EVALUATION OF RARE SUGAR CONTENTS IN EDIBLE MUSHROOM

Pornpimol Muangthai [*], Wareerut Chouesain and Khanidtha Tosuntiku[1]

ABSTRACT

There are many types of mushroom, some can used as food for human known as edible mushroom but some are toxic mushroom. An edible mushroom is one of a popular vegetable for vegeterian people since it contains higher protein content than most vegetable and also riches in many minerals and vitamins. In this work,trehalose and psicose were the interested rare sugar which were separated from three popular edible mushroom such as *Lentinus edodes* (Shitake mushroom) , *Volvariella volvacea* (Straw mushroom) and *Auricularia polytricha* (Cloud ear fungus).Both rare sugars were analysed by TLC qualitative test and determined their contents by HPLC-RID.The separation was studied in standard trehalose , standard psicose ,standard glucose systems by 5 mobile phase system such as system1 acetonitrile : water (70:30) , system 2 n-butanol : ethanol :water(50: 30:20), system 3 ethyl acetate : acctic acid :methanol: water (60:15:15:10) system 4 n-propanol : NH_3 conc.:water (50:30:20) and system 5 n-butanol :pyridine: water(60:40:20) and using 4 spraying agent systems such as 20% H_2SO_4 , H_2SO_4 : methanol(1:1) , 5% $AgNO_3$ and 3,5-dinitrosalicylate.The extracted solutions from those mushrooms were analysed the trehalose , psicose and glucose by TLC and HPLC using Lichrosorp-NH_2 as separated column and using acetonitrile : water (70:30) as mobile solvent with1ml/min flow rate with Refractive index detector.However, the boiled mushroom (at 100 ^oC) were also analysed the sugars content.The result showed that, the best mobile system for TLC qualitative test was system 1 and detection of spot on chromatogram with 20% H_2SO_4. The trehalose and glucose sugar cleary presented in the chromatogram of each fresh mushroom but psicose was not showed clear spot. From the HPLC work, psicose could be detected and also lowest content in each mushroom. The trehalose and psicose contents presented in all fresh mushroom less than found in cooked mushroom.

Keywords: Rare sugar , edible mushroom

[*]Department of Chemistry, Faculty of Science, Srinakharinwirot University,Sukhumvit 23 Bangkok ,10110 , Thailand

[*]Corresponding Author, E-mail : pornpi @swu.ac.th

INTRODUCTION

There are many kinds of mushroom in Thailand , some of them are cultivated for human foods but others may be natural growthing in forest known as wild edible mushrooms. However, cultivated mushroom such as *Lentinus edodes* (Shitake mushroom) , *Volvariella volvacea* (Straw mushroom) and *Auricularia polytricha* (Cloud ear fungus) are the mushroom that found in many foods. All refered mushrooms are available throughout the year and sold in every market in Thailand. There are many reports about the advantage of those nutrition mushroom especially Shitake mushroom (Manzi, 2001). The production of Shitake mushroom has increase faster in Thailand since the expensive price of it. Straw mushroom and Cloud ear fungus are the common mushroom with cheaper price than Shitake mushroom and frequently used to cook in many food.Each mushroom differences in their size color and taste. The taste component of Straw mushroom has been reported by many researcher (Diez and Alverez 2001), (Crisan and Sand, 1978). Several studies have been carried out on the chemical composition and nutrition quality of edible mushroom (Maga, 1981; Yang, et al., 2001; Lillian, 2007). Trehalose is one of the sugar that reported in many mushroom (Aletor, 1995) but there was no report about psicose sugar.In this work, the rare sugar content as trehalose and psicose were separated from three mushrooms such as *Lentinus edodes*, *Volvariella volvacea* and *Auricularia polytricha*. and qualitatively checked by Thin Layer Chromatography (TLC) and analysed their contents by High performance liquid chromatography with refractive index detector. The studies done in the raw mushroom and boiled mushroom to studied the variation on sugar content.

MATERIALS AND METHODS

Part 1 Qualitative Analysis of Trehalose and Psicose in edible mushroom by TLC

The three mushrooms were purchased from the fresh market in Bangkok, the mushroom were chopped and weighed 10.0000 g. and placed in the 25 % of ethanol solution (pure ethanol AR grade purchased from Merck)and left at room temperature for 1 hr.The residue mushrooms were filtered out and the filtrates were spotted on TLC plate (commercial plate from Chromatographia) and compared with standard sugar such as glucose fructose trehalose (purchased from Fluka) and psicose (donated from Professor Dr. Ken Izumori and Professor Dr. Shigeru Hayakawa from Rare Sugar Center). The TLC plate was placed in the developing tank by using 5 mobile phase system such as system1 acetonitrile : water (70:30) , system 2 n-butanol : ethanol :water(50: 30:20), system 3 ethyl acetate : acctic acid :methanol: water (60:15:15:10) system 4 n-propanol : NH_3 conc.:water (50:30:20) and system 5 n-butanol :pyridine: water(60:40:20) and using 4 spraying agent systems such as 20% H_2SO_4 , H_2SO_4 : methanol (1:1), 5% $AgNO_3$ and 3,5-dinitrosalicylate. The extracted solvent from mushroom was studied by variation of ethanol concentration to 50, 75 and 100 % and change the extraction solvent to be 25 ,50 , 75 and 100 % acetone too.

