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Product development of mushroom beverage with high β-glucan content from local mushrooms

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Abstract Mushrooms are fungi with a high nutritional value. β-Glucan is an interesting functional component of mushrooms. This compound is showed powerful medicinal properties. The local edible mushrooms with high β-glucan content was reported. Result showed that fifteen species of native mushroom were analyzed for their β-glucan content. It was found that the highest β-glucan content was exhibited in *Schizophyllum commune* Fr. (39.08 ± 5.82 %w/w). However, there was only a non-significant difference ($p \leq 0.05$) from *Pleurotus ostreatus* (Fr.) Kummer and *Pleurotus sajor-caju* (Fr.) Sing (37.01 ± 1.35 and 34.02 ± 3.06 %w/w, respectively). These species were chosen to demonstrate product development of high β-glucan content in mushroom drink. Results found that treatment 1 (*S. commune* Fr: *P. ostreatus* (Fr.) Kummer: *P. sajor-caju* (Fr.) Sing) showed the highest significance ($p \leq 0.05$) of β-glucan content. It is strongly suggested that the levels of phenolic compounds, β-glucan content and radical scavenging antioxidant activity depended on the amount of *S. commune* Fr. It concluded that *S. commune* Fr. could be expressed as a potential medicinal mushroom drink. Furthermore, this investigation provides a guideline for the selection of native mushrooms to develop a new functional drink in the future.

Keywords: β-Glucan, Mushroom , *Schizophyllum commune* Fr., Product development

Introduction

Mushrooms have been used as food and medicine in different countries for a long time, especially in Japan, China and Korea (Manzi and Pizzoferrato, 2000; Hesham *et al*., 2002). Thailand has cultivated various mushrooms. Previously research reported that mushrooms are an important source of novel bio-active compounds (Rathore *et al*., 2017). Moreover, many studies informed that mushrooms become attractive as a functional food and ~~as~~ a source to develop drugs and nutraceuticals. Recently, many studies have illustrated that mushrooms are shown to be the medicinal properties. These studies attempted

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to highlight the immunomodulating, antitumor, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, hepatoprotective, antidiabetic effects antioxidants and prebiotic activity(Aida *et al*., 2009; Wasser, 2010; Mirfat *et al*., 2014). Also, they are potentially reported as a great nutritionally functional food source and a beneficial non-toxic medicine (Wasser and Weis, 1999). In addition, Manzi *et al*. (1999) described mushrooms as offering nutritional values much more than texture and flavor. Vitamins, such as thiamin, riboflavin and ascorbic acid and minerals are presented a significant values of mushrooms (Breene, 1990). As for their nutritional values, edible mushrooms have been found to be low in calories and fat, but rich in proteins, minerals and dietary fibre (Cheueng, 2013; Rathore *et al*., 2017). They are also a potential source of functional components of dietary fibre and β-glucans. β-Glucans are groups of polysaccharides that are composed of glucose units linked together with β-glycosidic bonds, associated with health benefits (Klis *et al*., 2002). β-Glucans also exhibit medicinal properties, such as antitumor, antimicrobial and antioxidant activities, mycotoxin absorption (Manzi and Pizzoferrato, 2000; Chen and Seviour, 2007) in the stimulation of the immune response in animals and the reduction of blood cholesterol and glucose levels (Nicolosi *et al*., 1999).

Okamura *et al*. (2001) reported to produce wine by mushroom fermentation, which indicated mushroom wine seemed to be a functional food and possible a preventative effect against cancer and thrombosis. In terms of our research, we studied the product development of fruit tea mixed with “Hed Krang” (*S. commune* Fr.) in 2013 and assessed the product development of *Garcinia cowa* Roxb tea mixed with high β-glucan content from edible mushrooms in 2016 (Mongkontanawat, 2013; Mongkontanawat and Phuangborisut,2016). From our previous research, The eleven local mushrooms *Schizophyllum commune* Fr.(Hed Krang), *Pleurotus ostrearus* (Hed Pao Hea), *Lentinus polychrous* (Hed Kon), *Pleurotus ostreatus* (Hed Nang Rom Hanggari), *Pleurotus djamor* (Hed Nang Nuan), *Hypsizygus marmoreus* (Hon-Shimeji) (Hed Shimeji Dum), *Auricularia fuscosuccinea* (Hed Hunu Dum), *Pleurotus sajor-caju* (Hed Nang Fa), *Hypsizygus marmoreus* (Buna-Shimeji) (Hed Shimeji Kaw), *Flammulina velutipes* (Hed Kem Thong) and *Lentinus edodes* (Hed Hom) were all brought from a local farm and from Krating market in Kao Kitchakut District, Chanthaburi province, Thailand and transported to the laboratory for analysis of their β-glucan content (Mongkontanawat and Wongekalak, 2015). However, there are no reported on the product development of a high β-glucan mushroom drink. Therefore, the aim of research work was to determine β-glucan content of edible local mushrooms. The high β-glucan content mushrooms were selected for product development

for drinking, and the physical, chemical and sensory evaluation of the drink was investigated.

