method of Harada et al.,[10] with some modifications. At first, 1 gm of dried powder of mushroom fruit body or mycelial tissue was dissolved in 40ml of 80 % ethanol and was incubated at 80 ºC for 30 min. The resulting suspension was centrifuged at 15,000 gm for 10 min. The pellet was discarded and the supernatant was transferred to another tube and was concentrated at 60 ºC under reduced pressure. It was then defatted with 10 ml ethyl ether thrice. Finally, it was concentrated at 40 ºC to solid residues and was dissolved in 5 ml sterile water.

Sugar composition determination by HPLC analysis: The sugar standard solution was prepared using ten sugar standards at 5 mg / ml. Arabinose, trehalose, glucose, sucrose, maltose, mannose, rhamnose, xylose, fructose and mannitol were used as standards. Soluble sugars were detected by RID-10A detector and with a LC column (Luna 5 µm NH2 100 Aº, 250 mm X 4.6 mm column). HPLC system (UFLC Shimadzu) was equipped with LC 20AT prominence liquid chromatographic pump and SIL prominence auto sampler injector. Acetonitrile / deionized water were used as the mobile phase at 7:3 (v/v) ratios at 1 ml / min flow rate. Chromatogram of sugars was generated by using LC solution software. Sugars were identified by comparing the retention times of the unknown sample peaks with standard sugar solution and concentration of sugar in unknown sample was calculated from chromatographic peak area and expressed in gm / 100 gm dry weight.

Fatty acid profile study by GC-MS

Fatty acid extraction and fame preparation: Fatty acid was extracted from the powdered sample using Soxhlet apparatus. 10 gm powdered mushroom sample was extracted with 100 ml petroleum ether. To the petroleum ether extracted sample 1.25 ml toluene was added and mixed well. Next 2.5 ml methanol and 1.25 ml sulphuric acid was added to the above sample. The mixture was incubated in the water bath for 12 h at 50 ºC, 160 rpm. After incubation 3 ml of deionised water was added and votexed for phase separation. 3ml of n-Hexane was added to the vortexed sample and two separated phases became clear. Upper phase was collected in a tube and the water in the sample was eliminated by passing it through a micro-column containing anhydrous sodium sulphate. The fatty acid methyl esters (FAME) was recovered by filtration using 0.2 µm nylon filter and was collected in Teflon vial.

Fatty acid analysis by GC-MS: The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the derived FAME was done using GC (Trace GC Ultra, Thermo Fisher Scientific India Pvt. Ltd.). The fatty acid sample was passed through a capillary TR-WaxMS polar column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) and detected by Electron Ionization Detector (EI). Following temperature conditions were used for oven: Initial hold at 50 ºC for 2 min, then temperature was raised from 50-240 ºC at 20 ºC per min and final hold of 11 min. 1 µl of Fatty acid sample was injected in the GC and helium carrier gas was supplied at the flow rate of 1 ml per min. The individual fatty acids present in the supplied sample was separated by GC and was identified by comparing the retention time and peak area of the unknown sample with FAME standards (Sigma) and by NIST Library (version 2.0, 2008).

Statistical analysis

All the nutritional variations of hybrid and parent mushrooms were analyzed and evaluated using Graph pad prism 5 by One way ANOVA analysis and Turkey Post hoc tests, followed by the comparison of mean, standard deviation and level of significance ($p < 0.05$).

III. RESULTS AND DISCUSSION

Macronutrient content

In the present study, we compared the macronutrient profile of two parent mushrooms (*Agaricus bisporus* and *Calocybe indica*) with the hybrid mushrooms obtained by PEG-mediated protoplast fusion technique. In the Table 1 a comparison of different macronutrients (carbohydrate, protein, fat, ash and moisture) of hybrid and parents are summarised. The moisture content in the *A.bisporus* parent was high (90.86 g / 100 g) than the *C. indica* parent 85.38 g/ 100g. Among the hybrids *AbCi* 8 strain showed highest moisture content (90.23 g/ 100g) and lowest in *AbCi* 6 (82.46 g/ 100g). Usually, mushrooms are also high in moisture content. Mostly 85–90% and 10–12% of moisture content is found on fresh and air dried mushrooms respectively [11, 12]. In *AbCi* hybrids moisture content ranged between 90.23 -82.46 g , was same as the parent strain.
Carbohydrate content of hybrids and parent strains as calculated by difference, ranged between 20.29 - 35.75 g. Hybrid *AbCi* 8 showed highest carbohydrate content of 35.75 g/ 100g while *AbCi* 6 depicted lowest content of 20.29 g/100 g. This result

shows that carbohydrates are also abundant macronutrients in mushrooms. Previous reports also shows that carbohydrate is the predominant macronutrient found among the edible mushroom fruit bodies [13, 14].

