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Product Development of *Garcinia cowa* Roxb Tea Mixed with High β-Glucan Content from Edible Mushroom

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Garcinia cowa Roxb has been used in traditional folk medicine for a long time ago. Moreover, β-glucan from mushrooms also exhibited various medicinal properties. However, no previous researches in product development of *G. cowa* Roxb leaves mixed with high β-glucan content from local edible mushrooms have been found. Therefore, the objective of this research aimed to develop the *G. cowa* Roxb tea substituted with high β-glucan content from local edible mushrooms. From a previous research, the result found that blanched *Pleurotus ostreatus* was selected based on high β-glucan content and its cheap price. Product development of the tea was determined using randomized complete block design (RCBD), which was composed of six treatments– *G. cowa* Roxb leaves and *P. ostreatus* powder proportions as follows: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, respectively. The result of sensory testing by 50 panelists using 9-point hedonic scale found that the most appropriate ratio of *G. Cowa* Roxb: *P. ostreatus* was the 80:20. The highest average liking scores of 4 attributes (colour, aroma, taste, and overall liking were 7.10, 6.16, 6.02, and 6.70, respectively. The physical and chemical characteristic of the product were: 9.22 lightness (L) , 0.56 redness (a), 0.58 yellowness (b), 3.00 °Brix total soluble solid , 2.09 pH , and 0.45 a_w. Viable plate count, yeast and mold count exhibited less than 10 CFU/g. For the percentage of β-glucan increase, the levels were increased depending on the mushroom content. Interestingly, from the result of consumer testing, most of consumers (35%) rated "extremely like" (7.88 out of 9-point). Ninety-one percent of consumers recognized the new product had more nutritional value added than other marketed tea;76% purchased the new product. In addition, 52% of the consumers accepted the price at 50 baht/package. In summary, this herbal tea could be developed as a healthy food choice in the future.
Keywords: tea, *Garcinia Cowa* Roxb, mushroom, β-glucan

Introduction

Mushrooms have been used as medicinal food in Asian region for a long time in Japan, China, and Korea (Manzi and Pizzoferrato, 2000) wherein Thailand, they have also been found and cultivated. Previous researches reported that mushrooms have been an important source of novel bio-active compounds (Hawksworth, 1995). It has also a great potential as a nutritionally functional food and a source of physiologically beneficial and

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non-toxic medicines (Wasser and Weis, 1999). Moreover, many other researchers supported the idea that mushrooms have become attractive as a functional food and as a source for drugs development and nutraceuticals. Furthermore, recent studies reported that mushrooms have shown medicinal properties. They were reported to have various immunological and anti-cancer properties. They also found other potentially important therapeutic properties including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-tumor, anti-viral, anti-microbial, and other beneficial or therapeutic health effects without any significant toxicity (Breene, 1990; Miles and Chang, 1997; Wasser and Weis, 1999; Salahuddin, 2008). As for their nutritional value, the edible mushrooms are low in calories and in fat but are rich in proteins, minerals, and dietary fiber. In addition, Manzi *et al.* (1999), described that mushrooms are much more for their texture and flavor. Mushrooms also contain significantly amounts of vitamins such as thiamin, riboflavin, ascorbic acid, as well as minerals (Breene,1990; Miles and Chang, 1997). They are also a potential source of interesting functional components dietary fibre: β-glucan. β-Glucan is a group of polysaccharides that are composed of glucose units linked together with β-glycosidic bonds (Klis *et al.*, 2002). β-Glucan also exhibits medicinal properties such as antitumor, antimicrobial, and antioxidant activities plus mycotoxin absorption (Manzi and Pizzoferrato, 2000; Chen and Seviour, 2007) as well as uses in stimulation of the immune response in animals and the reduction of blood cholesterol and glucose levels (Nicolosi *et al*., 1999).

