Total Sugar Content in Healthy Drinks of Oyster Mushroom (Pleurotus ostreatus) as Beta-Glucan Resources

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ABSTRACT

Many oyster mushroom cultivation in several regions in Indonesia. To increase the value added necessary post-harvest effort that has commercial value. Fruit bodies of oyster mushrooms (*Pleurotus ostreatus*) was characterised as a source of polysaccharides, as one of the alternative good nutritionally for food, relatively inexpensive, can be as immunomodulators or immune system enhancer. Given some of these properties, Team Mushroom, Centre of Bioindustrial Technology-BPPT have conducted experiments based drinks manufacturing of oyster mushrooms plus natural fruit flavorings and sweeteners sucrose. The purpose of this experiment was to determine the proximate and total sugars content contained therein. The mushroom extract done manually, ie 500 mesh filter cloth, while the total sugar analysis by Anthrone method. Result analysis of the healthy drink bottle 140 ml volume, the energy contained 37, 24 kcal, protein levels of 392 mg, 8918 mg levels of carbohydrates, dietary fiber 70 mg, and 1380 mg of beta-glucan, while the results analysis of total sugar with Anthrone method is 8.8% (w / v). Recomendation of this experiment were re-optimization in formulations, especially in sweetener.

Key words: *Pleurotus ostreatus*, healthy drinks, proximate, beta glucan, Anthrone method, the total sugar

INTRODUCTION

More than 2000 species of mushrooms exist in nature but only approximately 22 species are intensively cultivated, for commercial purposes (Manzi et al., 2001). Alongside the mushrooms' long history as a food source is an equally long history of beliefs about their curative abilities in traditional medicine systems—both the folk medicine of the western world and traditional medicine of the orient. Although there are limited direct human intervention trials, there is a rapidly growing volume of in vitro and in vivo animal trials describing a range of possible health benefits including immunomodulatory, anti-tumor, anti-microbial effects and hypocholesterolemic effects (Roupas et al., 2012).

Preliminary reports indicated that diet containing 4-10 % dried fruiting body of Pleurotus species show more reduction in the arterial pressure and blood cholesterol level when compared to normal diet in rabbits and rats. Lovastatin, a drug, used in the lowering blood cholesterol level, produced by cells Pleurotus ostreatus was approved by FDA in 1987. When dried mushroom mixed in the diet of experimental animal acted as accelerator of HDL (high density lipoprotein), reduced production of VLDL (very low density lipoprotein), .LDL (low density lipoprotein), cholesterol, reduced cholesterol absorption and reduced HMG-CoA reductase that acted activity in the liver (Patel et al., 2012)..

Some of the more efficacious compounds in mushrooms are 1,6-branched 1,3-β-glucans which have been reported to inhibit tumor growth by stimulating the immune system via activation of macrophages, via balance of T helper cell populations and subsequent effects on natural killer (NK), cells and also via cytokine production (Hetland et al, 2011). Other work has implicated polysaccharides with varying sugars such as beta- and alpha-glucans (Borcher et al., 2008). Such mushroom polysaccharides are beginning to be evaluated as adjuvant cancer therapy compounds alongside conventional cancer treatments (Standish et al., 2008). Beta glucan on oyster mushrooms (Pleurotus ostreatus) can be as enhancing the immune system (Widyastuti, 2014).

Among the 1,300 known types of citrus, lime (Citrus aurantifolia), have the most benefit. In 100 g of lime, there are calories 51 calories, 0.9 g protein, 0.2 g fat, 11.4 g carbohydrate, 0.5 g minerals, calcium 33 mg, phosphorus 23 mg, iron 0.4 mg and sour 49 mg ascorbate (Vitamin C) efficacious antioxidant. Lime also contains citric acid, amino acids (tryptophan, lysine), essential oils, resins, glycosides, citric acid, fat, sulfur, vitamin B1. In Indonesia, lime is often used to solve various kinds of diseases such as dysentery, constipation, hemorrhoids, cough, body odor, flu, fever, too fat, tonsillitis,

nosebleeds, nasal inflammation. Lime is also effective in preventing the onset of kidney stones. Citrus aurantifolia (Christm) is the most widespread significant cropped and consumed lime species in Iran. This fruit which is found as a healthy fruit for a long time has antioxidative activity (Miyake et al., 1997).

