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Development and evaluation of the formula for healthy mushroom beverage with high β-glucan prepared from *Schizophyllum commune* Fr. in Thailand

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Abstract *Schizophyllum commune* Fr., an edible macrofungus, and is a fungus with the unique taste and high β-glucan content. Therefore, the beverage with high β-glucan was evaluated the qualities of the beverage formula. Results was exhibited that the formula of *S. commune* beverage was prepared by the ratio of the dried powder of 1-day-old fruting bodies of *S. commune* with water at a ration of 1:15, and varied the amount of honey and lime juice. The criterion used for selecting the best formula was the amount of β-glucan content and antioxidant properties. The formulation contained with 10% honey and 1 % lime juice received the highest the sensory scores of most acceptable (7.03±1.00), with total phenolic compound of 1.23 ± 0.11 mg/ml and amount of β-glucan content of 7.24 ± 0.31 % v/v as well as high potential antioxidant activity of 82.43 ± 0.61 DPPH % and 91.91 ± 0.23 ABTS % ($p < 0.05$). Then, the formula development of beverage by using ratio profile test (RPT) which found to be closedly related to an ideal formula contained 10% honey and 1.5% lemon juice, which had 7.99 ± 0.31% (v/v) β-glucan and potential antioxidant activity of 82.92 ± 0.43 DPPH %, and 92.55 ± 0.33 ABTS % which was higher than the control formula. The physical and chemical characteristic of the product was 2.25± 0.03 lightness (L*), (-1.25)± 0.07 redness (a*), 2.35± 0.07 yellowness (b*), 14.40 ± 0.10 °Brix total soluble solid, 3.61± 0.01 pH, 89.86 ± 0.06 % moisture content, 0.36 ±0.01 % protein content, and 8.67 ± 0.07 % carbohydrate content. Viable plate count, yeast and mold count exhibited less than 10 CFU/ml. The study resulted to be benefit to the local beverage industry in Thailand.

Keywords: *S. commune* Fr., Mushroom, β-glucan, Product development

Introduction

Mushrooms and macrofungi have been used as medicinal foods in Asian region for a long time ago (Manzi and Pizzoferrato, 2000; Chandrawanshi *et*

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al., 2017). Theu have become attractive as a functional food and as a source for drugs development and nutraceuticals as reported by many researchers (Wasser and Weis, 1999; Mayakrishnan *et al*., 2013; Arbaayah and Umi, 2013). *Schizophyllum commune* Fr. or split gill macrofungus is local name "Krang", and native macrofungus grows on logs in Thailand's forests. *S. commune* Fr. has long been acknowledged for its medicinal properties, consumed and cultivated in many parts of Thailand. The fruting body of *S. commune* Fr. has the good unique taste, high nutritional value, and high bioactive compounds (Klaus *et al*., 2011; Antunes *et al*., 2020; Basso *et al*., 2020).

Additionally, *S. commune* Fr. is expressed to be medicinal properties as inhibiting inflammation and boosting the immune system due to phenolic compound and β-glucanin in high content (Rattanadilok Na Phuket *et al*., 2019; Abd. Razak *et al*., 2018). The β-glucan in *S. commune* Fr. is a water-soluble triple-stranded helix known as schizophyllan, and developed as an anti-cancer drug. From the study of anti-proliferation and anti-migration of *S. commune* Fr. extracts on Human Cholangiocarcinoma cell line, it is found that the extract from the extraction *S. commune* Fr. with hot water suppressing KKU-M213 cell migration (Menakongka *et al.*, 2019).

From the interesting properties of *S. commune* Fr. As mentioned above, product development of fruit tea mixed with *S. commune* Fr. is evaluated from our previously research (Mongkontanawat, 2013). In addition, *S. commune* Fr. is found highest β-glucan content when compared with fourteen edible mushrooms in the markets in Thailand (Mongkontanawat and Phuangborisut, 2019). For this reason, *S. commune* is selected for study. Therefore, the aim of research was to develop and evaluate the healthy beverage with high β-glucan from *S. commune* Fr. available in Thailand.

