Int.J.Curr.Microbiol.App.Sci (2019) *8*(8): 2972-2978

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.808.343

Assessing the Degradation Ability and Nutritional Quality of Different Strains of Milky Mushroom

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Keywords

Degradation ability, Nutritional quality, Milky mushroom, Carbohydrates, Digestible crude protein

Article Info

Accepted:
22 July 2019
Available Online:
10 August 2019

A B S T R A C T

The experiment was carried Measuring activity of enzyme like exoglucanase, endoglucanase and xylanase and the nutritional quality of different strains of milky mushroom namely *viz.*, CI-1, CI-2, CI-3, MG-4, CI-5 and APK-2 in the study. Among the five strains tested, CI-3 recorded maximum activity of endoglucanase, exoglucanase, and endoxylanase (1.50, 1.02, 1.66 µ moles sugar/min/mg protein,) followed by strain APK-2 (1.22, 0.23and 0.82 µ moles sugar/min/mg protein respectively). The nutrient range of different strain of milky mushroom were found to contain 86.42 to 88.9 per cent moisture, 42.32 to 55.32 per cent total carbohydrates, 18.20 to 24.50 per cent digestible crude protein,3.58 to 4.74 per cent crude fat, 7.91 to 9.84 per cent crude fiber and 8.38 to 9.86 per cent ash content on dry weight basis

Introduction

Milky mushrooms are with great source quality protein, minerals and various vitamins. From the age old period of time mushroom were used as a food materials without knowing its medicinal values. It is an important food item concerning human health, nutrition and disease prevention. Gruen and Wong (1982) indicated that edible mushrooms were highly nutritional and compared favourably with meat, egg and milk food sources. Different strains of *Calocybe indica* ability to adopt wide range of the temperature, pH levels and yield potential depending on the substrates used. It is more attractive with excellent shelf-life, grows on several agricultural wastes and on wide range of temperatures (Singh *et al.*, 2015). *C. indica* has great demand in many parts of the country due to its attractive milky white sporocarp, typical wild mushroom taste, fibrous feel of the texture, long shelf. This mushroom was first collected in wild form from West Bengal (India) by Purkayastha and Chandra in 1974. Production technology of *Calocybe indica* has been introduced by Purkayastha and Nayak in 1979 which was improved by Purkayastha and Nayak in 1981.Milky mushroom cultivation has proved its economic strength

and ecological importance for efficient utilization, value-addition and biotransformation of agro-industrial residues (Zervakis and Philippoussis 2000). Commercial mushroom production is yet another efficient and relatively short biological process of food protein recovery from unutilized lignocellulosic materials through enzymatic degrading capabilities of mushroom fungi (Chiu and Moore, 2001).

Materials and Methods

Estimation of enzymes

Endoglucanase

Celluloytic enzymes namely, Endo-β-D-1, 4-glucanase activity was measured by estimation of the reducing sugars released during incubation of the substrate with enzyme extract according to method Mandels *et al.* (1976). The reducing sugars were estimated using DNS reagent (Miller, 1959).

Reagents

1) 0.1 M citrate buffer (pH 4.8)
A. 0.1 M Citric acid
B. 0.1 M tri sodium citrate

23 ml of A and 27 ml of B and volume made upto 100 ml with distilled water.

2) Carboxy Methyl Cellulose solution (CMC)
1 g of Cellulose solution was dissolved in 90 ml of 0.1 M citrate buffer of pH 4.8 and volume was made to 100 ml.

3) Dinitrosalicylic acid (DNS) solution

10 g of DNS and 0.5g sodium sulfite in 500 ml of 2% NaOH solution already prepared in distilled water were added. The solution was allowed to cool, 2 g of phenol was dissolved in it and final volume was made to 1000 ml. The solution was filtered and stored in dark bottles in refrigerator.

4) Sodium potassium tartarate solution (40%) (Rochelle salt)

40 g of sodium potassium tartarate was dissolved in 100 ml distilled water. The solution was filtered and stored at room temperature.