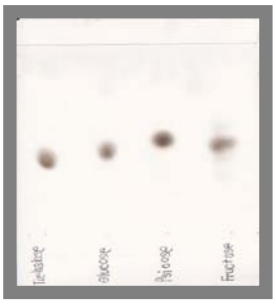
From the above condition, the mushrooms were boiled for 5 min. and extracted with appropriate solvent as 25% acetone .Then 5 µl of each extracted solution were spotted in TLC and developing chromatogram by the best condition .

Part 2. Quantitative Analysis of Trehalose and Psicose in edible mushroom (Ferreira, 1997)

The raw mushrooms and boiled mushrooms were extracted by acetone or ethanol, the filtrate were filtered through 250µm of cellulose membrane filter before injected to the Lichrosorb-NH_2 column (300 mmx 25 mm()purchase from Phenomenex) and detected with refractometer detector.The column was controlled temperature at 50 ^oC and eluted with acetonitrile: water at 70 : 30 by isocratic system at 1 ml/min flow rate. The chromatogram of raw and boiled mushroom were recored and calculated the contents of each sugar by compared with standard glucose , standard trehalose and standard psicose.

RESULTS AND DISCUSSION

From part 1 ,the appropriate mobile phase system in separation was studied and detection with 4 systems, the result showed that TLC chromatogram from the developing mobile system 1 (acetonitrile : H_2O = 70:30) by using 4 detection method showed in figure 1 – 4.



1 2 3 4

Figure 1 Chromatogram of standard Sugar by spraying with methanol : H_2SO_4 (1:1)

1 2 3 4

Figure 2 Chromatogram of standard Sugar by spraying with 20% H_2SO_4

1 2 3 4

Figure 3 Chromatogram of standard Sugar by spraying with 5% $AgNO_3$

1 2 3 4

Figure 4 Chromatogram of standard Sugar by spraying with3,5dinitrosalicylate

Note: 1 = trehalose standard 2 = glucose standard

3 = psicose standard 4 = fructose standard

From the above chromatogram , showed the R_f value of the sugar spots as in table 1

Table 1 R_f values of standard sugars

Sample type	Rf
trehalose	0.31
glucose	0.41
fructose	0.45
psicose	0.50

The result of sugar separation from mushroom showed in figure 5.

a b c d e f g h i j k l m n o p Q r s t u v w x y 1 2 3

Figure 5 Chromatogram of separated sugar from 3 mushrooms.

Note: a - j = raw cloud ear mushroom extract – boil cloud ear mushroom extract

k – s = raw straw mushroom extract – boil straw mushroom extract

t - y = raw shitake mushroom extract – boil shitake mushroom extract

ı = trehalose standard 2= glucose standard 3 = psicose standard

From part 2 ,trehalose and psicose sugar showed the peak at the retention time of each standard on HPLC chromatogram and calculated their contents as showed in figure 6. After mushroom were boiled at 100 ^{o}C for 5 – 60 mins , the sugar content in each condition showed as in figure 7 -9.