Garcinia cowa Roxb is commonly known as Cha-muang in Thailand. It is widely distributed all over Malaysia, Thailand, and Myanmar. The fruit and young leaves are edible with a sour taste. The leaves are glossy, deep green in color, oblong shaped, and up to 6-15 cm in length and 2.5-6.0 cm in width (Ritthiwigrom *et al,* 2013). Many parts of *G. cowa* Roxb have been used in traditional folk medicine. For example, fruit and leaves have been used for indigestion and improvement of blood circulation and as an expectorant (Panthong *et al,* 2009; Ritthiwigrom *et al,* 2013).

Asian countries are known to be rich source of medicinal mushrooms. In addition, *G. cowa* Roxb is a local herb in Thailand. However, no study on product development of *G. cowa* Roxb tea mixed with native edible mushrooms has been reported. Therefore, the objective of this study was to determine the optimum amount of selected high β-glucan mushroom; *Pleurotus ostreatus* (local name: Hed Nang Rom Hanggari) on *G. cowa* Roxb tea production. The best treatment was selected to evaluate for its physical properties, chemical properties, and microbiological characteristic. Finally, the percentage of β-glucan increase, the suitable time for making tea, and consumer testing were also studied.

Materials and method

Materials

From the previous research, *Pleurotus ostreatus (*local name: Hed Nang Rom Hanggari*)* was selected based on high β-glucan content and its cheap price per gram(Mongkontanawat *et al*., 2015). The mushrooms used in this study were purchased from a local farm. *G. cowa* Roxb leaves were collected from the local area of Khao Kitchakut district in Chanthaburi province, Thailand and was transported to the laboratory.

Sample preparation

The *P. ostreatus* were sliced in a small size (1 cm^3). A sample of 50 grams was dipped in blanching fluid at 100 ℃ for 5 minutes and then dipped suddenly in cold water. Then, the sample was dried using a hot air oven (Binder, FD115, Germany) at 70˚C for 24 hours. The dry sample was ground using a blender. For *G. cowa* Roxb, the leaves were cut in a small, crosswise shape (1 cm width). Then, the leaves were dried using a hot air oven (Binder, FD115, Germany) at 60˚C for 4 hours.

Product development of tea

The first section, the optimum quantity of *P. ostreatus* for the *G. cowa* Roxb tea production was determined by randomized complete block design (RCBD), which is composed of the ratios of *G. cowa* Roxb leaves and *P. ostreatus* powder (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, respectively). The sensory evaluation, physical properties, and chemical characteristic were done as described below.

Sensory evaluation

Six treatments of *G. cowa* Roxb leaves with *P. ostreatus* tea mixture were sensory evaluated with 50 untrained panelists from the staff and students of Department of Product Development and Management Technology at Rajamangala University of Technology Tawan-ok Chanthaburi Campus. The tea samples were soaked in hot water with a ratio of 1:10 (fruit tea:hot water). 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 best treatment was selected to evaluate for its chemical composition and microbiological characteristic, respectively.

Physical properties determination

The six treatments composed of the proportioned tea mixture were conducted 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* indicates the lightness on a 0 to 100 scale from black to white; a* (+,-) indicates the redness or greenness, respectively; b* (+,-) indicates yellowness and blueness, respectively.

Chemical properties determination

The tea solution for the six treatments was prepared as described above. The total soluble solid was determined using a hand refractometer (Atago, Japan) while pH by a pH-meter (Subtex, Taiwan). The tea powder was measured through its water activity (a_w) using Pa$_w$kit water activity meter (Decagon, N/A 2000, USA).

The precentage of β-glucan increase

β-Glucan contents in the six treatments of *G. cowa* Roxb and *P. ostreatus* tea mixture were analyzed using a Yeast Beta-Glucan Assay Kit (Megazyme, Ireland).

