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# Development of Healthy Snack from Sa-med Mushroom (*Boletus griseipurpureus* Corner)

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#### Abstract

This study investigated alpha-glucosidase and angiotensin-converting enzyme inhibitory activities of Sa-med mushroom powder. Healthy snack had been developed by varying 3 levels of Sa-med mushroom powder at 10, 12 and 14 % (w/w) and determined its physico-chemical properties and sensory evaluation. The results showed that anti-alpha-glucosidase activity and anti-angiotensin-converting activities of Sa-med mushroom powder were at 59 and 93 %, respectively. As the results of healthy snack, lightness ( $L^*$ ) and extended ratio were decreased, but redness ( $a^*$ ) and bulk density were increased with higher amounts of Sa-med mushroom powder in the formulation (p < 0.05). However, hardness and crispiness were not significantly different. For sensory evaluation, healthy snack with 14 % Sa-med mushroom powder had the highest overall liking score of 7.6 (like moderately to like very much). Finally, the nutritional values of developed snack revealed that protein and fiber were increased by 3 and 18 times, while carbohydrate was decreased by 15 %. In addition, the mushroom snack could be claimed as a "source of protein", "high fiber" and "low sodium". This snack also had phenolic compounds, antioxidant activities and alpha-glucosidase, and angiotensin-converting enzyme inhibitory activities. According to consumer acceptability test (n = 300), the acceptability level was 95 %, in which around 64 % of the subjects were interested in buying the product.

Keywords: Sa-med mushroom, Healthy product, Snack, Enzyme inhibition, Consumer acceptability

#### Introduction

*Boletus griseipurpureus* Corner is a wild edible ectomycorrhizal mushroom which is widely found in eucalyptus forest in the Southern and Northeastern part of Thailand [1-4]. It is called as "Hed Sa-med". *B. griseipurpureus* can germinate only 1 - 2 times a year during raining season [5]. Moreover, it is most frequently associated with *Melaleuca leucadendron* (Sa Med Khao or cajeput tree), *Acacia mangium*, *Acacia auriculiformis, Eucalyptus spp* and *Casuarina equisetifolia*. The characteristics of *B. griseipurpureus* consists of (1) macroscopic features, including pileus 3.0 - 6.0 cm across, convex to plane, gray to purple or light purple, surface covered with gray black short soft hairs when young; fresh white and firm when young, later softer, unchanging when injured; tube small and firm, pallid white when young, later pinkish to pale brown; and stipe 3.0 - 6.0 cm long, 1.5 - 3.0 cm across, purple, cylindrical to clavate, increasing in thickness to clavate toward the base, sometimes with pale brown net at the apex, and (2) microscopic features, encompassing Basidia 24.0 -  $40.0 \times 8.0 - 10.5 \mu$ ; clavate; basidiospores 3.1 - $4.9 \times 7.8 - 10.7 \mu$ , cylindric, pinkish pale brown and smooth [6]. *B. griseipurpureus* fetches a higher price (200 - 300 baht/kg) because wild mushrooms fruit in a specific season and demand exceeds supply [5]. Although it has a bitter taste, but it is rich in protein and low in fat and carbohydrate. In the previous study, the proximate composition of *B. griseipurpureus* was compared with 3 other Thai edible fungi, including Phlebopus portentosus, another sought after ectomycorrhizal bolete, protein concentration in B. griseipurpureus was higher than in many ectomycorrhizal fungi and fat concentration in B. griseipurpureus was lower than in P. portentosus but similar to C. bibarius (0.7 %). The concentration of carbohydrate of B. griseipurpureus (33 %) was lower than other wild edible Thai mushrooms which ranged from 42 to 65 % [2]. Moreover, some researchers have found that the antioxidant compounds, such as phenolic compounds, tocopherols, ascorbic acid, carotenoids and enzyme inhibition activities, have the potential to fight against non-communicable diseases (NCDs), including obesity, diabetes, hypertension, and Alzheimer's disease from Boletus griseipurpureus Corner [5,7,8]. Therefore, this mushroom can be used for diet as health food and the additive and synergistic effects of all the bioactive compounds can stand against oxidative processes as natural potential antioxidants [9,10]. However, mushrooms are rapidly perishable commodity, and they start deteriorating immediately within a day after harvest. After harvesting, moisture loss, shrinkage, and rapid spoilage in terms of color and texture takes place. The shelf life of mushroom is only about 2 to 5 days depending upon the species [11]. In view of their highly perishable nature, the fresh mushrooms have to be processed to extend their shelf life for the off-season use. The drying process is a common technique for preservation with many advantages. including longer shelf life, ready usage, and easy delivery, thereby increasing the economic value of mushrooms.