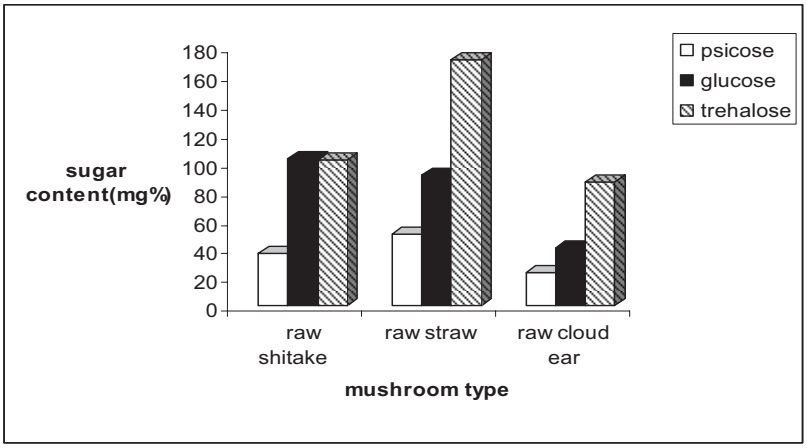


Figure 6 sugar contents in raw mushroom

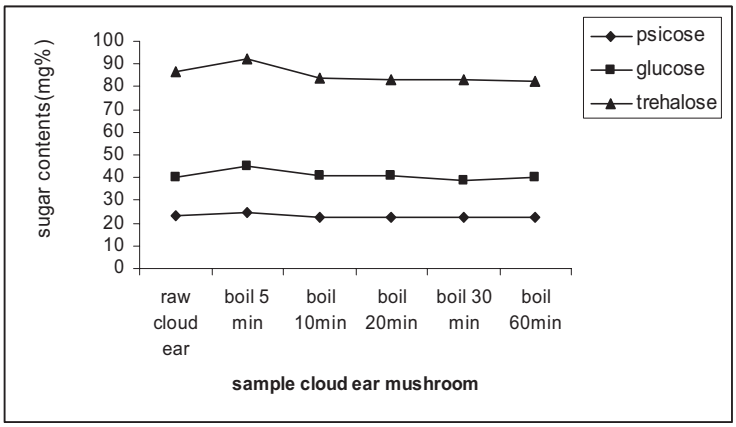


Figure 7 sugar contents in raw cloud ear mushroom and boil mushroom

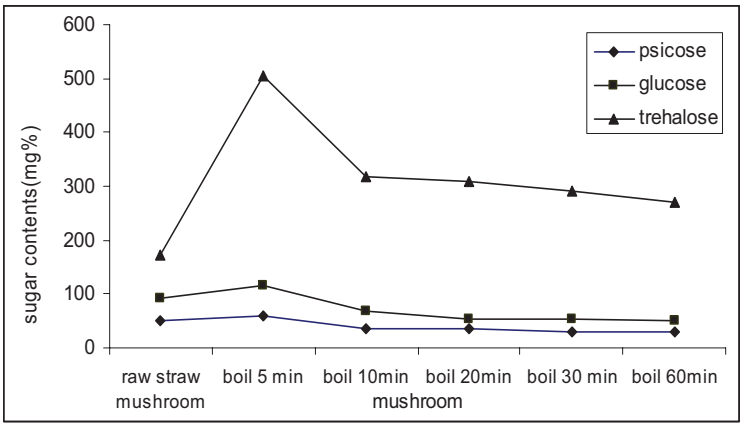


Figure 8 sugar contents in raw straw mushroom and boil mushroom

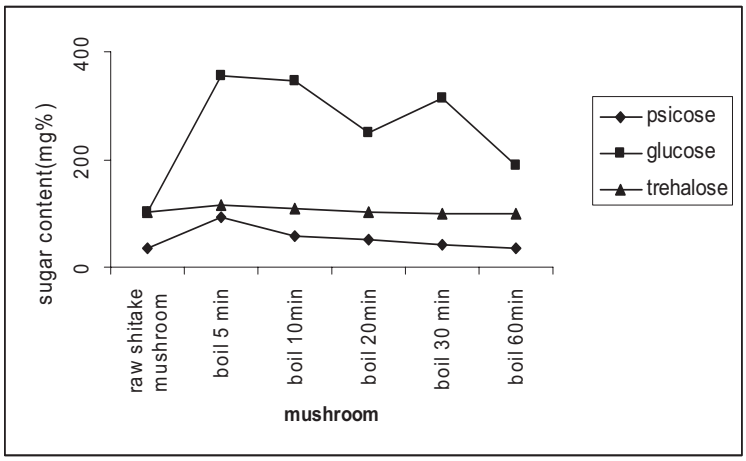


Figure 9 sugar contents in raw shitake mushroom and boil mushroom

CONCLUSION

From part 1, the best condition for sugar detection was the sytem 1 that used the mobile phase as acetonitrile : water (70: 30) with the 20 % H_2SO_4 as the spraying solvent for spot detection. Since the above condition showed the best clearly spot with difference R_f value of each standard sugar. However, the spot of fructose could not be detected by all experiment conditions.After the extracted sugar from each mushroom were treated with the best condition as in figure 5, the spot of sugar clearly showed only at R_f of trehalose.This present that the qualitative test only gave the data about trehalose but the psicose could not present spot. The result of quantitative experiment in part 2 by HPLC –RID technique showed that there were all three types of sugar in each mushroom as shown in figure 5.The trehalose contained the most content about 90-178 mg% in all raw mushrooms and the psicose sugar found in the range of 30 – 50 mg%. After the boiling or cooking process by heating the raw mushrooms at 100^{o}C for 5 – 60 minutes, all sugars trended to change their contents as showed in figure 7-9. However, after heated mushrooms for 5 minutes, the three sugars contained the highest content but all sugars content trend to decrease after heating at 10 min.The result from this experiment presented that the cooking mushroom for 5 minutes gave the maximum content of trehalose and psicose sugar but cooking for along time the valuable rare sugar will lost from the mushroom.

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