Materials and methods

Materials

From the results of previously our research, the 1-day fruiting bodies *S. commune* Fr. (local name: Hed Krang) from the Chanthaburi mushroom farm, Thailand, was used in this study. The sample was sliced into small sizes (1 cm^3). Next, the sample was dried by using hot air oven (Binder, FD115, Germany) at the temperature of 70 $\pm$ 5 ^{0}C until the moisture content was reduced to the level of 2.50 $\pm$ 0.02%. The dry samples were then finely ground, sifted through an 80 mesh sieve, and stored at room temperature in aluminum bags for further study.

Product development of healthy mushroom beverage

For develop the formulation of healthy beverage, there were two steps. The first step was selection of suitable formula. Five formulas of the beverage were prepared by using the solution from mixing powder of *S. commune* with water in a ratio of 1:15 (mixed M/W) and varied the honey content and lime juice content in Table 1. The mixture was boiled at 100 ^{o}C for 1 h, and then filtered through white cheesecloth. The clear part was put into the sterilized PP (polypropylene) bottles, lid immediately, and kept at 4 ^{o}C for selecting the best formula by using 3 criteria, namely: sensory score, β-glucan content, and potential antioxidation activity. Sensory was evaluated from five beverage formulas, elucidating 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 evaluated the sample using a nine-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) (Watts *et al*., 1989). Each panelist evaluated the samples for colour, flavor, taste, and overall acceptability. Then, the beverage from five formulas of *S. commune* was evaluated by β-glucan content, potential antioxidation activity, chemical composition, and physical characteristic, respectively.

The second step was developed the healthy beverage of *S. commune*. The suitable formula from the step 1 was selected for continued improvement of the product so that it can be accepted by the consumers as much as possible, based on the ideal of each factor preferred bythe consumer as the guidelines for the formula development. The formula was evaluated by 30 consumers who are familiar with the product using Ratio Profile Test (RPT). The β-glucan content, antioxidation properties (ABTH % and DPPH %), physical properties, chemical characteristic, and microbiological properties of the beverage were done as described below.

Table 1. The five formulas of beverage from *S. commune*

Formula No.	Mixed 1 Dried M/ 15W (%)	Honey (%v/v)	Lime juice (%v/v)
1(Control)	100	-	-
2	95	5	-
3	94	5	1
4	90	10	-
5	89	10	1

Physical properties determination

The beverage samples of *S. commune* were evaluated for colour using a colour meter (Nippon Denshoku, ZE-2000, Japan). The equipment was

calibrated with a standard plate. Colour measurements were expressed in: L* indicating the lightness on a 0 to 100 scale from black to white; a* (+,-) indicating the redness or greenness, respectively; b* (+,-) indicating yellowness and blueness, respectively.

Chemical properties determination

The total soluble solid was observed by using a hand refractometer (Atago, Japan) while pH by a pH-meter (Subtex, Taiwan). B-Glucan content, antioxidant activity, total phenolic compounds and cytotoxicity to the tumor cell line were assessed as explained below.

Glucan content determination

The glucan contents was determined by using a β-Glucan Assay Kit (Megazyme© International Ireland Ltd., 2013). The principle of the β-Glucan (Yeast & Mushroom) Assay Kit is based on the determination of total glucan, which consists of α-glucan and β-glucan linkages. The bonds of (1→3,1→6) -β - D-glucan, (1→3) -β-glucan and α-glucan were dissolved and cut by concentrated hydrochloric acid at 100 ^{0}C for 2 h, and then the solution was incubated with exo-1,3-β-glucanase and β-glucosidase in order to obtain complete D-glucose for analysis of total glucan content. For α-glucan, it was digested to be glucose with amyloglucosidase plus invertase, using the GOPOD reagent to measure glucose content.

