

Research Article

Evaluation of rare sugar content in edible mushroom

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Abstract

There are many types of mushroom, some can be used as food for human consumption and are known as edible mushrooms, while there are also toxic mushrooms. Edible mushrooms are a popular vegetable for vegetarian people since they contain higher protein content than most other vegetables and are also rich in many minerals and vitamins. In this work, trehalose and psicose were the interested rare sugars of interest which were separated from three popular edible mushrooms; *Lentinus edodes* (Shitake mushroom), *Volvariella volvacea* (Straw mushroom) and *Auricularia polytricha* (Cloud ear fungus). Both rare sugars were analysed by TLC qualitative test and their contents determined by HPLC-RID. The separation was studied in standard trehalose, standard psicose and standard glucose systems by 5 mobile phase systems. These were; System 1 acetonitrile : water (70:30), System 2 n-butanol : ethanol : water (50: 30:20), System 3 ethyl acetate : acetic acid : methanol : water (60:15:15:10), System 4 n-propanol : NH₃ conc. : water (50:30:20) and System 5 n-butanol : pyridine : water (60:40:20) and using 4 spraying agent systems such as 20% H₂SO₄, H₂SO₄ : methanol (1:1), 5% AgNO₃ and 3,5-dinitrosalicylate. The extracted solutions from those mushrooms were analysed for trehalose, psicose and glucose by TLC and HPLC using Lichrosorp-NH₂ as separated column and using acetonitrile : water (70:30) as mobile solvent with 1ml/min flow rate with Refractive index detector. The boiled mushrooms (at 100°C) were also analysed for sugar content. The result showed that the best mobile system for TLC qualitative test was System 1 and detection of spot on chromatogram with 20% H₂SO₄. The trehalose and glucose sugar were clearly presented in the chromatogram of each fresh mushroom but psicose was not shown as a clear spot. From the HPLC work, psicose could be detected and also the lowest content in each mushroom. The trehalose and psicose contents presented in all fresh mushroom were less than found in cooked mushroom.

Keywords: *Lentinus edodes*, *Volvariella volvacea*, *Auricularia polytricha*, HPLC-RID, TLC, trehalose, psicose, glucose, Thailand

Introduction

There are many kinds of mushroom in Thailand, some of them are cultivated as food while others may grow naturally in forests and are harvested as food and are 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 some of the more common mushrooms found in many food products. All three are available throughout the year and are sold in almost every market in Thailand. There are many reports about the nutrition advantages of these mushrooms, especially Shitake [1]. The production of Shitake mushroom has increased at a faster pace in Thailand due to its expensive price. Straw mushroom and Cloud ear fungus are the more common mushrooms with cheaper price than Shitake and are frequently used in cooking. Each mushroom differs in their size, colour and taste. The taste component of Straw mushroom has been reported by a number of researchers [2, 3]. Several studies have been carried out on the chemical composition and nutritional quality of edible mushroom [4, 5, 6]. Trehalose is one of the sugars reported as being found in many mushrooms [7], but there has been no report regarding psicose sugar. In this work, the rare sugar content, trehalose and psicose, were separated from these three edible mushrooms and qualitatively checked by Thin Layer Chromatography (TLC) and their contents analysed by High Performance Liquid Chromatography with refractive index detector. The studies were undertaken with both fresh mushroom and boiled mushroom to determine the variation in sugar content.

Methodology

Qualitative analysis of trehalose and psicose in edible mushroom by TLC

The three mushrooms were purchased from the fresh market in Bangkok, then chopped and weighed at 10.0000g and placed in a 25% ethanol solution (pure ethanol AR grade purchased from Merck) and left at room temperature for 1 hr. The mushroom residues were filtered out and the filtrates were spotted on TLC plates (commercial plate from Chromatographia) and compared with standard sugar such as glucose, fructose, trehalose (purchased from Fluka) and psicose (donated by the Rare Sugar Center, Japan). The TLC plates were placed in the developing tank by using 5 mobile phase systems; System 1 acetonitrile : water (70:30), System 2 n-butanol : ethanol : water(50:30:20), System 3 ethyl acetate : acetic acid : methanol : water (60:15:15:10), System 4 n-propanol : NH₃ conc. : water (50:30:20) and System 5 n-butanol : pyridine : water (60:40:20) and using 4 spraying agent systems such as 20% H₂SO₄, H₂SO₄ : methanol (1:1), 5% AgNO₃ and 3,5-dinitrosalicylate [10]. The extracted solvent from the mushrooms was studied by variation of ethanol concentration to 50, 75 and 100% and changing the extraction solvent to be 25, 50, 75 and 100% acetone.