For total-glucan content, 100 mg of milled mushrooms were placed in a test tube added with 1.5 ml of 37% hydrochloric acid. The solution was mixed and incubated at 30 ℃ for 45 minutes (with interval mixing every 15 minutes). Then, 10 ml of distilled water was added; remixed, and re-incubated at 100 ℃ for 2 hours before adding 10 ml of 2 N KOH. The solution was then taken; volume was adjusted to 100 ml with sodium acetate buffer pH 5, and was mixed again. After that, the mixtures were centrifuged at 1,500×g for 10 minutes. Samples (100 μl) were taken to each test tube (in duplicates) before adding 100 μl of a mixture of exo-1, 3-β-glucanase plus β-glucosidase and then incubated at 40 ℃ for 60 minutes. Finally, 3 ml of glucose oxidase/peroxidase were added and incubated at 40 ℃ for 20 minutes. The absorbance was measured at 510 nm with a spectrophotometer (Celli, CE1011, England). The concentration of glucose in the sample was calculated from the assay kit procedure.

For α-glucan content, 100 mg of milled mushrooms was placed in test tubes and then added with 2 M KOH (2 ml). 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) were added to the mixture. Then, amyloglucosidase plus invertase (200 μl) were also added, incubated at 40 ℃ for 30 minutes and mixed by a vertex stirrer. After that, the mixture was centrifuged at 1,500×g for 10 minutes. Supernatant (100 μl) were then taken to another test tubes (in duplicates). Glucose oxidase/peroxidase (3 ml) were added to each tube and incubated at 40 ℃ for 20 minutes. The absorbance was measured at 510 nm with a spectrophotometer (Celli, CE1011, England). The concentration of glucose in the sample was calculated from the assay kit procedure. The amount of β-glucan content was calculated by the total-glucan substract α-glucan (Megazyme, Ireland; Mongkontanawat *et al*., 2011). Finally, the percentage of β-glucan increase was calculated.

Microbiological properties determination

The best treatment was examined 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.

Suitable time for preparation

The best treatment was selected to determine the suitable time for preparation as to compare with the control group. It was conducted using the phenol-sulfuric acid method (DuBois *et al*. 1956). Two ml of a sample solution was mixed with 1 ml of 5% solution of phenol in a test tube. Next, 5 ml of concentrated sulfuric acid was added rapidly to the mixture. They were vortexed for 30 seconds and placed in a water bath for 20 minutes at room temperature for colour development. Finally, light absorption at 490 nm was recorded on a spectrophotometer.

Consumer testing

The highest overall acceptability treatment was prepared as described above; it was then determined for final consumer acceptability with 200 untrained panelists by CLT (Central Location Test) (Boutrolle *et al*., 2007).

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 and discussion

Sensory evaluation of *G. cowa* Roxb tea mixture: the sensory score associate with *G. cowa* Roxb tea made from the various ratio of *G. cowa* Roxb leaves and *P. ostreatus* powder using randomized complete block design (RCBD) as mentioned above is presented in Table 1. The mean sensory scores of 6 treatments differed significantly ($p \leq 0.05$) in colour, aroma, taste, and overall liking. The colour, aroma, taste, and overall liking all ranged between “nearly like”, “slightly”, and “moderately like”. From the result, it could be proposed that the high amount of *P. ostreatus* powder effected to decrease all parameters. Treatment 2 (the ratio of 80:20; *G. cowa* Roxb and *P. ostreatus* powder) was mostly accepted among the samples, however non-significantly difference ($p \leq 0.05$) from treatments 1 and 3. This could be because this treatment exhibited slight musty smell of mushroom. This condition could then be taken into consideration in terms of consumers’ acceptability need. Afterwards, all treatments were determined for some of its physical and chemical properties as shown in Tables 2.