Total glucan content

For the total glucan content, 10 ml of solution from *S. commune* was placed in test tubes, then 0.15 ml of 37% hydrochloric acid was added. The solution was mixed and incubated at 30 ^{0}C for 45 min (vortexed every 15 min). Then, 1 ml of distilled water was added, mixed and incubated at 100 ^{0}C for 2 h before adding 0.5 ml of 4 M KOH. The 200 µl solution was taken and adjusted for volume to 1 ml with sodium acetate buffer pH 5 (800 µl) and mixed. After that, the mixtures were centrifuged at 13,000 x g for 5 min. Samples (20 µl) were placed into each well (in duplicates) before adding 10 µl of a mixture of exo-1,3-β-glucanase plus β-glucosidase and then incubated at 37 ^{0}C for 90 min. Finally, 200 µl of glucose oxidase/peroxidase was added followed by incubation at 37 ^{0}C for 30 min. The absorbance was measured at 510 nm with a spectrophotometer. The amount of total glucan content was calculated with Equation (1.1).

Total glucan (%w/w) = ΔE x F/W x 90 (1.1)

Where ΔE is the absorbance,
F is the factor to convert the absorbance to µg of D-glucose, and
W is the weight of sample (g)

α-glucan and β-glucan content

For the α-glucan content, 100 ml of solution from *S. commune* was placed in test tubes. 2 M KOH (2 ml) was added and the pellets were stirred with a magnetic stirrer in ice bath for 20 min, and after that, 8 ml of 1.2 M sodium acetate buffer (pH 3.8) was added to the mixture. Then, 1 ml of the sample was placed into an Eppendorf tube, and 20 µl of amyloglucosidase plus invertase was added, followed by incubation at 40 ^{0}C for 30 min. Next, the mixture was centrifuged at 13,000 x g for 5 min. Supernatants (20 µl) were placed into the microtiter plate. Glucose oxidase/peroxidase (200 µl) was added to each well and incubated at 37 ^{0}C for 30 min. The absorbance was measured at 510 nm with a spectrophotometer. The amount of α-glucancontent calculated from Equations (1.2) or (1.3) depends on the α-glucan content. The β-glucan content was calculated from total glucan minus α -glucan, as shown in Equation (1.4).

α-glucan> 10% (w/w); α-Glucan (%w/w) = ΔE x F/W x 90 (1.2)

α-glucan< 10% (w/w); α-Glucan (%w/w) = ΔE x F/W x 9.27 (1.3)

Where ΔE is the absorbance,
F is the factor to convert the absorbance to µg of D-glucose, and
W is the weight of sample (g)

β-Glucan(%w/w) = Total Glucan (%w/w) –α-glucan (%w/w) (1.4)

Total phenolic compounds determination

The total phenolic content was analyzed with a modified method from Iqbal *et al.* (2005). Briefly, 3 ml of solution from *S. commune* was mixed with 30 ml of 80% ethanol (v/v). Then, the supernatant was filtered through Whatman filter paper No. 1. The reaction mixture contained 50 µl of clear soluble in 950 µl of deionized water, 200 µl of freshly prepared diluted Folin-Ciocalteu reagent from Merck, and 0.5 ml of 7.5 % sodium carbonate. The final mixtures were incubated in dark at room temperature for 2 h to complete the reaction, and then, the absorbance was measured at 760 nm. The gallic acid was used as a standard. The total phenolic content of the sample was calculated as gallic acid equivalents per g dry weight of extraction. The reaction was conducted in triplicate and the results were averaged.

ABTS free radical scavenging assay

The ABTS radical scavenging activity was modified from the assay method of Goh *et al.* (2003). To prepare the ABTS radical cation, 1.23 mM of potassium persulfate (Merck, Germany) aqueous solution was added with 5 mM ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); Sigma-Aldrich, Germany) aqueous solution in equal quantities. The mixtures were kept in dark at room temperature for 24 h to complete the reaction. Then, 1 ml of the samples was added and diluted for 10 times with 3 ml ethanol and incubated for 1 h. Next, 0.1 ml of the extraction solution was added to 2 ml of $ABTS^{+*}$ solution and kept in the dark at room temperature for 10 min to complete the reaction. The absorbance was measured at 734 nm. The scavenging effect of the ABTS free radicals was calculated as follows:

ABTS scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of control reaction and A_{sample} is the absorbance of the sample.