Starch-based snacks from corn, rice, and wheat are widely consumed, by children and young people. They are high in fat and carbohydrates and low in protein [12]. Starch-based snacks have been traditionally puffed by deep-frying; however, high oil content in these products can cause health problems and short shelf life [13]. Air-puffing or baking were applied to snacks for health-conscious consumers for convenient operation. Addition of dietary fiber, protein from plant sources and natural antioxidants were also used to improve the nutritional and health benefits of the snacks [15,16]. Thus, the study aimed to investigate  $\alpha$ -glucosidase and angiotensin-converting enzyme inhibitory activities of Sa-med mushroom powder. Then, the healthy snacks with Sa-med mushroom powder were developed using sensory evaluation. Physical properties, nutritional values, total phenolic contents, antioxidant activities,  $\alpha$ -glucosidase and angiotensin-converting enzyme inhibitory activities of the developed healthy snacks were observed.

# Materials and methods

#### **Raw materials**

Sa-med mushroom was harvested from Dong Yai community forest, Hua Taphan district, Amnat-Charoen province, Thailand. Glutinous rice flour and tapioca flour were purchased from Bangkok Inter Food Co., Ltd, Thailand. Other ingredients used for snack production included palm oil (Morakot Industries PCL, Thailand), salt (Thai Refined Salt Co., Ltd, Thailand) and sugar (Mitr Phol Sugar Corporation, Ltd, Thailand). Thai sweet chili sauce was obtained from Ampol Food Processing, Ltd, Thailand.

#### Sa-med mushroom powder preparation

Sa-med mushroom was prepared following the method of Pornpitakdamrong, Aung-aud-chariya & Sudjaroen with some modifications [17]. Briefly, mushrooms were cleaned and boiled in 5 % (w/v) salt solution at the ratio of 1:2 for 1 h. The boiled Sa-med mushrooms were dried in a hot air oven at 60 °C for 4 h or until the moisture content was below 8 %. The dried Sa-med mushrooms were ground and sieved through a 120-mesh screen. The Sa-med mushrooms powder was stored in an aluminum foil bag at 4 °C until required for use.

# Preparation of Sa-med mushroom snacks

Sa-med mushroom powder was used to replace mixed flour (glutinous rice flour and tapioca flour) by 10, 12 and 14 % (w/w) while other ingredients were the same. All ingredients (glutinous rice flour and tapioca flour, mushroom powder, palm oil, salt, and sugar) were mixed with water (58.4 %) on pan. The heating was used to swell the starch granules. The dough was formed into the square shape with a

thickness of 2 mm and cut in to  $4.5 \times 5$  cm<sup>2</sup>, and then dried at 70 °C for 3 h or until the moisture content was below 8 %. All dried dough was puffed in an oven at 150 °C for 3 min. The snacks were coated with Thai sweet chili sauce and dried to reduce the moisture content again at 120 °C for 3 min. Finally, the snacks were kept in an aluminum foil bag until required for use. The snacks without mushroom powder added were used as the control formula. The experimental design was a completely randomized design (CRD) with triplicates.

# Physical properties of Sa-med mushroom snacks

Color measurement: the color values of the sample were determined by using Hunter Lab Digital Colorimeter (COLORFLEX 4510 model, USA). The CIE color values were recorded as  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness).

Expansion Ratio: Expansion ratio of snacks was assessed by using the modified method of Segnini *et al.* [18]. A ratio of the difference between the final volume and initial volume was observed.

Bulk Density: Bulk density of snacks was evaluated by using the sesame seed displacement method described by Sahin & Sumnu [19]. Bulk density (p) was calculated as:

#### Bulk density = Mass of snacks/Volume of snacks

(1)

Textural Properties: Hardness and crispiness were examined on a Texture Analyzer (TA-XT2, Macro stable systems, UK) with 5-blade Kramer shear cell and 5.0 kg load cell. Hardness value was considered as maximum peak force (kg) and crispiness value was determined by linear distance (kg s). The study was conducted at a distance of 20 mm with test speed at 2 mm/s. An average of 10 measurements was reported.

# Proximate analysis

The moisture content, ash, protein, fat, carbohydrate, dietary fiber, and sugar were determined by using standard methods of AOAC (2016) [20]. The calorie content was calculated based on the contents of protein, fat, and carbohydrate.