Table 1 Mean sensory scores of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

Treatments	*G. cowa* Roxb: *P. ostreatus* powder	Parameter			
		Colour	Aroma	Taste	Overall liking
1	100:00	7.80^{a}	6.90^{a}	6.18^{a}	6.80^{a}
2	80:20	7.10^{b}	6.16^{b}	6.02^{a}	6.70^{a}
3	60:40	6.24^{c}	5.66^{bc}	5.92^{ab}	6.50^{ab}
4	40:60	5.64^{d}	5.16^{c}	5.56^{ab}	6.00^{bc}
5	20:80	5.60^{d}	4.82^{d}	5.18^{b}	5.64^{c}
6	0:100	6.14^{cd}	5.48^{c}	5.50^{ab}	5.60^{c}

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

Physical properties and chemical properties of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder: the results of the physical and chemical properties of tea are shown in Table 2. The level of L*(lightness), a*(redness/greenness) and b* (yellowness/blueness) were found to vary among 50.66-61.25, -0.18–2.96, and 6.47–16.64 for all treatments, respectively. Treatment 6 (the ratio of *G. cowa* Roxb leaves and *P. ostreatus* powder (0:100) was found to have the highest level of L* and a*: 61.25 and 2.96, respectively and non-significantly different ($p \leq 0.05$) from other treatments. From this results, it could be proposed that the substitution of mushroom increased the level of L* (lightness), a* (redness) and b* (yellowness). Generally, this could be due to the yellow colour of mushroom. Mixing it with *G. cowa* Roxb, therefore, would give light colour and yellowness. The level of water activity (a_w) and pH were varied between 0.45-0.50 and 1.91-6.21, respectively. In addition, the increasing substitution of mushroom indicated an increase in the water activity and pH. This result could be generally due to the moisture and pH of mushroom which was higher than *G. cowa* Roxb, so after the mixing process, the water activity and pH slightly increased. For the total soluble solid, the increasing substitution of mushroom did not affect to the total soluble solid in all treatments. The same numbers were exhibited (3 °Brix) as well as the non-significantly difference ($p \leq 0.05$). From the result, it could be proposed that all treatments had high acidity and low moisture content which indicated that the product can be stored for a longer time period.

Table 2 Physical and chemical properties of of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

Treatments	*G. cowa* Roxb: *P. ostreatus* powder	Colour (powdered mixture form)			Colour (tea mixture with hot water)			Aw	Total soluble solid	pH
		L	a	b	L	a	b		(°Brix)^{ns}	
1	100:00	50.66^{b}	0.18^{e}	6.47^{d}	8.33^{d}	0.82^{a}	0.98^{a}	0.46^{d}	3.00	1.91^{f}
2	80:20	51.09^{b}	0.25^{e}	9.75^{c}	9.22^{c}	0.56^{ab}	0.58^{b}	0.45^{d}	3.00	2.09^{e}
3	60:40	54.84^{ab}	0.52^{d}	11.76^{b}	10.25^{b}	0.40^{bc}	0.32^{c}	0.46^{cd}	3.00	2.39^{d}
4	40:60	58.88^{ab}	1.71^{c}	15.04^{a}	11.65^{a}	0.08^{d}	0.29^{c}	0.47^{bc}	3.00	2.89^{c}
5	20:80	60.82^{a}	2.18^{b}	16.90^{a}	11.42^{a}	0.12^{cd}	0.16^{d}	0.49^{ab}	3.00	3.80^{b}
6	0:100	61.25^{a}	2.96^{a}	16.64^{a}	11.72^{a}	0.45^{b}	0.07^{d}	0.50^{a}	3.00	6.21^{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.

Based on the percentage of β-glucan increase, the amount of β-glucan in the six treatments was increased depending on the amount of *P. ostreatus* powder (Figure 1). Highest percentage of β-glucan increase

(12.99%) was found in treatment 6 (the ratio of 0:100; *G. cowa* Roxb and *P. ostreatus* powder). Lowest percentage of β-glucan increase (0%) was found in treatment 1 (the ratio of 100:0: *G. cowa* Roxb and *P. ostreatus* powder). The result could be explained that β-glucan is generally located in the cell wall of a mushroom (Manzi and Pizzoferato, 2000). Therefore, the amount of percentage of β-glucan increase depended on the amount of mushroom added. However, according to sensory evaluation, treatment 2 (the ratio of 80:20: *G. cowa Roxb* and *P. ostreatus* powder) was selected to produce the tea; chemical composition and microbiological characteristic were determined as presented in Tables 3 and 4, respectively. The moisture content, mineral, lipid, protein, fiber, and carbohydrate were exhibited as follows: 1.71±0.05, 1.51±0.07, 0.14±0.01, 1.42±0.06, 49.65±0.20, and 45.57±0.25, respectively. The viable plate count, yeast and mold count were less than 10 CFU/g.