Assay

The test tubes containing a mixture of 0.5 ml CMC solution and 0.5 ml of appropriately diluted enzyme extract were incubated at 50ºC for 30 minutes in water bath. Controls devoid of enzyme extract were also run simultaneously. Reducing sugars produced during this reaction were measured by using DNS method. 3 ml of DNS was added to each tube and kept in boiling water bath for 15 mins. While still hot, 1 ml of sodium potassium tartarate solution was added, the contents were cooled to room temperature followed by addition of 2 ml of distilled water in each test tube. The percent light absorbance by the resulting solution was recorded at 575 nm in a spectronic 20. The corresponding enzyme activity was calculated from the standard curve.

Exoglucanase

Cellobiohydrolase activity was determined by the method reported by Mandels and sternberg (1976).

Reagents

1) 0.1 M citrate buffer (pH 4.8)
2) DNS solution
3) Sodium potassium tartarate solution (40%)
4) Filter paper strips (Whatmann no.1, 1x 6 cm)

Assay

The test tube containing 0.5 ml enzyme extract and 1 ml of citrate buffer and a filter paper strip were incubated at 50ºC for 1 hour in water bath. Controls were run simultaneously. The reducing sugars produced during the reaction were estimated by DNS method as described in section 3.16.1.

Preparation of standard curve

A standard curve for measurement of reducing sugars was prepared under the same conditions Miller (1959), as described above using standard solution of glucose from 0.1-0.5 mg/ml concentration.

Enzyme units

Enzyme activity of endoglucanase and exoglucanase was expressed as µmoles glucose/min /ml of culture filtrate or /g straw. Specific activity was expressed as µ moles glucose /min/mg protein.

Endoxylanase

Xylanase (hemicellulase) activity was assayed according to the method of Erikson andBucht (1968)

Reagents

1) Xylan solution

1 gm of xylan was dissolved in 0.05 M citrate buffer, pH 4.8 and volume made to 100 ml.
2) DNS reagent
3) Sodium potassium tartarate solution (40%)

Procedure

1 ml of sample of approximately diluted culture was mixed with 1 ml of 1% xylan solution and incubated for 30 minutes at 50ºC. Reducing sugars were measured as xylose equivalents by DNS method as described in 3.16.1.

Units of enzyme activity

One unit of enzyme activity is expressed as amount of xylanase catalyzed the formation of 1 µmol xylose/min/ml of culture filtrate or /g straw. Specific activity was expressed as µmol xylose/min/mg protein.

Nutritive value of different strains of milky mushroom

The various constituents in the sporophores of different strains of milky mushroom(CI-1, CI-2, CI-3, CI-5and var. APK-2) were analyzed on dry weight basis by the following methods.

Determination of moisture content

The moisture content of the sporophore was estimated by drying 25 g of fresh sporophore in an oven at 80°C for three consecutive days. It was cooled in a desiccator and weighed. The moisture content was calculated as,

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Determination of total carbohydrates

The anthrone method described by Hedge *et al.* (1962) was followed to determine the total carbohydrates.

One hundred mg of the sample was hydrolysed in a boiling tube by keeping it in a boiling water bath for three hrs with five ml of 2.5 N HC1 and cooled to room temperature. This was neutralised with solid sodium carbonate until the effervescence ceased. Solution was centrifuged after making the volume upto 100 ml. From the

supernatant, 0.5 and 1 ml of aliquots were drawn for analysis. Volume was made up to one ml using distilled water and added four ml of anthrone reagent. Heated for 8 mins in a boiling water bath and rapidly cooled. Green colour obtained was read at 630 nm by using Spectronic-20. Amount of total carbohydrates present was calculated from the standard graph.

Determination of crude protein

The total nitrogen content was estimated by the Micro-kjeldahl method.

One g of powdered sample was taken in a digestion tube to this 0.05 g of digestion mixture (selenium dioxide, copper sulphate, potassium sulphate (1: 8: 40), ground separately and mixed well) was added followed by 10 ml of conc. sulphuric acid.