Materials and Methods

Materials

Four species of mushroom samples, *Agrocybe cylindracea* (Hed Kon Yipun), *Ganoderma Lucidum* (Hed Lin Jue), *Hericium erinaceus* (Hed Hua Ling) and *Volvariella volvacea* (Hed Fang) were analyzed for β-glucan content. These were brought from a farmer in the local area in Kao Kitchakut District, Chanthaburi province, Thailand.

Sample preparation

The mushrooms samples were sliced into small sizes (1 cm^3) and blanched. A sample (50 gram) was dipped in the blanching fluid at 100 ℃ for 5 minutes and immediately dipped in cold water. Then, all samples were dried using a hot air oven (Binder, FD115, Germany) at 70 ˚C for 24 hours. The dried samples were ground in a blender.

β-Glucan contents determination

The β-Glucan content in the mushroom powder was analyzed using a Yeast Beta-Glucan Assay Kit (Megazyme, Ireland), as follows: to find the total glucan content, 100 mg of milled mushrooms were placed in a test tube, then 1.5 ml of 37 % hydrochloric acid was added. The solution was mixed and incubated at 30 ℃ for 45 minutes (mixed every 15 minutes). Then, 10 ml of distilled water was added, mixed and incubated at 100 ℃ for 2 hours before adding 10 ml of 2 N KOH. The solution was taken, and the volume adjusted to 100 ml with sodium acetate buffer pH 5 (800 μl) and mixed. After that, the mixtures were centrifuged at 1,500 ×g for 10 minutes. Samples (100 μl) were taken in each test tube (in duplicates) before being added with 100 μl of a mixture of exo-1,3-β-glucanase plus β-glucosidase and incubated at 40 ℃ for 60 minutes. Finally, 3 ml of glucose oxidase/peroxidase was added and incubated at 40 ℃ for 20 minutes. The absorbance was measured at 510 nm using a spectrophotometer. For α-glucan content, 100 mg of milled mushrooms were placed in test tubes and then 2 M KOH (2 ml) was added. The pellets were stirred with a magnetic stirrer in an ice bath for 20 minutes. Next, 8 ml of 1.2 M sodium acetate buffer (pH 3.8) was added to the mixture.

Amyloglucosidase plus invertase (200 µl) was added, incubated at 40 ℃ for 30 minutes and mixed by a vertex stirrer. The mixture was centrifuged at 1,500 ×g for 10 minutes. Supernatant (100 µl) was taken in a test tube (in duplicates). Glucose oxidase / peroxidase (3 ml) was added to each tube and incubated at 40 ℃ for 20 minutes. The absorbance was measured at 510 nm using a spectrophotometer. Finally, the β-glucan content was evaluated by the difference between the total glucan and α-glucan (Mongkontanawat *et al.*, 2013).

Effect of mushrooms on the physical and chemical properties of mushroom drink

Three species of local mushrooms were selected for product development of the drink. Mixture design was used for determination. Seven treatments were set up by using diffferent ratios of the three species with high β-glucan mushroom (60:20:20, 40:40:20, 20:60:20, 20:40:40, 20:20:60, 40:20:40 and 33.33:33.33:33.33, respectively). The production of the mushroom drink, all samples were made as previously explained, sterilized at 100 ℃ for 1 hour and filtrated through a sieve once. Completely Randomized Design (CRD) was used for the experiment. Samples were monitored for their physical and chemical properties. Physical property was evaluated by, measuring a solution of mushroom drink for color using a color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. The color measurement was expressed in L*, indicating the lightness on a 0 to 100 scale from black to write. a* (+,-) indicated the redness or greeness, b* (+,-) indicated yellowness and blueness. The chemical property was examined using mushroom drink sample for analyses of the β-glucan content, protein content, total carbohydrates, total soluble solids, total phenolic compounds, pH and antioxidant activity. β-Glucan content was also measured as previously described. The method of Bradford (1976) was modified for the protein content evaluation. One ml of sample was added with 4 ml of 0.0125 % w/v of coomassie blue G-250. Then, the solution was mixed and incubated for 60 minutes until a blue color appeared. Next, the absorbance was detected at 595 nm using a spectrophotometer. Protein content concentrations was calculated from the standard curve of Bovine Serum Albumin (BSA) with concentrations of 0, 0.10, 0.20, 0.30, 0.40, 0.50 and 0.60 ug/ul, respectively. The phenol-sulfuric acid assay of Dubois *et al.* (1956) was modified to measure the total carbohydrates. Firstly, a sample (0.10 ml) was added to a test tube, and the volume was adjusted to 1 ml using distilled water. Secondly, 5 % w/v phenol solution (1.00 ml) and 5 ml of 96 % v/v sulfuric acid were mixed together for 10