Given some of these properties, Team Mushroom, Centre of Bioindustrial Technology-BPPT have conducted experiments based drinks manufacturing of oyster mushrooms plus natural fruit flavorings and sweeteners sucrose. The purpose of this experiment was to determine the proximate and total sugars content contained therein. The mushroom extract done manually, ie 500 mesh filter cloth, while the total sugar analysis by Anthrone method.

MATERIALS AND METHODS

Materials incorporated were healthy drink, a mixture of lime extract (*Citrus citrifolia*) with beta-glucans from extracts of oyster mushroom (*Pleurotus ostreatus*), and lump sugar (sucrose). Oyster mushroom from CV Asa Agro Corporation, Cianjur, West-Java and lime from Serpong traditional market.

Equipments used were scales (Radwag digital WAS/C/2), blender (Philip Philips HR 2071, autoclave (Merk. ALP), spectrophotometer (Hitachi U-2001), cuvettes, erlenmeyer, glass beaker,measuring cup, stir, and separator centrifuge. Total procedure Anthrone Sugar Method (Apriyanto *et al.* 1989) materials were 200 ppm standard solution of glucose (20 mg in 100 ml of distilled water) and 0.1% anthrone reagent (0.1 g in 100 ml of concentrated sulfuric acid).

Preparation of Standard Curve

Into a test tube with a lid, pipette standard stock solution of 200 ppm glucose by 0.2; 0.4; 0.6; 0.8; and 1 ml, then diluted so that the total volume of each tube was 1 ml. Create a blank solution containing 1 ml of distillate water. Into each standard glucose solution and the blank, quickly add 5 ml of anthrone reagent and closed. Vortex and shake until evenly distributed. Heat the test tube above 100^{o}C water bath for 12 minutes. Once cool transfer the solution into a cuvette and read the absorbance using UV-Vis spectrophotometer with a wavelength of 630 nm. Create a standard curve plot.

Measurement of health drinks oyster mushroom samples

A total of 1 ml of sample was added to 5 ml of reagent anthrone quickly and closed. Vortex and shake until evenly distributed. Heat the test tube above 100^{o}C water bath for 12 minutes. Once cool transfer the solution into a cuvette and read the absorbance using UV-Vis spectrophotometer with a wavelength of 630 nm.

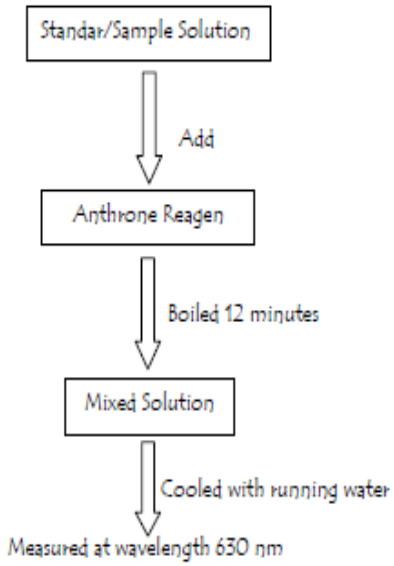

Figure 1. Anthrone analysis procedure

Content Determination and Calculation

Luo and Peng (2012) stated that in the experiment get all the samples got from preprocessing, and each sample needs three parallel experiments of which each parallel experiment needs three repeatitions. Take nine dry clean tubes and number them; then add sample solution, tri-distilled water, anthrone reagent in turn into the tubes and put them in boiling water for 12 minutes. After that, move them quickly into icy water to cool down, then put them in the dark for 12 minutes to test the absorbance in wave length of 630 nm. Absorbance obtained included a standard curve equation to obtain the total concentration of the sugar in the healthy drink of oyster mushroom .According to the formula (*w*: sugar mass fraction (%); *C*: sugar mass fraction from standard curve (mg/ml); *V*: the sample's volume after dilution (ml); *m*: the sample quality (mg)), calculate the sugar content in each sample.100%

$$W = \frac{C \times V}{m} \times 100\ \%$$

Proximate analysis

Levels of Water (Apriyantono *et al*., 1988)

Previously empty bowls oven at 105^{o}C for about 1 hour, put into a desiccator for 15 minutes, then weighed as heavy empty bowls. 1 gram sample is weighed, then oven at 105^{o}C for 5 hours, input to decicator to be cooled for 15 minutes, then weighed to determine the weight of the sample after oven. Calculated water content was 130.505 g (93.2176 % w/w).