The mixture was digested till it turned to colourless solution. The digest was then cooled and the volume was made upto 100 ml with distilled water. 10 ml of aliquot was taken, distilled and titrated against 0.1 N HC1 by using Micro Kjeltech unit (Vapodest, version 45).

A reagent blank with an equal volume of distilled water was run and the titration value was subtracted from the sample titrate value.

Nitrogen content estimated as N in g kg^{-1} =

$$\frac{(\text{ml HCL} - \text{ml blank})\ \text{Normality} \times 14.01}{\text{Weight (g)}}$$

The digestible crude protein content was obtained by multiplying the total nitrogen value with the factor 4. 38 (Crisan and Sands, 1978).

Determination of crude fat

The crude fat in the sporophores was estimated by Soxhlet method (Lees, 1975) . In the pre-weighed extraction flask, two g of the dried sporophore was kept in the extraction thimble. The thimble was placed in the extractor for the extraction of crude fat using 100 ml of petroleum ether (b. p. 49 - 60°C) by heating over a water bath. After six to eight siphoning, the petroleum ether was evaporated in a water bath. The fat content was recorded after cooling in a desiccator. Percentage of crude fat was calculated as,

$$= \frac{(\text{Weight of flask + ether extract}) - \text{Weight of flask}}{\text{Sample weight}} \times 100$$

Determination of crude fiber

The crude fiber content in the sporophore was estimated following the method of De (1965). The residue in the thimble, after extracting crude fat was transferred to a beaker and boiled for 30 mins with 200 ml of 1.25 per cent sulphuric acid.

The mixture was filtered through a muslin cloth and the residue was washed for free of acid with water. The residue was transferred to a beaker containing 200 ml of 1.25 per cent sodium hydroxide and boiled for 30 mins. The solution was filtered through muslin cloth and washed with 25 ml of boiling 1.25 per cent sulphuric acid, 350 ml portions of water and 25 ml alcohol. Residue was transferred to silica dish and dried fortwo hrs at 130 ± 2˚C. Dish was cooled in a desiccator, weighed and ignited at 600+15 °C for 30 mins, cooled and weighed.

Per cent crude fiber content =

$$\frac{\text{Loss in weight on ignition}}{\text{Weight of the sample}} \times 100$$

Determination of ash content

A quantity of five g of dried and powdered sporophore was ignited in a silica dish for five h at 6000˚ C till a white ash was obtained, cooled and weighed (Raghuramulu *et al.*, 1983).

Determination of energy value

The energy value of oyster mushroom species was estimated based on the content of crude protein, crude fat and total carbohydrates in the mushroom using the factor 2.62, 8. 37 and 4.2 k cal per gram of each component respectively(Crisan *et al.*, 1978).

Energy value (k cal/100 g dry weight) = 2. 62 (% N x 6. 25) +8.37 (% fat) + 4.2 (% total carbohydrates)

Results and Discussion

Enzyme activity

Production of enzyme is of prime importance for efficient degradation of substrates and utilization of nutrients. The enzymes such as cellulase and laccase are responsible for degradation of cellulose and lignin content present in the substrates. The efficiency of the enzyme production positively correlates with the yield of mushroom.

Among the five strains tested, CI-3 recorded maximum activity of endoglucanase, exoglucanase, and endoxylanese (1.50, 1.02, 1.66 µmoles sugar/min/mg protein,) followed by strain APK-2 (1.22, 0.23and 0.72 µmoles sugar/min/mg protein). The least level of endoglucanase activity recorded in both the strains CI -2 and CI -5 (0.20 and 0.17 µmoles sugar/min/mg protein). Minimum level of exoglucanase activity was measured in strain CI-5 (0.06 µmoles sugar/min/mg protein). CI-1 and CI-5 recorded the low level of endoxylanase activity (0.51 and 0.54. µmoles sugar/min/mg protein) which correlates withRamkumar *et al.* (2011) revealed that $CaCO_3$ (2percent) amended Czapeck's Dox liquid medium recorded the high level of lignocellulolytic enzyme production *viz*., exo-β-1,4 glucanase 2.31 and endo β-1,4 glucanase 1.59, β glucosidase (1.79), xylanase (1.94), laccase polyphenol oxidase(0.82) in *Lentinuseddodes*. (1.85) and