minutes. The resultant solution was incubated in a water bath at 30 ℃ for 20 minutes until a yellow color was achieved. Next, the absorbance was detected at 490 nm using a spectrophotometer. Glucose content concentrations was calculated from the standard curve of glucose with the concentrations of 0, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg/ml, respectively. The total soluble solids were analyzed by hand refractometer (Atago, Japan). The total phenolic compound content was modified by the method of Iqbal *et al*. (2005). Briefly, 3 ml of the sample was extracted with 30 ml of 80% ethanol and the solution was shaken for 24 hours. The obtained solution was filtrated by whatman number 1. Then, 50 ul of the filtrated solution was mixed with 950 ul of distilled water, folin-ciocalteu phenol reagent and $NaCO_3$ 7.5%. The solution was mixed and incubated at 37 ℃ for 2 hours. The absorbance was detected at 760 nm using a spectrophotometer. Finally, the concentration of the total phenolic compound was calculated by using standard curve of gallic acid. The pH was measured with a pH meter (Subtex, Taiwan). Antioxidant activity, was done using radical scavenging effect which analyzed by DPPH radical scavenging assay modified the method of Yen and Hsieh, (1997). 2.00 ml of sample was mixed with 2.00 ml of 0.16 mM Diphenyl picryhydrazyl (DPPH) in a test tube. Then, 2.00 ml of the sample was mixed with 2.00 ml of 95 %ethanol in anothor test tube. Next, 2.00 ml of 95 %ethanol was mixed with 2.00 ml of 0.16 mM Diphenyl picryhydrazyl (DPPH) in the last test tube. The mixture was shaken vigorously and incubated in dark conditions for 30 minutes. Finally, the absorbance was measured at 517 nm using a spectrophotometer. % Scavenging effect was calculated using the equation below.

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_{sample}-A_{blank})/A_{control}] \times 100$$

A_{sample} = the absorbance of test compound

A_{blank} = the absorbance of test and blank reaction

$A_{control}$ = the absorbance of control reaction

Effect of boiling time on the chemical properties of mushroom drink

The highest β-glucan content and antioxidant activity, were selected to evaluate the effect of boiling time on physical and chemical properties of the mushroom drink. These properties included β-glucan content, protein content, total carbohydrates, total soluble solids and yield.

Effect of extraction methods on the physical and chemical properties of mushroom drink

Conventional boiling and autoclave (121 ˚C) 15 min were used as the most economical extraction methods. The resultant solutions from two methods

were compared for their physical and chemical properties. Physical properties were measured by color parameters (L*, a* and b*). Chemical properties was determined by, β-glucan content, protein content, total carbohydrates and total soluble solids and yield.

Effect of sugars types on the physical, chemical properties and sensory evaluation of mushroom drink

The extraction method of high amount of β-glucan, and high yield was choosen to determine the effect of sugar types on the physical, chemical properties and sensory evaluation of the mushroom drink. First, the best treatments and combinations of high β-glucan mushrooms were selected to prepare the drink in 250 ml durams with water added at the ratio of mushroom mixture: water (1:15). Then, 1 % w/v of pandanus leaf was added. The 5 % w/v of various types of sugar were added as, a standard refined sugar, honey, fruit syrub, xylitol, and a control sample where no sugar was used. Next, the solutions were stertilized using the best extraction method. The solution was collected to monitor the physical, chemical properties and for sensory evaluation. Physical properties was measured by, color parameters. Chemical properties were done to determine β-glucan content, protein content, total carbohydrates, total soluble solids, total phenolic compound, pH and radical scavenging assay. Sensory was evaluated, from five treatments of the mushroom drink with various types of 5 % w/v of sugar and elucidated the sensory with 50 untrained panelists from the staff and students of the Department of Product Development and Management Technology at Rajamangala University of Technology Tawan-ok Chanthaburi campus. The panelists rated the samples using a nine-point hedonic scale ranging from 1 (extremely disliked) to 9 (extremely liked) (Watts *et al*., 1989). Data were analysed and subjected to analysis of variance (ANOVA) ($p \leq 0.05$). Mean with significant differences was separated by Duncan's multiple range test (DMRT) using computer software.

Results

β-Glucan contents investigation

Eleven species of local mushrooms from our previous study (Mongkontanawat and Wongekalak, 2015) combinded with the four species used in the current evaluation were measured for their β-glucan content. The results are given in figure 1. As indicated by figure 1, the highest β-glucan

concentration was found in *S. commune* (Hed Krang) at 39.08±5.82 %w/w. The value of β-glucan, was not significantly different ($p \leq 0.05$) from *P. ostreatus* (Hed Nang Rom Hanggari) at 37.01±1.35 %w/w and *P. sajor-caju* (Hed Nang Fa) at 34.02±0.06 %w/w.