Levels of Protein, Kjeldahl Method (Apriyantono *et al.*, 1988)

0.1 grams of sample plus 0.4 grams of a mixture of concentrated sulfuric acid and selenium as much as 5 mL, were then destructed for 2.5 hours. Results of destruction cooled, then added 30% NaOH by 20 mL. Sample distilled until the distillate volume reaches approximately 100 mL, which previously had been given a container of distilled 10 mL of 2% boric acid has been added BCGMR indicator (*Brom Cresol Green-Methyl Red*). Distillate is then titrated with 0.01 N HCl until the pink color (pink). Calculate the volume of HCl is used to titrate the solution sampel. Calculate its protein content. Levels of protein is 392 mg.

Dietary Fiber

The definition of dietary fiber (DF) proposed by American Association of Cereal Chemists (AACC) defines DF being made up of edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine as well as having beneficial physiological effects such as laxation, blood glucose attenuation and/or blood cholesterol attenuation. More specifically, dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in humans.

These non-digestible carbohydrate (NDC) polymers should occur naturally in the food as consumed and have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities. Dietary fiber and high-fiber food products have attracted great attention because of their significant health benefits to consumers. Mushrooms are valuable resources for food, medicine and nutraceuticals. Edible mushroom is considered as a novel source of dietary fiber. The dietary fiber content and composition in edible mushroom vary greatly with its morphological stages including fruit body, mycelium and sclerotium (Cheung, 2011). Dietary fiber (AOAC 985 29 2005 method) is 70 mg.

Levels Carbohydrate Proximate Methods

Carbohydrate levels can be determined by proximate, that is calculated by the following formula: Carbohydrates = 100% - (water + protein + fat +ash) = 8918 mg.

Levels Energy

Energy = (4 Kcal/g x carbohydrate content) + (9 Kcal/g x fat content) + (4 Kcal/g x protein content) = (4 Kcal/gx8.918 g) + (9 Kcal/g x0) + (4 Kcal/g x 0.392) = 37.24 Kcal.

Beta Glucan

Digital scales Kris Chef, Philips blender to smooth mushrooms, pot to boil, stir, filter cloth of 500 mesh to take the filtrate. Healthy drink from the filtrate Oyster mushroom, suspense enzyme exo-

1,3-_- glucanase and _-glucosidase, an enzyme amiloglucosidase, the enzyme invertase, glucose reagent, glukosaoksidase, peroxidase, 4- aminoantipyrine, control _-glucan yeast Megazyme, sodium acetate, potassium hydroxide. The compound beta-glucan obtained by boiling based on the principle of heating with distilled water at a temperature of 100 ° C for 1 hour, followed by precipitation by ethanol at 4 ° C for 24 hours andends with drying freeze drying. The resulting cake is then extracted three times over until the extract. The dry extract obtained compounds measured levels of beta-glucan with Enzymatic Kits Megazyme method. Levels of beta glucan 1380 mg.

RESULTS AND DISCUSSION

The use of methods for the analysis of total carbohydrate Anthrone began to grow since first use by Dreywood in 1946 for a qualitative test. The basis of this reaction is the ability of carbohydrates to form furfural derivatives in the presence of acid and heat, which is then followed by reaction with Anthrone that produce blue-green. Anthrone, $C_6H_4COC_6H_4CH_2$, are derivatives of anthraquinone. These compounds are produced by the catalytic reduction of anthraquinone by the presence of hydrochloric acid with tin. These compounds may exist in the keto or enol forms, each of which is known by the name Anthrone and anthranol.