Mushrooms are considered as an excellent choice for low fat containing food. As compared to the level of carbohydrate and proteins the fat content in mushrooms is very low. In our study the fat level ranged between 2.01g in *C.indica* to 3.26 g in *AbCi* 6 [15]. In mushrooms all classes of lipids like free fatty acids, glycerids, sterols and phospholipid are found [16]. Earlier report shows mushroom contain less than 1% to as high as 15–20% fat of its dry weight with 2–8% average fat [17]. Mushrooms are beneficial for heart and diabetic patients due to low fat content [18].

Mushrooms are good source of proteins as they are highly rich in proteins. It is the second most abundant macronutrient found in mushrooms. The level of proteins in mushrooms depends on the different growing stages and species of mushroom along with the surrounding environment [19]. In our study the *AbCi* 8 showed highest protein content 21.54 g among all the hybrids and parent strains. The protein content ranged between 18.65 – 21.54 g with lowest level in *AbCi* 6 hybrid. Different reports suggest different protein levels in mushroom some report about 12-29% [19] while others suggest 54-59% [13].

Ash is actually the inorganic residue which remains after the removal of organic matter and water with the help of oxidising agents. Ash is rich in minerals. In our analysis the ash content ranges between 4.89-7.92 g/ 100g of dry weight with highest value in *AbCi* 8.

The chemical composition and the nutritional content of the mushroom fruit bodies are highly influenced by growth compost composition [16]. Other factors like developmental stages along with the pre and post-harvest conditions also affect the mushroom quality. The atmospheric conditions, age of species, growth condition etc. also influence the nutritional composition, accumulation of metal ions and water content [20, 21].

Table 1: Comparison of Nutritional value of parent and hybrid mushrooms (mean ± SD).

	A. bisporus	***AbCi* 6**	***AbCi* 7**	***AbCi* 8**	***AbCi* 9**	***C. indica***
Moisture (g/100g)	90.86 ± 0.37	82.46 ± 0.42	89.65 ± 0.58	90.23 ± 0.83	88.62 ± 0.28	85.38 ± 0.48
Carbohydrate (g/100g)	29.09 ± 0.83	20.29 ± 0.65	28.02±0.68	35.75±0.49	34.42 ± 0.08	33.99 ± 0.42
Crude Protein (g/100g)	20.89 ± 0.66	18.68 ± 0.95	19.99 ± 0.28	21.54 ± 0.86	20.78 ± 0.06	18.65 ± 0.89
Fat (g/100g)	2.92 ± 0.42	3.26 ± 0.22	2.44 ± 0.48	2.49 ± 0.22	3.02 ± 0.42	2.01 ± 0.31
Ash (g/100g)	5.62 ± 0.26	4.89 ± 0.48	5.99 ± 0.26	7.92 ± 0.62	5.02 ± 0.25	4.89 ± 0.26
Energy (kcal/100g)	**226.2 ± 9.72**	**185.22 ± 8.38**	**214 ± 6.12**	**251.57 ± 7.38**	**247.98 ± 4.34**	**228.65 ± 7.58**

The values of Carbohydrate, Protein, Fat, Ash, Moisture and Energy are means ± SD of 3 repeated experiments
Results of One way ANOVA $P < 0.05$

Sugar composition by HPLC

HPLC analysis of four fruit body bearing *AbCi* hybrids and parents, *A. bisporus* and *C. indica*, was done for sugar composition determination. Results of the five sugars i.e., Fructose, Rhamnose, Mannitol, Sucrose and Trehalose are shown in the Table 2. Among the different sugars the concentration of mannitol and trehalose was found greater. Mannitol level was found higher in the fruit bodies of hybrid *AbCi* 8 (23.45 g /100 g dry weight) compared to all other strains. *A. bisporus* had higher mannitol content than *C. indica* but trehalose content was higher in *C. indica*. Amount of trehalose dominated in *AbCi* 7 (8.65 g /100 g dry weight) and *AbCi* 6 (6.62 g /100 g dry weight). Sucrose was detected in only *A. bisporus* (0.269 g /100 g dry weight) and hybrids *AbCi* 6 (0.04 g / 100 g dry weight). Rhamnose content was found in very small amount in *C. indica* and *AbCi* 9 compared to *AbCi* 6. In all the hybrids and parent strains fructose was detected with highest amount in *C. indica* and lowest in *AbCi* 7. Overall sugar content was found higher in *AbCi* 8 (26.64 g / 100 g dry weight) and *AbCi* 9 (25.77 g / 100 g dry weight) Fig. 2.