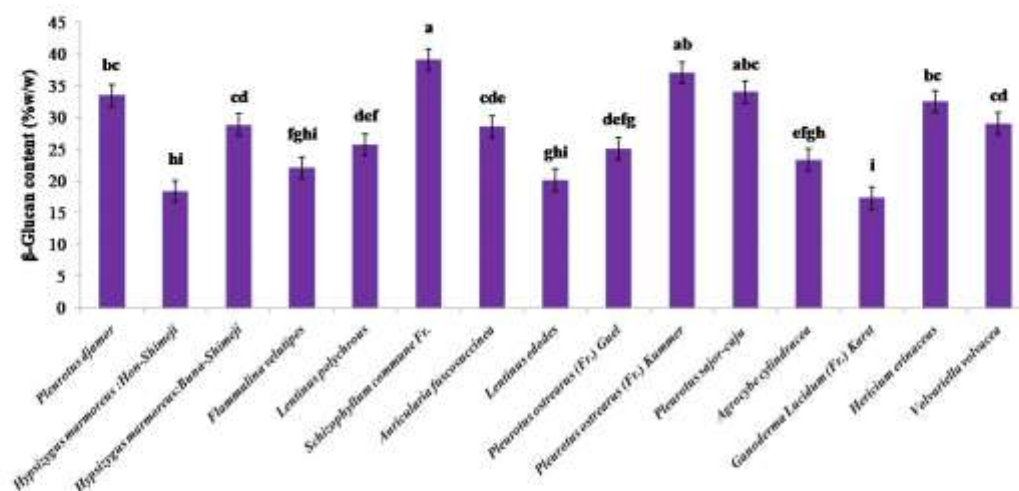

Figure 1. β-Glucan content of native edible mushroom species. Bars represent standard deviation from triplicate determination

Effect of mushrooms on physical and chemical composition of mushroom drink

Result showed that treatment 5 (ratio of *S.commune*:*P. ostreatus*:*P. sajor-caju* at 20:20:60) exhibited significantly ($p \leq 0.05$) highest lightness (L*) with the value 3.84±0.08. For a*, treatment 2 (ratio of *S.commune*:*P. ostreatus*: *P. sajor-caju* at 40:40:20) showed the highest value (0.59±0.29). However, there was not significant difference from treatment 1, 6 and 7. For b*, treatment 5 was significantly the ($p \leq 0.05$) highest (5.82±0.39) compared to other treaments. Overall, mushroom drink in all treatments indicated in gold color. The chemical properties of the mushroom drink gave significantly highest β-glucan content (7.18±1.96 %w/w) which found in treatment 1(ratio of *S.commune*:*P. ostreatus*:*P. sajor-caju* at 60:20:20. The finding suggested that the amount of β-glucan concentration corresponded with the quantity of *S.commune.* Protein content in treatment 1 demonstrated high protein content (30.43 ± 0.38 mg/ml) but there was not significant difference from treatments 2,3,4,6 and 7 as shown in figure 2.

Table 1. Effect of mushrooms on color parameter of mushroom drink

Treatments	*S.commune*: *P. ostreatus*: *P. sajor-caju*	Color parameter		
		L*	a*	b*
1	60 : 20 : 20	1.39±0.09 [d]	0.36±0.13 [a]	2.18±0.35 [cd]
2	40 : 40 : 20	2.41±0.15 [c]	0.59±0.29 [a]	2.64±0.19 [c]
3	20 : 60 : 20	2.81±0.20[b]	-0.51±0.64[b]	4.15±0.33[b]
4	20 : 40 : 40	2.52±0.10 [c]	-0.42±0.04[b]	4.25±0.72[b]
5	20 : 20 : 60	3.84±0.08 [a]	-0.34±0.29[b]	5.82±0.39 [a]
6	40 : 20 : 40	1.51±0.40 [d]	0.48±0.31 [a]	2.01±0.65 [d]
7	33.33 : 33.33 : 33.33	1.37±0.14 [d]	0.42±0.32 [a]	1.77±0.75 [d]

L* (lightness) 0 = black, 100 = white, a*(redness/greeness) + = redness, - = greenness, b*(yellowness/buleness) + = yellowness, - = buleness. Each data represents mean of three replications with standard errer. Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's Multiple Range test.