DPPH scavenging assay

The DPPH radical scavenging activities were the ability to reduce the free radical 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma). The DPPH radical scavenging activity was modified from the assay method of Gulcin *et al.* (2003). Briefly, 2 ml of the sample solution were mixed with 2 ml of 0.16 mM DPPH in methanol and kept in dark at room temperature for 30 min to complete the reaction. Then, 2 ml of 80% methanol mixed with 2 ml of 0.16 mM of DPPH. All samples were kept in dark at room temperature. The absorbance was measured at 517 nm. The scavenging effect of DPPH free radicals was calculated as follows:

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

Where $A_{control}$ is the absorbance of control reaction and A_{sample} is the absorbance of the sample.

Cytotoxicity of mushroom beverage determination

Cytotoxicity assay by using MTT assay was measured by the Microbiology department, Faculty of Science, Chulalongkorn University in Bangkok, Thailand. This method was evaluated following the method reported by Senthilraja and Kethiresom (2015) with the slight modifications. In brief, seeding cell MDA-MB-231 cell was seeded at 1.5 X 10^4in 96 well plate for overnight (total volume 100 µl/well). For the cell treatment, beverage of *S. commune* was prepared by using six difference concentrations of bioactive

compounds which diluted in completed media and added to the well that contain the cell (100 µl/well). Supernatant was removed, mixed complete media that contain with sample solution or DMSO (vehicle control) and incubated for 24 h. For the measurement the cell cytotoxicity, MTT solution (conc. 5 mg/ml) 10 µl/well was added and incubated at 37 ℃ for 4 h in CO_2 incubator. The purple formazan was dissolved by using isopropanol with HCl (100 µl/well) and mixed. Finally, the absorbance was monitored at wave 540 nm by microplate reader.

Microbiological properties determination

The best treatment was assessed for its microbiological properties such as total microorganism, mold and yeast using total plate count on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA), respectively.

Data analysis

Property analysis was carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$) (Steel *et al*., 1997). Mean with significant differences was separated by Duncan's multiple range test (DMRT) using the computer software.

Results

Product development of healthy beverage from S. commune

In the first step, the 5 formulas of beverage with high β-glucan from *S. commune* Fr. were selected by selection criteria comprising sensory test scores, beta-glucan content, antioxidant properties, physical and chemical properties as shown in Tables 2 – 5. The results showed that formula No. 5 was chosen formula. The beverage of *S. commune* from 5 standard formulas contained β-glucan content, important bioactivity compound, between 6.74 – 7.27 % (v/v) and the beverage from formula No. 5 was optimal formula to develop in the second step due to the highest overall acceptability score (7.03±1.00), % DPPH (82.43±0.61%), total phenoloic content (1.23±0.11 mg gallic acid/ml sample), total glucan content (12.77±0.35% w/w), α-glucan content (5.53±0.05% w/w), and β-glucan content (7.27 + 0.31 % w/w) ($p \leq 0.05$).

Table 2. Sensory evaluation of five formula standards of mushroom beverage (Based on 9-pointhedonic scores)

Formula No.	Preference Scores ±Standard deviation			
	Colour^{ns}	Aroma^{ns}	Taste^{ns}	Overall Acceptability
1(Control)	6.03±1.22^{b}	5.30±1.18^{b}	4.73±1.17^{c}	5.10±1.12^{c}
2	6.23±1.14^{b}	6.23±1.14^{a}	5.87±1.07^{b}	6.23±1.04^{b}
3	6.53±0.86^{ab}	6.30±1.15^{a}	5.97±0.97^{b}	6.30±0.79^{b}
4	6.97±1.22^{a}	6.57±1.14^{a}	6.27±1.26^{b}	6.57±1.07^{ab}
5	7.03±1.13^{a}	6.87±1.28^{a}	7.37±1.22^{a}	7.03±1.00^{a}

The increasing of the amount of honey and lime juice trend to be increased the preference score of colour, aroma, taste and overall acceptability. It could be comcerned the concentration of honey and lime which affected to increase sweet and sour taste of beverage, therefore, the preference score of all treatments from consumer were then increased. Likewise, the supplementation of the amount of honey and lime trend to be increased L* a* b* and total soluble solid, while pH was decreased as presented in Table 3.