The principle of the determination of the total sugar content by the method of Anthrone namely carbohydrates in sulfuric acid will be hydrolyzed into monosaccharides which will then be dehydrated by sulfuric acid into HMF (Hydroxy methyl furfural), furfural compounds react with Anthrone (9,10-dihydro-9 oxanthracene) form blue-green compound, and then calculated the absorbance at a wavelength of 630 nm. The mechanism of the formation of color Anthrone with sugar have been investigated. Hurd and Isenhour (1932) and Wolfrom *et al.* (1948) postulated that the carbohydrates and their derivatives undergo ring formation in the presence of a strong mineral acid, as shown for glucose:

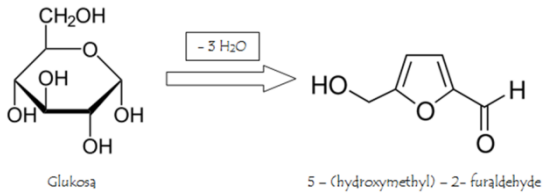

Figure 2. Breakdown of glucose to 5- (hydroxymethyl) -2-furaldehyde

Each stage is the breakdown of glucose to 5- (hydroxymethyl) -2-furaldehyde indicate dehydration either at the double bond or ring formation. The formation of the green color of the reaction depends on the presence Anthrone 5- (hydroxymethyl) -2-furaldehid, or compounds similar furfural, which formed by reaction of sulfuric acid on carbohydrates. Three moles of Anthrone react with 1 mole of glucose, which is described in the equation:

$$3C_{14}H_{10}O + C_6H_{12}O_6 \rightarrow C_{47}H_3O_{30} + 5H_2O + CH_2O$$

Anthrone test has advantages in terms of sensitivity and simple test. A little carbohydrate can provide color detected by using a spectrophotometer. Dreywood (1946) to test the specificity of the reaction and make a list of 18 types of carbohydrates, including some cellulose derivatives, which gave positive results. Disadvantages of the method were the instability of the reagent Anthrone (Anthrone dissolved in sulfuric acid), so we need a new reagent preparation every day.

The heat generated by the sulfuric acid leaching is an important part of the test. Significance of heat on Anthrone reaction showed that the amount of carbohydrate that is given, the intensity of the color varies with the amount of heat generated. Therefore the standard curve also needs to be made every day.

Drawing the Standard Curve D

Raw the standard curve (Figure 3) with concentration gradient as abscissa and peak area as ordinate, and get the regression equation: Y = 0,0004x+0,002, R2=0.997, and the chromatogram of caffeine standard curve is shown as Figure1.

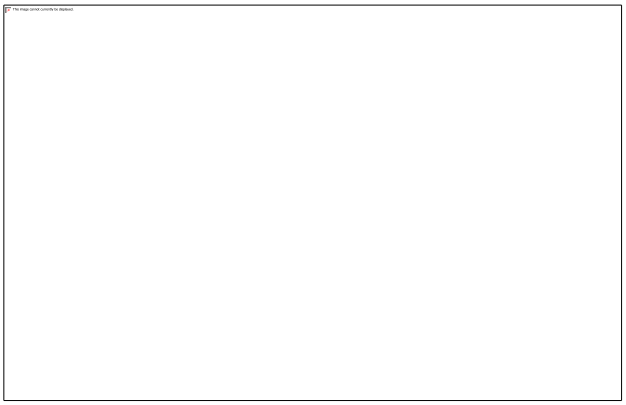

Figure 3. Absorbance data standard curve anthrone method

Table 1. Level total sugar healthy drink of oyster mushroom

Sample	FP	Absorbance	Consentration (ppm)	Level total sugar (% w/v)
Healthy drink of oyster mushroom	500	0.7775	88068	8.8

Result analysis of the healthy drink bottle of 140 ml volume, the energy contained 37. 24 KKcal, protein levels of 392 mg, 8918 mg levels of carbohydrates, dietary fiber 70 mg, and 1380 mg of beta-glucan, while the results analysis of total sugar with Anthrone method was 8.8% (w/v).

Figure 4. Discoloration reaction after the addition of reagent

Total sugar healthy drink of oyster mushrooms showed 8.8% w / w, compared with the minimum requirement of 6% (SNI 01-3143-1992). The results showed that no sign of contamination in the 1st and 2nd month.