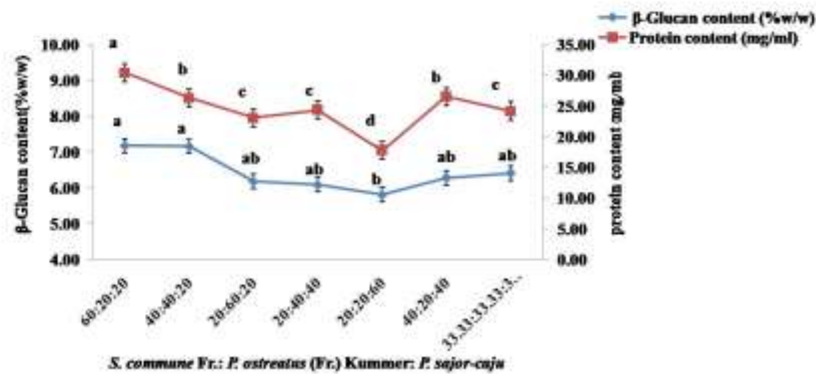

Figure 2. β-Glucan and protein content of the mushroom drink with combination of various concentrations of *S.commune*: *P. ostreatus*: *P. sajor-caju*. Bars represented standard deviation from triplicate determination

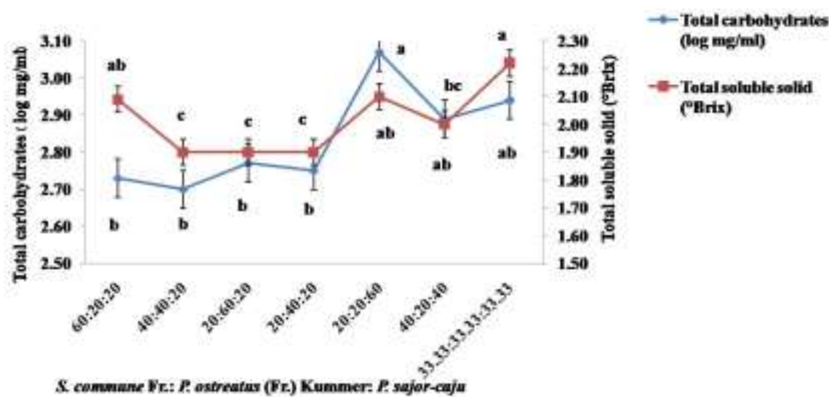

Figure 3. Total carbohydrates and soluble solids of the mushroom drink with a combination of various concentrations of *S.commune*: *P. ostreatus*: *P. sajor-caju*. Bars represented standard deviation from triplicate determination

The level of total carbohydrates and total soluble solids showed the same trend (figure 3). The highest value of sugar was presented in treatment 5 (ratio of *S.commune*: *P. ostreatus*: *P. sajor-caju*; at 20:20:60) with the number of 3.07±0.19 mg/ml and 2.10±0.12 °Brix, respectively. Total phenolic compound was the highest level (0.32±0.01 mg/ml) in treatment 7 (ratio of *S.commune*: *P. ostreatus*: *P. sajor-caju*; of 33.33:33.33:33.33. It was not significantly different from treatment 1, 2 and 6 sugesting that the total phenolic compound corresponded with the amount of *S.commune*. Antioxidant activity was the highest value scavening effect (89.42±0.31 mg/ml) in treatment 1 (ratio of *S.commune*:*P. ostreatus*:*P. sajor-caju* of 60:20:20. However, it was not significantly different from treatments 4, 5 and 6 as represented in figure 4.

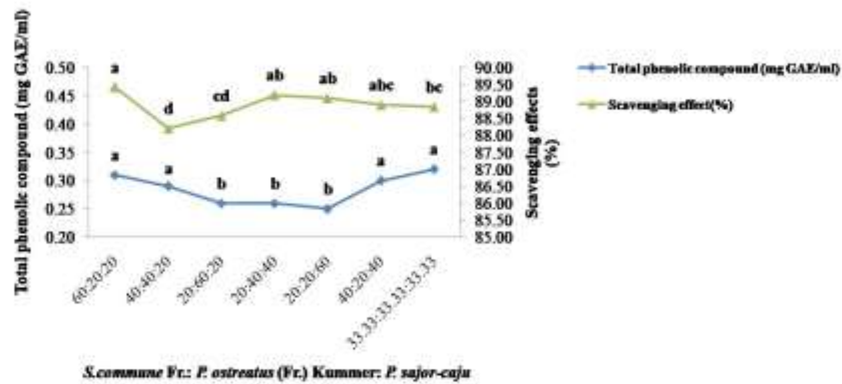

Figure 4. Total phenolic compound and radical scavening effect on DPPH of the mushroom drink with combinations of various concentrations of *S.commune*: *P. ostreatus*: *P. sajor-caju*. Bars represented standard deviation from triplicate determination