Table 3. Physical and chemical properties of five formula standards of mushroom beverage

Formula No.	pH	Total soluble Solid	Colour		
			L*	a*	b*
1(Control)	6.64±0.01^{a}	0.17±3.10^{e}	2.48±0.32^{a}	-0.65±0.18^{a}	2.16±0.09^{b}
2	6.09±0.01^{b}	0.10±7.07^{d}	2.49±0.07^{a}	-1.08±0.39^{b}	2.48±0.11^{a}
3	4.04±0.01^{d}	7.37± 0.08^{c}	2.37±0.13^{ab}	-1.34±0.29^{b}	2.39±0.21^{a}
4	5.26±0.01^{c}	14.00±0.00^{b}	2.35±0.28^{ab}	-1.75±0.32^{c}	2.15±0.13^{b}
5	3.75±0.01^{e}	14.20± 0.00^{a}	2.22±0.02^{b}	-1.27±0.33^{b}	2.35±0.02^{a}

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

Table 4. Total phenolic content and antioxidant properties of five formula standards of mushroom beverage

Formula No.	DPPH (%)	ABTS (%)	Total phenolic content (mg gallic acid/ml sample)
1(Control)	76.62±0.89 [c]	95.58±0.33 [b]	0.75±0.02 [b]
2	81.20±0.75 [b]	98.15±0.38 [a]	0.76±0.02 [b]
3	81.23±0.52 [b]	87.94±0.50 [d]	0.78±0.06 [b]
4	82.51±0.79 [a]	98.30±0.24 [a]	1.27±0.09 [a]
5	82.43±0.61[a]	91.91±0.23 [c]	1.23±0.11 [a]

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

The addition of honey tended to increase the antioxidant activity. Highest DPPH, ABTS and total phenolic acid were exhibited in formula 4 which was the combination of *S. commune* solution: honey at 90:10 as seen in Table 3. For glucan contents, highest the content of total-glucan, α-glucan and β-glucan were showed in formula 5 which the combination of *S. commune* solution: honey:lime at 89:10:1. In this study, the increasing of the concentration of honey and lime added total-glucan, α-glucan and β-glucan of *S. commune* beverage. Furthermore, the increasing of honey was significantly difference ($p \leq 0.05$) of α-glucan content. Overall, the supplemented of 10% honey and 1% lime juice were significantly ($p \leq 0.05$) difference increased the amount of β-glucan content (7.24 ±0.31%v/v) as presented in Table 5.

Table 5. Mean of glucan content of five formula standards of *S. commune* beverage

Formula No.	Total-glucan content(% v/v)	α-Glucan content (% v/v)	β-Glucan content (% v/v)
1(Control)	6.97±0.18[d]	0.23±0.01 [d]	6.74±0.43 [ab]
2	9.23±0.06 [c]	4.08±0.04 [b]	5.15±0.02 [c]
3	11.15±0.18 [b]	4.72±0.20 [b]	6.43±0.38 [b]
4	11.56±0.30 [b]	5.35±0.10 [a]	6.21±0.19 [b]
5	12.77±0.35 [a]	5.53±0.05 [a]	7.24±0.31 [a]

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

In the second step, from the selection of the standard formula in the step 1, it was found that the optimum combination parameter of healthy beverage withhigh β-glucan from *S. commune* Fr. consisted of 89% from the solution of the dried powder of 1-day-old fruting bodies of *S. commune* Fr. with water (1:15), 10% honey and 1% lime juice. The researcher applied the formula to further product development by conducting a descriptive sensory test, Ratio

Profile Test (RPT) by using30 consumers who are familiar with the product. (Table 6 and Figure 1).