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

Quantitative analysis of trehalose and psicose in edible mushroom [8]

The raw mushrooms and boiled mushrooms were extracted by acetone or ethanol and the filtrate filtered through 250µm of cellulose membrane filter before being injected into the Lichrosorb-NH₂ column (300 mmx 25 mm) (purchased from Phenomenex) and detected with refractometer detector. The column was controlled temperature at 50°C and eluted with acetonitrile : water at 70:30 [11] by isocratic system at 1 ml/min flow rate. The chromatogram of raw and boiled mushroom were recorded and the contents of each sugar calculated by

comparison with standard glucose , standard trehalose and standard psicose.

Results and Discussion

From the first experiment, the appropriate mobile phase system in separation was studied and detected with 4 systems, the result showed that TLC chromatogram from the developing mobile System 1 (acetonitrile : H₂O = 70:30) by using 4 detection methods as shown in Figures 1–4.

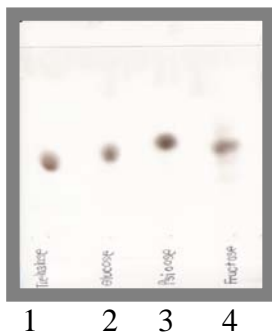


Figure 1. Chromatogram of standard sugar by spraying with methanol : H₂SO₄ (1:1)

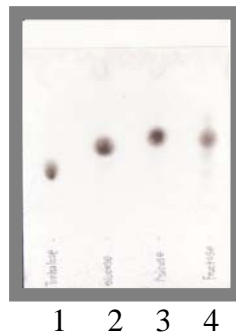


Figure 2. Chromatogram of standard sugar by spraying with 20% H₂SO₄



Figure 3. Chromatogram of standard sugar by spraying with 5% AgNO₃



Figure 4. Chromatogram of standard sugar by spraying with 3,5 dinitrosalicylate

Note: 1 = trehalose standard 2 = glucose standard
 3 = psicose standard 4 = fructose standard

From the above chromatogram, the R_f values of the sugar spots is as shown in Table 1.

Table 1. R_f values of standard sugars.

Sample type	R _f
trehalose	0.31
glucose	0.41
fructose	0.45
psicose	0.50

The result of sugar separation from the mushrooms is shown in Figure 5.



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
 1 = trehalose standard 2 = glucose standard 3 = psicose standard

From the second part of the experiment, trehalose and psicose sugar showed peak retention times at each standard on HPLC chromatogram and their contents were calculated as shown in Figure 6. After mushrooms were boiled at 100°C for 5 – 60 m, the sugar content at each condition is as shown in Figures 7-9.

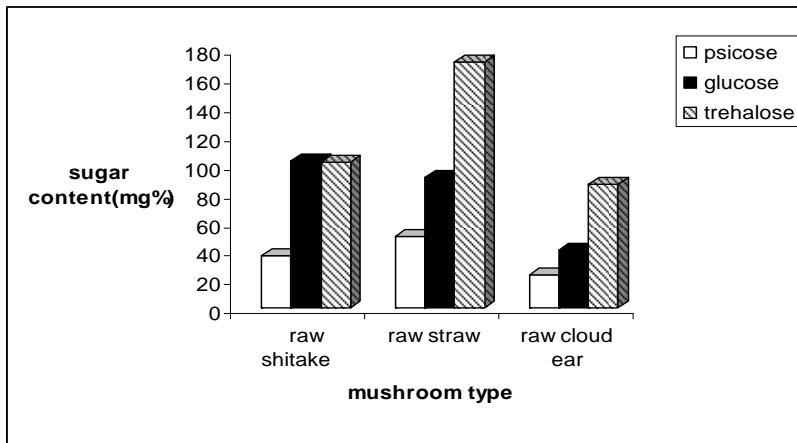


Figure 6. Sugar contents in fresh mushroom.