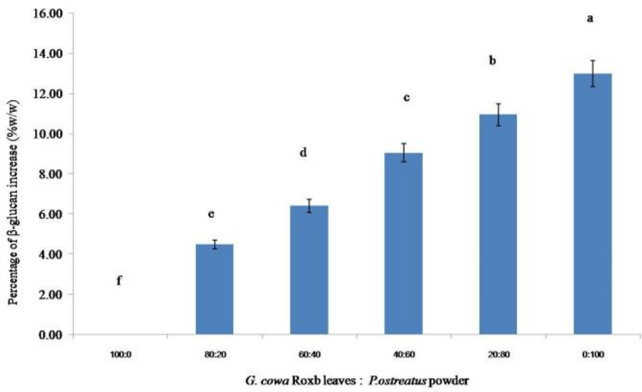

Figure 1 Percentage of β-glucan increase of *G. cowa Roxb* leaves mixed with *P. ostreatus* powder

Table 3 Chemical composition of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

Chemical composition	Content (% w/w)
Moisture	1.71±0.05
Mineral	1.51±0.07
Lipid	0.14±0.01
Protein	1.42±0.06
Fiber	49.65±0.20
Carbohydrate	45.57±0.25

Each data represents the mean of three replications with standard error.

Table 4 Microbiological characteristic of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

***G. cowa* Roxb leaves: *P. ostreatus* powder**	**Parameter**	
	Total plate count (CFU/g)	Yeast and mold count (CFU/g)
80:20	< 10	< 10

For the suitable time to prepare the tea, the result found that the amount of polysaccharide of *G. cowa* Roxb leaves was reached high at 40 minutes, then fell dramatically at 60 minutes (Figure 2).

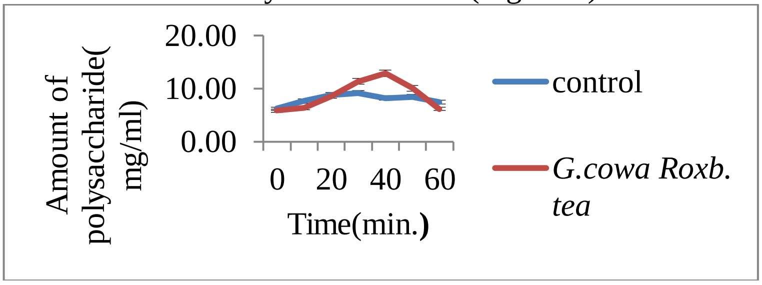

Figure 2 Amount of polysaccharide of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder in different preparation time

Table 5 Demographic information about consumers participating in the sensory analyses of the *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

Characteristic	Category	Panel (percent)
Gender	Female	69.50
	Male	30.50
Age	<20	4.50
	20-30	14.50
	31-40	21.00
	41-50	20.50
	51-60	24.50
	>61	15.00
Education	Lower primary school	28.00
	Primary school	7.50
	Secondary school	10.50
	Diploma	8.00
	Bachelor's Degree	36.50
	Higher Bachelor's Degree	9.50
Career	Student	3.00
	University undergraduate	13.00
	Official/ state enterprise	20.50
	Employee	6.50
	Private business	13.00
	Farmer	24.00
	Others	20.00
ncome	<2,000 baht	11.00
	2,001-5,000 baht	10.00
	5,001-10,000 baht	18.50
	10,001-15,000 baht	12.50
	15,001-20,000 baht	6.50
	>20,000 baht	41.50

From the consumer testing by Central Location Test (CLT), the demographic information of the consumers was presented in Table 5. Most of consumers were female (69.5%), aged 51-60 years old (24.50%), with a bachelor's degree education (36.50%), farming occupation (24%), and with an income more than 20,000 baht/month. The results found that most of consumers (35%) rated "extremely like" (7.88). Ninety-one percent recognized that the new product had more nutritional value added than other marketed tea; while 76% of the consumers purchased the new product. In addition, 52% of the consumers accepted the price at 50 baht/package, as shown in Figures 3-6, respectively.