Table 6. The results of the organoleptic quality test of the selected standard formula by using Radio Profile Test method

Sensory characteristics	Ideal value (I/I)	Aceptance score (S/I)
Colour	1.00±0.00	1.16±0.39
Aroma of mushroom	1.00±0.00	1.06±0.26
Aroma of honey	1.00±0.00	1.01±0.15
Aroma of lime	1.00±0.00	0.99±0.23
Sweet taste	1.00±0.00	1.04±0.17
Sour taste	1.00±0.00	0.98±0.12

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

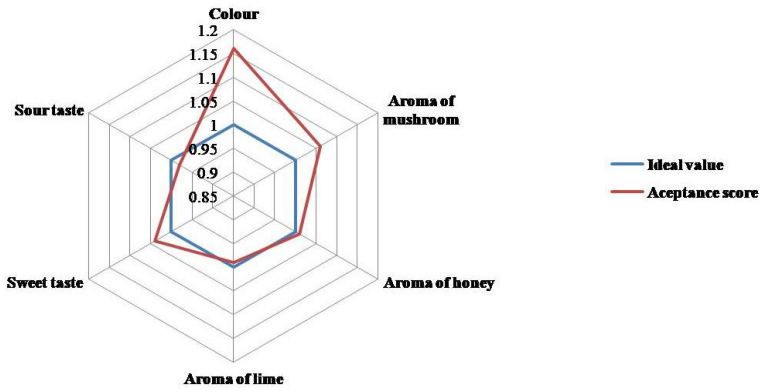

Figure 1. The spider web graph shows the outline of the selected standard formula by using Radio Profile Test method

From the Radio Profile Test method, the aceptance score from the familiar consumers showed that they want to add the colour, aroma of *S. commune*, aroma of honey and sweet taste with the score 1.16±0.39, 1.06±0.26, 1.01±0.15 and 1.04±0.17, respectively as shown in Table 6 and Figure 1. Thus, we were further developed by studying the ratio of the solution from the dried powder of 1-day-old fruting bodies (dried MB) of *S. commune* Fr with water (1:10), honey, and lime juice suitable for high β-glucan beverage production at the ratio shown in Table 7. The properties of 3 treatments were evaluated by using sensory test, physical properties, chemical properties, total phenolic content, antioxidant properties, and glucan content, of which the results are showed in Table 8- Table 11, respectively.

Table 7. The ratio of the developed high β-glucan beverage production from *S. commune*

Treatment No.	Ratio (% v/v)		
	The solution of dried MB with water (1:10)	honey	lime
1	88.50	10.00	1.50
2	89.50	9.00	1.50
3	89.00	9.00	2.00

Table 8. Sensory evaluation of developted beverage formulas from *S. commune*

Formula No.	Preference Scores ±Standard deviation			
	Colour^{ns}	Aroma^{ns}	Taste	Overall Linking^{ns}
1	7.10 ±0.84	7.17 ±0.83	7.83 ±0.83^{a}	7.63 ±0.56
2	7.00 ±0.74	7.10 ±0.71	7.47 ±0.86^{ab}	7.37 ±0.67
3	7.03 ±0.81	7.13 ±0.78	7.23 ±0.77^{b}	7.33 ±0.66

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.
^{ns} mean no significant difference ($p \geq 0.05$)

The highest of colour, aroma, taste and overall linking were founded in treatment 1 which the combination of *S. commune* solution: honey:lime; at the ratio of 88.50:10.00:1.50 (Table 8). In addition, the highest significantly difference ($p \leq 0.05$) of taste was shown in treatment1with the score 7.83 ± 0.83. However, for the overall linking was still highest in treatment1 but did not significantly difference.

Table 9. Physical and chemical properties of developed beverage formula from *S. commune*

Treatment No.	pH	Total Soluble Solid	Colour		
			L*	a*	b*
1	3.610.01±^{a}	14.400.10± ^{a}	2.25±0.03^{b}	-1.25±0.07 ^{a}	2.35±0.07 ^{a}
2	3.560.01± ^{b}	13.000.10± ^{b}	2.29±0.03 ^{ab}	-1.41±0.16 ^{b}	2.26±0.16^{b}
3	3.400.01± ^{c}	13.000.00± ^{b}	2.30±0.03^{a}	-1.27±0.10^{ab}	2.35±0.07 ^{a}

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

The significantly lowest pH was exhibited in treatment 3 (the combination of *S. commune* solution: honey:lime at the ratio of 89.00:9.00:2.00 with the value 3.40 ±0.01 (Table 9). This finding suggested that the level of acidity dependent on the amount of lime juice. The higest of total soluble solid was presented in treatment 1 which the combination of *S. commune* solution: honey: lime at the ration of 88.50:10.00:1.50 and It is valuable responded to the amout of honey.