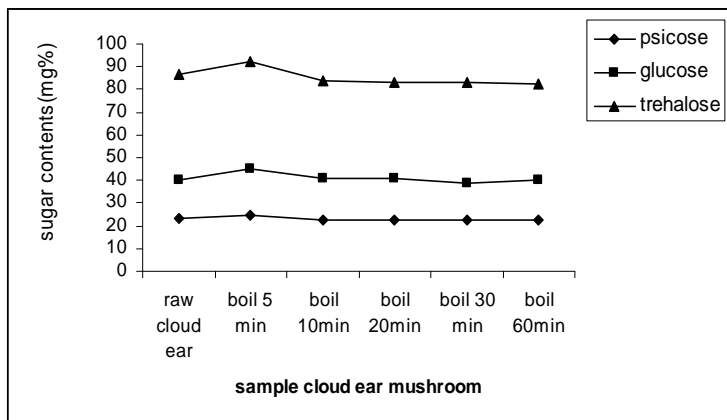


Figure 7. Sugar contents in fresh cloud ear mushroom and boiled mushroom.

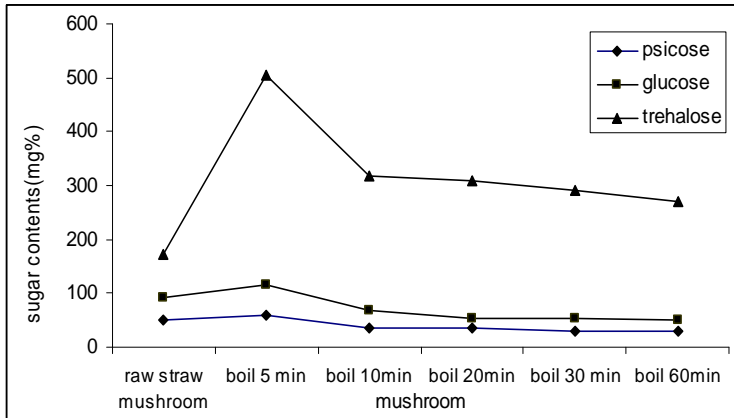


Figure 8. Sugar contents in fresh straw mushroom and boiled mushroom.

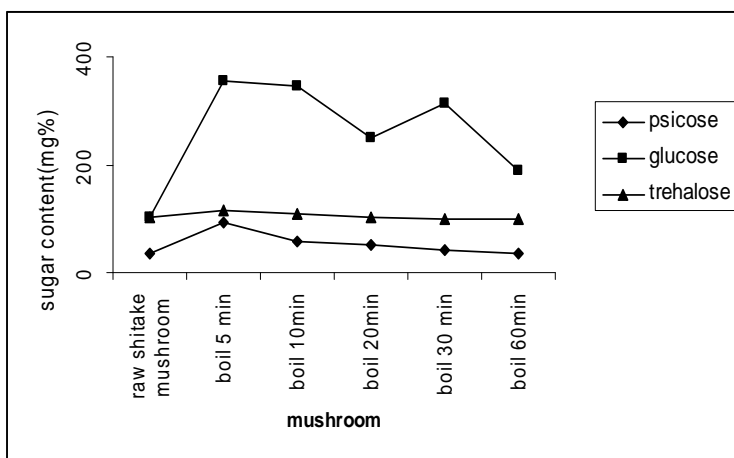


Figure 9. Sugar contents in fresh shitake mushroom and boiled mushroom.

Conclusions

From the initial analysis, the best condition for sugar detection was Sytem 1 that used the mobile phase as acetonitrile : water (70:30) with 20% H₂SO₄ as the spraying solvent for spot detection, since the above condition showed the best clear spots with different R_f values of each standard sugar. However, the spot of fructose could not be detected by all experiment conditions. After the extracted sugar from each mushroom was treated with the best condition as in Figure 5, the spot of sugar clearly showed only at R_f of trehalose. This shows that the qualitative test only provided data for trehalose, while spots for psicose could not be detected. The result of quantitative experiment in the second analysis by HPLC –RID technique showed that there were all three types of sugar present in each mushroom as shown in Figure 5. The trehalose had the highest content at about 90-178 mg% in all raw mushrooms and the psicose sugar was found in the range of 30–50 mg%. After the boiling or cooking process by heating the raw mushrooms at 100°C for 5–60 minutes, all sugars tended to change their contents as shown in Figures 7-9. However, after heating the mushrooms for 5 minutes, the three sugars showed peak content, but all of these tended to decrease after heating for 10 minutes. The results from this experiment indicated that cooking the mushrooms for 5 minutes gave the maximum content of trehalose and psicose sugars, while cooking for a longer time will result in the loss of the valuable rare sugars from the mushroom.

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