### Total phenolic contents and antioxidant activities

Approximately 20 mg of sample was mixed with 1 mL distilled water. After thoroughly mixing (1 min) with Vortex mixer, the mixture was heated in the water bath for 8 h at 50 °C. The residue was extracted after it was re-mixed and centrifuged at 4600 rpm for 15 min at 4 °C. The clear supernatant was separated for the analysis of total phenolic compounds using the Folin-Ciocalteu colorimetric method [21] and the results were expressed in gallic acid equivalents per g dry weight (mg GAE/g DW) of sample. Antioxidant activities were evaluated by using the DPPH-radical scavenging assay [22], ORAC (oxygen radical absorbance capacity) assay [23] and FRAP assays [24] and the results were expressed as Trolox equivalents per g dry weight (TE/g DW).

# $\alpha$ -glucosidase inhibitory assay

The  $\alpha$ -glucosidase inhibitory activity was performed according to the protocol of You *et al.* [25]. The assay consisted of 50 µL mushroom extract (5 mg/mL well<sup>-1</sup>), 100 µL *Saccharomyces cerevisiae*  $\alpha$ -glucosidase (0.5 mg well<sup>-1</sup>), and 50 µL *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG, 0.5 mM well<sup>-1</sup>) in 50 mM KPB (pH 7.0). The reaction was monitored at a wavelength of 405 nm using the 96 well microplate readers. The percentage of inhibition was calculated as;

% inhibition =  $100 \times (1 - ((B-b) / (A-a)))$ ,

(2)

where, A is the initial velocity of the control reaction with the enzyme (control), a is the initial velocity of the control reaction without the enzyme (control blank), B is the initial velocity of the enzyme reaction

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with mushroom extract (sample) and b is the initial velocity of the reaction with mushroom extract but without the enzyme (sample blank).

# Angiotensin-converting enzyme (ACE) inhibitory assay

The ACE inhibitory assay was performed according to Schwager *et al.* [26]. The assay was initiated by mixing rabbit lung ACE (0.75  $\mu$ g), hippuryl-L-histidyl-L-leucine (HHL) (0.3 mM) and sample in assay buffer (100 mM KPB, pH 8.3) before incubating at 37 °C for 30 min. To the enzyme assay, NaOH (0.1625 M) was added to stop the enzyme reaction. Then, the product of the reaction was visualized by adding *o*-phthaldialdehye (0.3 mg), and the reaction was neutralized by adding HCl (0.25 M). The reaction was monitored using an excitation wavelength of 360 nm and an emission wavelength of 485 nm using the 96 well microplate readers. The percentage of inhibition was calculated as above.

#### Sensory evaluation

Sensory evaluation was conducted with 30 untrained panelists comprised of faculty members, staffs, and graduate students at the Institute of Nutrition, Mahidol University (INMU), Thailand. The test was performed in an individual testing booth under the daylight. Samples were coded using random 3-digit numbers. Panelists were provided with a glass of water and, instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate the products for acceptability based on its appearance, color, flavor, taste, texture and overall acceptability using 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) [27].

# **Consumer acceptability test**

Consumer acceptability test was done according to Kotler [28]. Consumers (n = 300) were tested and interviewed at each of 2 locations: Chon Buri province (n = 150) and Hua-Hin city (n = 150). The method of central location testing was used, and consumers were selected according to criteria of familiarity with the snack and age. Approximately 15 g of Sa-med mushroom snack was served to the consumers. Consumers were asked to score the acceptability of Sa-med mushroom snack using a 9-point hedonic scale which varied from "dislike extremely" to "like extremely". After testing the product, consumers were interviewed to obtain demographic information regarding age, education, gender, occupation, income. Moreover, the consumers were asked about their buying intention to if it was on market. The interviews took approximately 15 minutes. The protocol was approved by Mahidol University Central Institutional Review Board (MU-CIRB) (No.MU-CIRB 2018/097.0405). All participants were given written informed consent before joining the study.

#### Statistical analysis

The data were expressed as mean  $\pm$  standard deviation. Data were analyzed using an analysis of variance and t-test. Duncan multiple range test was used to determine significant difference among the various samples in triplicate. Data were analyzed by using Statistical Package for Social Science (SPSS) version 19 SPSS Inc., Chicago, II, USA at the < 0.05 level of significance.