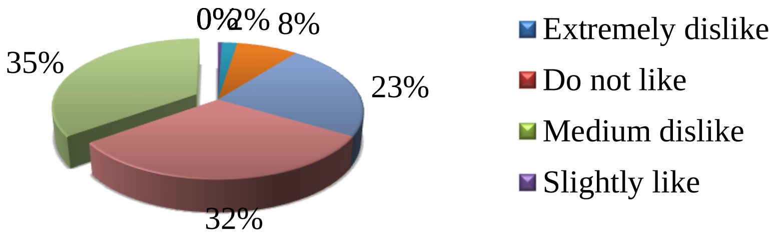

Figure 3 Sensory evaluation in developing *G. cowa* Roxb leaves mixed with *P. ostreatus* powder

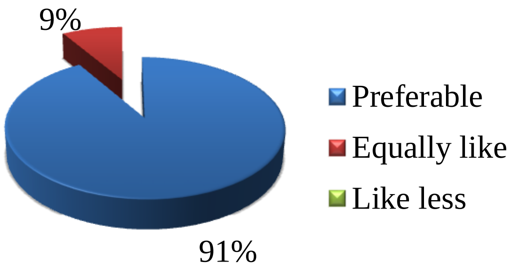

Figure 4 The opinion of the consumers to developing G. cowa Roxb leaves mixed with P. ostreatus powder

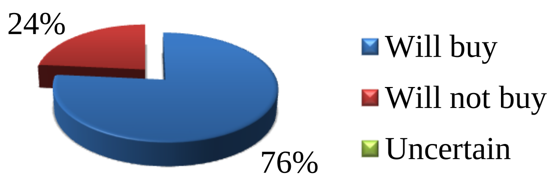

Figure 5 The opinion of the consumers to tea product availability

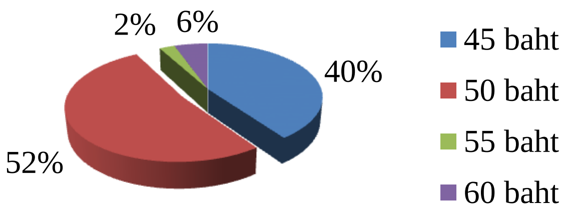

Figure 6 The opinion of the consumers to the optimum price of product

Conclusion

The results indicated that the optimum ratio of *G. cowa* Roxb leaves mixed with *P. ostreatus* powder for *G. cowa* Roxb tea production was 80:20. The 20% (w/w) amount of mushroom was the highest acceptable among the sensory evaluation. The physical, chemical, and microbiological properties of final product found that the L*, a*, and b* values were 51.09, -0.25, and 9.75, respectively. The herbal tea was greenish-yellow. Chemical properties showed total soluble solid, pH, and water activity at 3.00°Brix, 2.09, and 0.45, respectively. The overall liking of tea was rated as "moderately like" (6.70%). The total microorganism, yeast and molds had less than 10 CFU/g, respectively. Interestingly, from the results of consumer testing, most of consumers (35%) rated as "extremely like" (7.88 of 9-point). Ninety-one percent recognized the new product had more nutritional value added than other marketed tea; 76 % purchased the new product. In addition, 52% accepted the price at 50 baht/package. In conclusion, this research is a good preliminary study on the new tea product development. However, β-glucan should be first extracted out from the mushroom before mixing in with the *G. cowa* Roxb leaves in order to eliminate the musty smell, but still adding more nutritional value. Moreover, cytotoxicity to tumor cell and macrophage stimulation should also be studied to confirm the real product properties in further research.

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