Table 2: Composition of sugar (gm / 100 gm of dry weight of fruit bodies) of the *AbCi* hybrids and the parents, *A. bisporus* and *C. indica* (Mean ± SD).

Mushroom strains	**Fructose**	**Rhamnose**	**Mannitol**	**Sucrose**	**Trehalose**	**Total sugars (g/100g dry weight)**
A. bisporus	0.057 ± 0.04	nd	20.81 ± 1.3	0.269 ± 0.01	0.514 ± 0.03	21.65 ± 1.38
C. indica	4.60 ± 0.34	0.03 ± 0.00	15.25 ± 0.14	nd	5.65 ± 0.42	25.53 ± 0.9
AbCi 6	3.25 ± 0.54	1.6 ± 0.2	3.9 ± 0.37	0.04 ± 0.00	**6.62 ± 0.04**	15.41 ± 1.15
AbCi 7	0.01 ± 0.00	nd	14.40 ± 0.21	nd	**8.65 ± 0.25**	23.06 ± 0.46
AbCi 8	0.65 ± 0.01	nd	**23.45 ± 0.42**	nd	2.54 ± 0.08	**26.64 ± 0.51**

AbCi 9	3.49 ± 0.54	0.05 ± 0.03	16.8 ± 0.44	nd	5.43 ± 0.23	**25.77 ± 1.24**

nd =not determined

HPLC analysis of the sugar content in the *AbCi* hybrids revealed that the mannitol and trehalose are the most dominating sugars among all other sugars tested. In *AbCi* hybrids mannitol was dominated in *AbCi* 8 and trehalose in *AbCi* 6 and *AbCi* 7. Accumulation of mannitol and trehalose in the fruit bodies of mushroom species was reported previously [22, 10]. Sugar alcohol like mannitol functions to provide support and expansion of the fruit body. Mannitol has half the calories and is half as sweet, and because it is poorly absorbed by the body, it does not raise the insulin level as much as sugar and also does not promote tooth decay [23]. Trehalose is also an abundant sugar in mushroom fruit bodies and it is a non-reducing, an α-linked disaccharide [19].

IV.

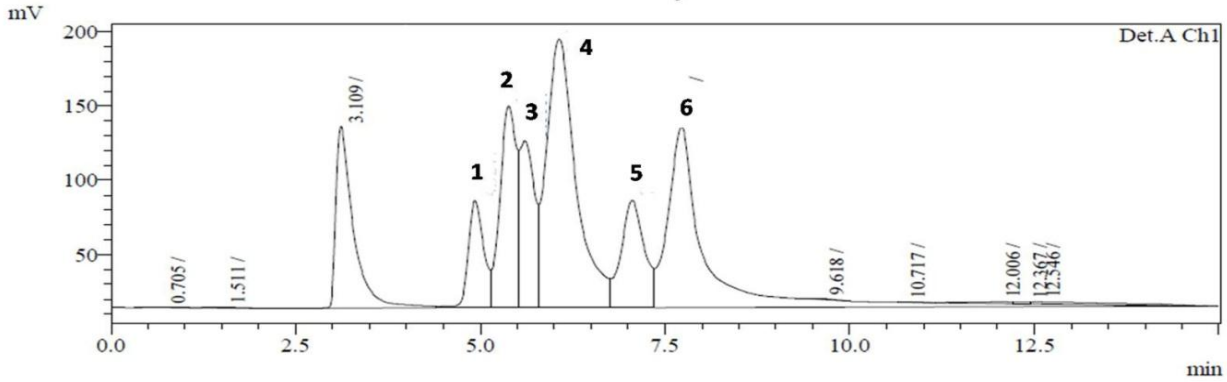

Figure 1: Individual sugar chromatogram of standard mix. 1- rhamnose, 2- arabinose, 3- fructose, 4- mannitol, 5- sucrose and 6- trehalose.