However, in the 3rd month indicated microbial contamination has been started. The result showed, at refrigerator temperature (4^0C) in the 3rd month = 9 x 10^2 cells / ml, and after 8 months in storage = 2.6 x 10^2 cells / ml. Requirements TPC (Total Plate Count) max 2.0 x 10^2. To increase, that the shelf life of healthy drinks oyster mushrooms further research to better sterilization is needed increased to reached 12 months or more.

Despite not having a problem with blood sugar, sugar consumption should also be restricted. The daily requirement of maximum daily sugar 4 tablespoons (52 grams) and a maximum of 1 tablespoon (13 grams) per day for people with uncontrolled diabetes. If diabetes is not controlled, then choose the

product that is naturally low in sugar, or which has the label No added sugar (no added sugar) or sugar free (sugar free). need for further research on the beverage sweetener that is safe for people with diabetes mellitus example is stevia to replace sucrose.

Based on the research results Taufik *et al*. (2012), showed that the higher the concentration of sucrose is added, the higher the total sugar content in bottled tea beverages. This is due to the sucrose is hydrolyzed by heat, sucrose is used in the manufacture of tea beverages dissolved in hot water so that some will break down sucrose into glucose and fructose or the so-called invert sugar.

Table 2.The quality requirements bottled tea beverages in accordance with SNI 01-3143-1992

No	Description	Unit	Requirements
1.	Status		
	1.1.Sightings		Clear
	1.2.Smell and taste		Typical tea
2.	Teina/caffeine		positive
3.	Tannins		positive
4.	Total sugar as sucrose, %, w/w		Min 6
5.	Food additives :		in accordance with SNI 0222-M and the Regulation of the Minister of Health No 722/Men.Kes/Per/IX/88
	5.1. Preservative		
	5.2.Artificial sweeteners		
6.	Metal contamination		
	6.1 Pb, mg/kg		Max 0,2
	6.2.Cu, mg/kg		Max 2,0
	6.3.Zn, mg/kg		Max 5,0
	6.4. Sn, mg/kg		Max 40,0
	6.5. Hg, mg/kg		Max 0,03
7.	As, mg/kg		Max 0,1
8.	Microbial contamination		
	8.1.Total plate count	Coloni/ml	Max 2,0 x 10^2
	8.2.Coliform bacteria	APM/100 ml	< 2,2
	8.3.E *coli*		Negative/100 ml
	8.4.Samonella		Negative/100 ml
	8.5.C *pertingens*		Negative/10 ml

This was confirmed by Desrosier (1988) which revealed that the total sugar content determination is the determination of sugar before inversion or reducing sugar and glucose measurements after inversion (sucrose). During the boiling solution of sucrose in the presence of acid hydrolysis process will occur produce reducing sugars (dextrose and levulose). Sucrose is converted into reducing sugar and the result is known as invert sugar. Speed is affected by temperature inversions, heating time and pH value of the solution. During heating a solution of sucrose into glucose and fructose due to the influence of the effect of heat and acid will increase the solubility of sucrose. With the increased solubility of sucrose will increase the total sugar content.

CONCLUSION

1. Preliminary reports indicated that diet containing 4-10 % dried fruiting body of *Pleurotus* species show more reduction in the arterial pressure and blood cholesterol level when compared to normal diet in rabbits and rats. Lovastatin, a drug, used in the lowering blood cholesterol level, produced by cells *Pleurotus ostreatus* was approved by FDA in 1987
2. Healthy drink of oyster mushroom experiments based drinks manufacturing of oyster mushrooms plus natural fruit flavorings (*Citrus aurantifolia),* and sweeteners of sucrose.
3. The proximate content bottle was 140 ml volume, the energy contains 37.24 Kcal, 392 mg of protein, 8918 mg of carbohydrates, 70 mg of dietary fiber, and 1380 mg of beta-glucan.
4. Anthrone test has advantages in terms of sensitivity and simple test. A little carbohydrate can provide color detected by using a spectrophotometer.
5. Total sugar healthy drink of oyster mushrooms showed 8.8% w/w, compared with the minimum requirement of 6% (SNI 01-3143-1992).

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