Table 10. Total phenolic content and antioxidant properties of developted *S. commune* beverage formulas

Treatment No.	DPPH[ns](%)	ABTS[ns](%)	Total phenolic content[ns] (mg gallic acid/ml sample)
1	82.49±0.77	92.25±0.29	1.18±0.10
2	82.21±0.70	92.22±0.18	1.13±0.07
3	82.14±0.54	92.10±0.32	1.15±0.09

[ns] mean no significant difference ($p \geq 0.05$)

The antioxidant activity (DPPH and ABTS) and total phenolic content were shown in the same trend (Table 10). Those parameters were founded slightly falling when the level of mushroom juice was decreased, but did not sigficantly difference.

Table 11. Mean of glucan content of developted *S. commune* beverage formulas

Treatment No.	Total-glucan content[ns] (% w/w)	α-Glucan content[ns] (% w/w)	β-Glucancontent[ns] (% w/w)
1	13.82±0.06	5.83±0.13 [a]	7.99±0.07
2	13.780.±08	5.81±0.05 [b]	7.97±0.05
3	13.50±1.30	5.82±0.03 [a]	7.69±0.33

[ns] mean no significant difference ($p \geq 0.05$)

Total-glucan, α-glucan and β-glucan were reduced when the level of *S. commune* solution decreased. Therefore, highest glucan content was founded treatment 1 but did not significantly difference (Table 8-11). It was founded that the optimal combination of *S. commune* beverage with high β-glucanconsisting of 88.50% the solution from the dried powder of 1-day-old fruting bodies (dried MB) of *S. commune* Fr. With water (1:10), 10% honey, and 1.5% lime juice had the highest taste score (7.83±0.83). Then, this healthy beverage were determined the properties by comparing with the control formula as shown in Table 12.

The properties of the developed healthy beverage with high β-glucan from *S. commune* Fr. in Thailand compared with the control formula were showed. It was found that the *S. commune* beverage from the developed formula has the highest β-glucan (7.99± 0.07 %w/w) and % DPPH (82.92± 0.43%) ($p \leq 0.05$).

Then, the final high β-glucan of *S. commune* beverage was observed for cytotoxicity to the breast cancer cell MDA-MB-231. Our result was revealed that the increasing of the concentration of *S. commune* beverage both control and developed formula tended to toxicity to the MDA-MB-231 cell. Interestingly, the healthy *S. commune* beverage product tended to be more

effective in viability of breast cancer cell than the control formula in the range 15-30% (v/v) concentration as shown in Figure 2.

Table 12. Properties of the developed healthy beverage with high β-glucan from *S. commune* Fr. in Thailand

Properties	Control(Standard formula No 1)	Developed formula
Total-glucan	6.97 ±0.18[b]	13.82 ±0.06 [a]
α-glucan	0.23 ±0.01 [b]	5.83 ±0.13 [a]
β-glucan	6.74 ±0.19 [b]	7.99 ±0.07 [a]
DPPH (%)	76.62 ±0.89 [b]	82.92 ±0.43 [a]
ABTS (%)	95.58 ±0.33 [a]	92.55 ±0.33 [b]
Moisture	91.58±0.41[a]	89.86±0.06 [b]
Protein[ns]	0.39±0.01	0.36±0.01
Fat[ns]	0.000005±0.00	0.000011±0.00
Ash	0.90±0.01 [a]	0.85±0.05 [b]
Fiber	0.30±0.11 [a]	0.27±0.03 [b]
Carbohydrate	6.85±0.50 [b]	8.67±0.07 [a]
Total plate count[ns] (CFU/ml)	<10	<10
Yeast and mold[ns](CFU/ml)	<10	<10

Mean with different letters in the row are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.