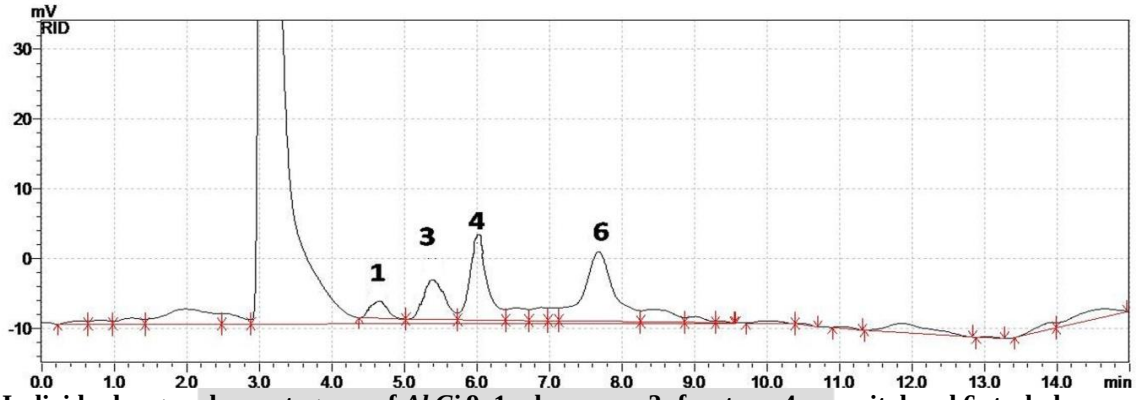

Figure 2: Individual sugar chromatogram of *AbCi* 9. 1- rhamnose, 3- fructose, 4-mannitol and 6- trehalose.

Fatty acid profile analysis by GC-MS

The fatty acid profile shows that percentage of unsaturated fatty acids (UFA) was high in the hybrids compared to the parent strains. SFA mainly dominated in the parent strains compared to the hybrid strains and it ranged from 15.0-30.18%. Highest SFA percent was observed in *A. bisporus* (30.18%) and minimum in *AbCi* 7 (15.0%). The percentage of MUFA in *A. bisporus* (12.7%) and *C. indica* (18.52%) was found lower compared to the hybrids *AbCi*7 (20.4%) and *AbCi* 8 (21.16%). PUFA ranged from 56.05-64.6% in hybrids and parent strains with highest concentration in *AbCi* 7 (64.6%) [24].
Among the SFA, 26.48 % Palmitic acid was found in *A. bisporus* and highest Stearic acid in *AbCi* 9 (7.78 %). Myristic acid (C14:0) was present in only *C. indica* parent and in hybrid *AbCi* 8. Major MUFA were Oleic acid- C18:1 (7.1-19.05 %) and Palmitoleic acid- C16:1 (0.4-3.9 %). In the hybrids *AbCi* 6, *AbCi* 7 and *AbCi* 8 Oleic acid percentage was higher compared to any of the parents. Oleic acid was found abundant in *AbCi* 8 (19.05 %) followed by *AbCi* 6 (18.57 %). Linoleic acid (C18:2) was the major PUFA in the hybrids and parent strains ranging between 60.4 - 53.3 %. Linoleic acid was found dominating in hybrid *AbCi* 9 (60.4 %) and *AbCi* 7 (59.95 %).

Table 3: Composition of Fatty acid in the *AbCi* hybrids and parent mushroom strains determined by GC-MS (presented as % w/w of the total fatty acid). [24]

Fatty acids	***A. bisporus***	***C. indica***	***AbCi* 6**	***AbCi* 7**	***AbCi* 8**	***AbCi* 9**
C14:0(Myristic acid)	nd	**0.8**	nd	nd	0.7	nd
C15:0(Pentadecylic	1.50	3.02	2.60	1.80	1.90	**3.20**

acid)						
C16:0(Palmitic acid)	**26.48**	16.03	15.55	12.45	14.50	13.20
C16:1(Palmitoleic acid)	0.90	2.20	0.40	3.66	**3.90**	2.50
C18:0(Stearic acid)	6.60	5.20	5.80	2.48	3.02	**7.78**
C18:1(Oleic acid)	7.10	15.24	18.57	16.24	**19.05**	11.02
C18:2(Linoleic acid)	54.80	56.11	53.30	59.95	54.69	**60.40**
C18:3(Linolenic acid)	2.00	1.40	**3.73**	3.50	2.24	1.90

nd = Not determined

Linoleic acid was the dominating PUFA and Oleic acid was the dominating MUFA in the fruit bodies. Both linoleic acid and oleic acids have been related to decrease risk of cardiovascular diseases and recommended for adding mushrooms in the diet of people with high blood cholesterol [25]. So from this perspective the *AbCi* hybrids would serve as a good source of PUFA and MUFA.

Nutritional parameters like moisture, protein, ash, carbohydrate and fatty acid content was analysed. Among the sugars mannitol and trehalose concentration was found higher in some of the hybrids compared to both the parent strains and this property will make these hybrids as a good dietary substitute. Along with this the concentration of unsaturated fatty acid was also found higher in the hybrids than their parental strains and consuming these hybrids will reduce the cholesterol level in body. The protein content was also high in some of the hybrids. All these properties will make these hybrid mushrooms superior as food and it will serve as nutraceutical in future along with this, these hybrids will act as the study material for understanding the genetics involved in the formation of hybrids.

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Conflict of Interest: The authors have no conflict of interest.

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