Bhupathi *et al.* (2017)recorded maximum level of xylanase at all the seven stages of mushroom growth followed by lipoxygenase activity. Maximum activity of xylanase was recorded in the pileus of APK-2. variety and CBE-TNAU-1523 wild strain (3.514 mols/min/g and 3.55 moles/ min/g respectively) when compare to stipe(Table 1).

Table.1 *In vitro* activity of endoglucanase, exoglucanase and xylanase production of different strains of milky mushroom

S.No.	Strains	Cellulase(µmoles glucose /ml)		Xylanase
		Endoglucanase	Exoglucanase	Endoxylanase(1µmol xylose/ml)
1.	*C.indica*- CI-1	1.09	0.19	0.72
2.	*C. indica*- CI -2	0.20	0.14	0.51
3.	*C. indica*-CI-3	1.50	1.02	1.66
4.	*C. indica*- CI-5	0.17	0.06	0.54
5.	*C. indica* -APK-2	1.22	0.23	0.82
	CD (P=0.05)	**0.05**	**0.01**	**0.04**

Table.2 Proximate composition of milky mushroom (percent dry weight basis)

S.No	Strains of milky mushroom	Moisture content	TotalCarbohydrate	Crude protein	Crude fat	Crude fiber	Ash	CalorieValue (k/100g)
1.	*C. indica*-CI-1	86.7	55.32	18.20	3.82	8.65	9.2	328.46
2.	*C. indica*-CI -2	88.9	50.12	19.39	4.01	7.91	8.38	310.26
3.	*C. indica*-CI-3	86.4	42.32	24.50	3.58	9.84	8.64	229.5
4.	*C. indica* - CI-5	88.0	48.50	19.85	4.74	9.32	9.86	305.98
5.	*C. indica*-var. APK-2	87.5	47.08	20.2	3.62	8.54	9.52	301.7
	CD(P=0.05)	**4.1**	**1.83**	**1.08**	**0.17**	**0.32**	**0.50**	

Proximate composition of milky mushroom

Mushrooms are considered as a one of world's greatest untapped resources of nutritious and palatable food (Subramanian *et al.* (2015). Pani *et al.* (2012) revealed that milky mushroom are rich in various nutrient source like protein, fiber, mineral, carbohydrate and more amount of essential amino acids.

The results registered that different strains of milky mushroom were found to contain 86.42 to 88.9 per cent moisture, 42.32 to 55.32 per cent total carbohydrates, 18.20 to 24.50 per cent digestible crude protein,3.58 to 4.74 per cent crude fat, 7.91 to 9.84 per cent crude fiber and 8.38 to 9.86 per cent ash content on dry weight basis. They possessed an energy value of 229.5 to 311.34 k cal/100 g. Alam*et al.* (2008) suggested that the 100 g of dried mushroom consists 24g of protein, 4.5g of lipid, 12.9g of fiber, 13.1g of ash, 48.5 % of carbohydrate and 87 % of moisture level. Doshi*et al*. (1988) reported that mature sporophore of *Calocybeindica*had soluble sugars (4%), starch (2.9%) and ash (7.4%) Dhakad *et al.* (2017) conducted an experiment on five different strains of milky mushroom to estimate the nutrient status and founded that highest level (0.14mg/g of fresh mushroom) of protein content in the strain CI-8,other strain CI-15,CI-13 and CI-14 had the protein content of 0.12 mg/ g, 0.09 mg/ g and 0.06 mg/ g respectively (Table 2).

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How to cite this article:

Venkatesh, P., N. Revathy and Kavi Bharathi, N. 2019. Assessing the Degradation Ability and Nutritional Quality of Different Strains of Milky Mushroom. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 2972-2978. doi: https://doi.org/10.20546/ijcmas.2019.808.343