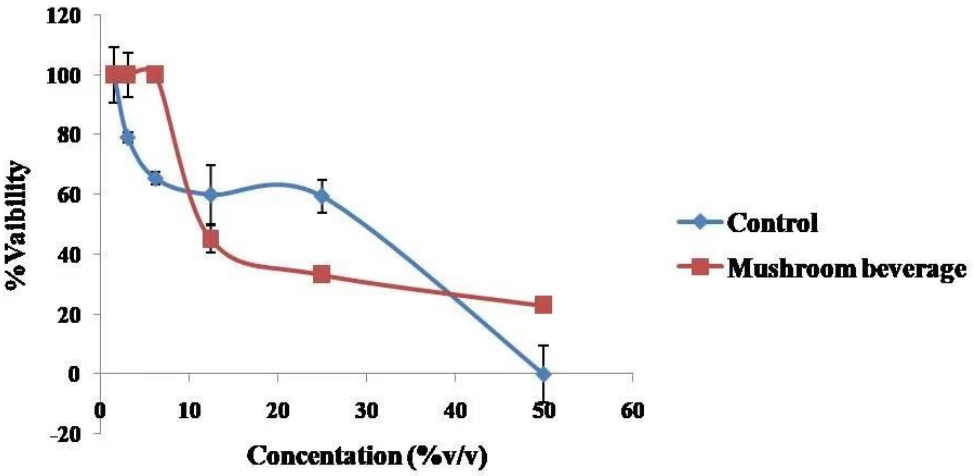

Figure 2. Effect of mushroom beverage to %viability ofbreast cancer cell MDA-MB-231

Discussion

In the development of healthy beverage with high β-glucan from *S. commune* Fr., it was found that the beverage from 100% 1-day-old fruting bodies (dried MB) of *S. commune* Fr with water (1:15) had not acceptable ordor by consumers. The mixed with honey and lemon juice, the favour of lime increased the sensory acceptance score and acidity of the *S. commune* beverage. The recipe No. 5 contained more honey and lemon juice than other recipes, it received the score of the sensory test in aroma, taste, and overall acceptability more than other recipes. The pH value of *S. commune* beverage in the rank 5.35-5.77 had no effect on the antioxidation properties (Mongkontanawat, and Phuangborisut, 2019), but the pH of developed formula was 3.61±0.01. The acid from lime juce increased DPPH, which is in consistent with Hajimahmoodi *et al*. (2012). This developed healthy beverage with high β-glucan from *S. commune* Fr. formula was suitable for general consumers and improved their health. *S. commune* has medicinal, gastronomic properties, and maintenance of healthy gut and gut microbiota (Muthuramalingam *et al.,* 2019).

When the researcher developed a healthy *S. commune* beverage product and used to test the viability of breast cancer cell MDA-MB-231. According to *S. commune* solution was suppressed the metastatic behavior of MDA-MB-231 by the inhibition of cell adhesion, cell migration and cell invasion (Jiang and Sliva, 2010). Thus, *S. commune* solution was reduced the number of breast cancer cell MDA-MB-231 as founded in this research. Similarly, it was founded the high concentration of *S. commune* juice increased toxicity to the breast cancer cells and cancer cells responded to *S. commune* juice at 15-30% concentrations because MDA-MB-231 breast cancer cells were triple negative breast cancer (TNBC) cells that did not have estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (Her2), resulted to increase sensitivity to the extracts of *S. commune* beverage in this concentration range(Yin *et al*., 2020). However, the cytotoxicity of *S. commune* beverge to the normal cell line could be evaluated for comparing in the further research.

In conclusion, It suggested this investigation is new product development with high β-glucan content from *S. commune* Fr. This beverage could be produced as a healthy drink for consumers in the future. On the orther hand, the health beneficial effect and futheractual production, the shelf life and the production cost could be monitored. The nutritional value, the addition of probiotic microorganism, dietary fiber and phytochemicals of *S. commune* beverage would be improved as the alternative way.

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