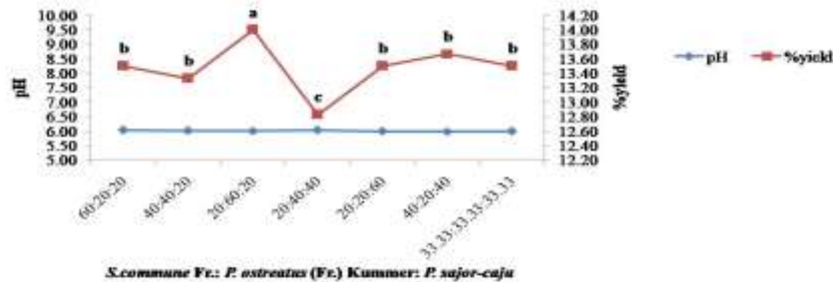

Figure 5. pH and % yield of the mushroom drink with combinations of various concentrations of *S.commune*: *P. ostreatus*: *P. sajor-caju*. Bars represented standard deviation from triplicate determination

All samples showed acidity with approximate value of 6.00. Treatment 3 (ratio of *S.commune*: *P. ostreatus*: *P. sajor-caju* at 20:60:20) exhibited the highest yield which significantly different from other treatments (figure 5).

Effect of boiling time on the chemical property of mushroom drink

The most effective treatment for achieving the highest β-glucan and antioxidant activity was treatment 1 (ratio of *S.commune*:*P. ostreatus*: *P. sajor-caju* at 60:20:20). Treatment 1 was choosen to evaluate the effect of boiling time (hours) on the chemical properties and yield of the mushroom drink (table 2).

β-Glucan content, protein content, total carbohydrates and total soluble solids increased when the boiling time increased (table 2). In contrast, the yield decreased when the boiling time increased. In economic terms, the conventional boiling method could not be considered a viable method for a high scale production environment. So, the physical and chemical properties of the drinks produced from two extraction methods were compared.

Table 2. Effect of boiling time on chemical properties and yield of mushroom drink

Treatments	Boiling time (h)	β-Glucan content (%w/w)	Protein content (mg/ml)	Total carbohydrates (log mg/ml)	Total soluble solid (°Brix)	%Yield (% v/v)
1	1	6.58±0.01 [b]	15.03±1.31 [e]	0.66±0.08 [c]	0.45±0.01 [d]	66.67±0.10[a]
2	2	6.38±0.01 [b]	20.60±0.24 [d]	0.76±0.06 [c]	0.55±0.01 [d]	54.44±0.06[b]
3	3	6.41±0.23 [b]	24.33±0.30 [c]	1.36±0.08 [b]	1.05±0.01 [c]	37.22±0.03[c]
4	4	6.28±0.29 [b]	32.28±0.60 [a]	1.36±0.08 [b]	1.25±0.01 [b]	18.89±0.06[d]
5	5	7.20±0.24 [a]	27.13±0.17 [b]	3.89±0.22 [a]	3.15±0.01 [a]	2.22±0.03[e]

Effect of extraction methods on the physical and chemical of mushroom drink

Result showed that the physical properties was the high pressure cooker method which produced significantly higher levels of L*, a* and b* than the conventional boiling method (figure 6).

Effect of sugars on the physical and chemical properties of mushroom drink

The mushroom drink was prepared with 5 % w/v of various type of sugar were mixed and sterilized in an autoclave at 121 °C for 15 minutes. The results were exhibited in table 4, 5 and 6, respectively.

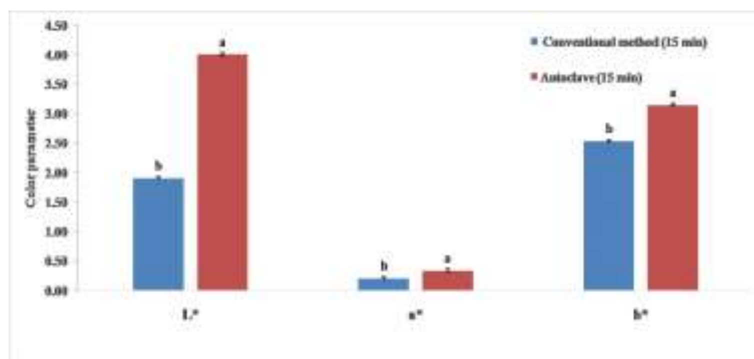

Figure 6. Effect of extraction method on color parameter of mushroom drink
L* (lightness) 0 = black, 100 = white, a*(redness/greeness) + = redness, - = greenness, b*(yellowness/buleness) + = yellowness, - = buleness. Bars represented standard deviation from triplicate determination. Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's Multiple Range test.

Table 3. Effect of extraction methods on chemical properties and yield of mushroom drink

Treatments	Extraction methods	β-Glucan content (%w/w)	Protein content (mg/ml)	Total carbohydrates (log mg/ml)	Total soluble solid (°Brix)	%Yield (% v/v)
1	Conventional method (15 min)	2.06 ± 0.14^{b}	20.58 ± 0.35^{a}	2.74 ± 0.09^{a}	1.23 ± 0.05^{a}	13.33 ± 0.04^{b}
2	Autoclave (15 min)	2.51 ± 0.13^{a}	15.55 ± 1.29^{b}	1.11 ± 0.04^{b}	1.03 ± 0.05^{b}	91.66 ± 0.08^{a}

Table 4. Effect of sugars on color parameter of the mushroom drink

Treatments	Type of sugars	Color parameter		
		L*	a*	b*
1	Control	3.34 ± 0.10^{b}	-0.99 ± 0.09^{b}	3.62 ± 0.09^{c}
2	Sugar	3.43 ± 0.02^{a}	-1.02 ± 0.02^{b}	3.61 ± 0.08^{c}
3	Honey	3.16 ± 0.03^{c}	-0.83 ± 0.07^{a}	4.06 ± 0.06^{b}
4	Fruit syrub	3.12 ± 0.02^{c}	-0.77 ± 0.03^{a}	4.22 ± 0.12^{a}
5	Xylitol	3.05 ± 0.02^{d}	-0.76 ± 0.10^{a}	4.10 ± 0.05^{b}

The lightness (L*) was the highest when 5% w/v of sugar was added. A high value of a* was found when adding 5% w/v of honey fruit syrub and xytitol (Table 4). The value of b* was highest when adding fruit syrub.

Table 5. Effect of sugars on β-glucan content, protein content, total carbohydrates and total soluble solids of the mushroom drink

Treaments	Type of sugars	β-Glucan content (%w/w)^{ns}	Protein content (mg/ml)	Total carbohydrates (log mg/ml)	Total soluble solid (°Brix)
1	Control	2.56±0.12	11.55±0.20 ^{a}	1.10±0.02 ^{d}	0.17±0.05 ^{e}
2	Sugar	2.50±0.16	10.15±0.10 ^{c}	5.15±0.01 ^{a}	5.00±0.06 ^{a}
3	Honey	2.51±0.06	11.10±0.16 ^{b}	5.04±0.00 ^{b}	3.67±0.12 ^{b}
4	Fruit syrub	2.54±0.12	9.15±0.12 ^{e}	5.02±0.01 ^{b}	2.93±0.12 ^{c}
5	Xylitol	2.52±0.16	9.80±0.04 ^{d}	4.99±0.01 ^{c}	2.33±0.12 ^{d}

Table 6. Effect of sugars on total phenolic compound, pH and antioxidant activity of the mushroom drink

Treatments	Type of sugars	Total phenolic compound (mg GAE/ml)^{ns}	pH	Scavenging effect(%)
1	Control	0.52±0.01	5.77±0.02^{a}	89.42±0.31
2	Sugar	0.54±0.01	5.68±0.02 ^{b}	89.39±0.13
3	Honey	0.54±0.02	5.56±0.01 ^{d}	89.57±0.61
4	Fruit syrub	0.54±0.01	5.35±0.01 ^{e}	89.25±0.22
5	Xytitol	0.54±0.01	5.64±0.01 ^{c}	89.80±0.04

Result reveled that the types of sugar did not affect β-glucan content, total phenolic compound and scavenging effect (table 5 and 6). However, lower protein content and pH were found after the different types of sugar were added. In addition, total carbohydrates and soluble solids were higher than the control group due to the addition of 5% w/v types of sugar. The sensory in treatment 2 (5% w/v of sugar) had the highest scores for taste, sweetness and acceptability. Nevertheless, it was non-significantly different from the mushroom drink with the additional 5% w/v of fruit syrub and xylitol (treatment 4 and 5) as indicated in table 7.

Table 7. Effect of sugars on mean sensory scores of mushrooms drink

Treatments	Type of sugars	Preference score±standard deviation				
		Color^{ns}	Aroma^{ns}	Taste	Sweet taste	Overall linking
1	control	6.86±0.64	6.80±1.07	6.02±1.08^{c}	6.30±0.91 ^{c}	6.10±1.02 ^{c}
2	sugar	6.80±0.57	6.70±1.31	7.00±0.99 ^{a}	7.06±0.89 ^{a}	6.70±0.54 ^{a}
3	honey	6.96±0.70	6.58±1.28	6.48±1.18 ^{b}	6.74±0.92 ^{ab}	6.34±0.66 ^{bc}
4	fruit syrub	6.85±1.07	6.57±1.45	6.58±1.23 ^{ab}	6.68±1.25 ^{abc}	6.41±1.20 ^{ab}
5	xytitol	6.88±0.66	6.72±0.93	6.48±0.89 ^{b}	6.54±0.95 ^{bc}	6.50±0.58 ^{ab}

Discussion

The higest β-glucan concentration was found in *S. commune* (Hed Krang). The value of β-glucan was not significantly different ($p \leq 0.05$) from *P. ostreatus* (Hed Nang Rom Hanggari) and *P. sajor-caju* (Hed Nang Fa). Consequently, these three species of mushroom were selected to produce a drink with a high level β-glucan content. Our findings indicated that the amount of β-glucan and protein concentration displayed the same trend. The levels of of β-glucan and protein concentration corresponded to the quantity of *S.commune* This could be because the amount of β-glucan of this mushroom was demonstrated to be concerned the three species of mushrooms used in this study. It had affeced in these parameters. The total carbohydrates and soluble solids showed the same trend. The highest value for sugar was found in treatment 5. Since the carbohydrate content of dry *P. sajor-caju* was higher than in *P. ostreatus* reported by Alam *et al*. (2008) which the levels of total carbohydrates and soluble solids were dependent on the amount of *P. sajor-caju* as found in our study. For the total phenolic compound appeared the highest level in treatment 7. Unfortunately, it was not significantly different from treatment 1, 2 and 6. This finding suggested that the total phenolic compound also corresponded with the amount of *S.commune*. In addition, the highest value of scavening effect. However, the results was not significantly different from treatment 4, 5 and 6 due to *S.commune* had the highest β-glucan content and phenolic compound, and the antioxidant activity would be the highest. Previous research concluded that the antioxidant activity of extract caused by both polysaccharides and polyphenols or by an interaction of both (Klaus *et al*., 2011). The conclusion was the findings by Chirinang and Intarapichet (2009), showed that water extraction of *P. ostreatus* produced a higher radical scavenging activity than the extraction of *P. sajor-caju*. Nonetheless, *S.commune* could be a powerful radical scavenging properties, β-glucan content and phenolic compounds, as it had already used for medicinal purposes reported by Hobbs (2005) and Rathore *et al* (2017). All samples showed the value of acididty of 6.00. The pH of mushrooms has usually found to be 6-7 as stated by Adedayo *et al*. (2010). Treatment 3 exhibited the highest values of yield and showed the highest β-glucan and antioxidant activity. Treatment 1 was choosen to evaluate the effect of boiling time on the chemical properties and yield of the mushroom drink resulted to increase β-glucan, protein, carbohydrate and soluble solid when the boiling time increased. Unfortunately, the yield decreased when the boiling time increased. These results could be explained that water evaporated during the boiling time increased and yield decreased while β-glucan, protein, polysaccharide and sugar increased. Our results was similar to Jaworska *et al*. (2011), who reported

that the effects of the production process on the amino acid content of frozen and canned *P. ostreatus* mushrooms. Although representing high economic value, the concentional (boiling) method may not be involved in a good choice for high scale production. A further comparison of the physical and chemical properities of the resultant drinks obtained from the two extraction methods. The high pressure cooking (autoclave) method increased the level of L*, a* and b*and high temperature (121 ℃) could potentially destroy some nutritional components such as protein, vitamins, sugar and others. Moreover, mailard reaction, resulted in significant increases in the color parameters, in the levels of lightness, greenness, and yellowness as presented in this evaluation (Chen *et al.*, 2018). Furthermore, the polyphenolic content and antioxidant activities increased as demonstrated by Choi *et al*. (2006). For the effect of sugars types, lightness was highest when 5% w/v of sugar was added. High values of a* were found when adding 5% w/v of honey, fruit syrub and xytitol. The value of b* was highest when adding fruit syrub and all samples presented a similar color for greenness and yellowness due to the color of the mushrooms and pandanus leaf, and the small amount of sugar. The sugar types did not affect the level of β-glucan, phenolic compound and radical scavenging activity. However, the differences of sugar were indicated to decrease protein content and pH. In addition, total carbohydraates and soluble solids increased more than control group. Treatment 2 had the highest score for taste, sweetness and overall acceptability. It could be concerned the consumers affinity with the taste of sugar which resulted to be high acceptability demonstrated in this study. It is concluded that the highest β-glucan content was exhibited in *S. commune* while there was not significant difference from *P. ostreatus* and *P. sajor-caju*. These species were selected to develop the high β-glucan content mushroom drink production. Our results found that *S. commune*: *P. ostreatus*: *P. sajor-caju* at a ratio of 60:20:20 showed the highest significance of β-glucan content and radical scavening activity. Interestingly, the trend of phenolic compound, radical scavening activity and β-glucan content were dependent on the amount of *S. commune*. The highest level of β-glucan content and the greatest economic value and consumer acceptability were found in mushroom drink produced with *S. commune*: *P. ostreatus*: *P. sajor-caju* at the ratio of 60:20:20 and sterilized by using autoclave for 15 minutes with an additional 5 % sugar. *S. commune* could be used as a potential medicinal mushroom drink. Additionally, this investigation would be provided a guideline to select native mushrooms for producing a new nutritional drinks in the future. However, the taste could be improved to increase the acceptabitity of the product. Furthermore, the shelf life and biological activity of the final product could be examined to ensure the health benefits of the functional mushroom drink. The adding probiotic

microorganisms may be an alternative way to improve the nutritional value of the functional drink.

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