#### **Results and discussion**

#### α-glucosidase and ACE inhibitory activities of Sa-med mushroom powder

The  $\alpha$ -glucosidase and ACE inhibitory activities of Sa-med mushroom powder are shown in **Table 1**. Type II diabetes is 1 major health problem in Thailand. Diabetes medicine is targeted to controlling carbohydrate degradation through inhibition of the key enzymes, such as  $\alpha$ -glucosidase. Our results revealed that Sa-med mushroom powder exhibited the  $\alpha$ -glucosidase inhibitory activities of 59.52 %. Samed mushroom powder showed the percentage of  $\alpha$ -glucosidase inhibitory activities lower than previous studies. Suttisansanee *et al.* [5] reported 78.8 %  $\alpha$ -glucosidase inhibitory activities in Sa-med mushroom. This might be due to the different time of harvest, the concentration of mushroom extract and the extraction solvents. However, the percentage of  $\alpha$ -glucosidase inhibitory activities was in range (20 - 87 %) of other water extracts of 7 Japanese edible mushrooms (*Agaricus bisporus*, *Hypsizigus marmoreus*, *Pleurotus ostreatus*, *Lentinus edodes*, *Pleurotus eringii*, *Grifola frondosa and Flammulina velutipes*) [29]. Therefore, Sa-med mushroom might promote health benefits through anti- $\alpha$ -glucosidase activities. For ACE inhibitory activities, the result showed 93.19 percentage of enzyme inhibition which was higher than the results reported by Suttisansanee et al. [5]. ACE belongs to the class of zinc metal proteases that catalyzes the conversion of angiotensin I to a potent vasoconstrictor angiotensin II and also promotes the degradation of the vasodilator bradykinin [30]. Therefore, this multifunctional enzyme plays a key role in the control of blood pressure, since the inhibition of its activity leads to a decrease in the angiotensin II concentration and an increase in the bradykinin level, and consequently in the reduction of hypertension that is one of the major causes of chronic diseases and a high-risk factor of cardiovascular diseases worldwide [31]. A wide variety of synthetic drugs have been extensively used in the treatment of hypertension and most of them have an ACE inhibitory activity, however, these drugs can cause certain adverse side effects [32]. Therefore, Sa-med mushroom extract could be a natural source with ACE inhibitory property for preventing hypertension or used as a functional ingredient in food industries.

**Table 1** The percentage of  $\alpha$ -glucosidase and ACE inhibitory activities of Sa-med mushroom powder<sup>1</sup>.

Mushroom	Percentage of enzyme inhibition	
	α-glucosidase	Angiotensin-converting enzyme (ACE)
Sa-med	$59.52 \pm 4.52$	$93.19 \pm 4.30$

Legand: <sup>1</sup>All data were represented as mean  $\pm$  SD.

# Effect of Sa-med mushroom substitution on physical properties of snacks

Among the snacks made by substitution composite flour with Sa-med mushroom powder at 10, 12 and 14 %, the results disclosed that the increase of Sa-med mushroom powder resulted in the decrease of brightness ( $L^*$ ) while the redness ( $a^*$ ) was increased. Moreover, there was no significant difference on vellowness  $(b^*)$  between the snack samples (Table 2), which might be due to the small molecules produced by thermal degradation of starch and proteins induced the pronounced Maillard reactions during baking [33]. The snack with 14 % Sa-med mushroom powder showed less expansion ratio because of higher protein content. Nelson [34] and Berrios et al. [35] reported that increasing the protein levels will lead to decrease in expansion ratio of the puffed snack. The snack made from 10 % Sa-med mushroom powder showed the least bulk density (0.25 g/cm<sup>3</sup>). As bulk density is inversely related to expansion ratio, the corresponding result was evidenced. The puffed snacks with least bulk density had the maximum expansion ration. A study done by Pracha & Chulaluk [36] and Pawar et al. [37] showed decrease in expansion ratio on increase in bulk density in corn-based extruded snack. Results of texture measurement reported that there were no significant differences on hardness and crispiness. However, the hardness and crispiness seemed to be increasing along with the increase in Sa-med mushroom powder. This might be caused by the high content of protein and fiber in the product, which enhanced its hardness and crispiness. This result is in agreement with that obtained by Sudha et al. [38,39] who reported the hardness and crispiness increase with the increase in the level of sorghum flour and guar gum and Anton et al. [40] who found that increase in expansion ratio candecrease hardness.

Physical properties	Sa-med mushroom powder (%)		
r nysicai properties	10	12	14
Color L*	$37.02 \pm 0.56^{a}$	$35.29 \pm 0.38^{b}$	$35.82 \pm 0.16^{b}$
<i>a</i> *	$4.00 \pm 0.11^{b}$	$4.21\pm0.03^{\text{a}}$	$4.20 \pm 0.13^{a}$
$b^*$	$13.37 \pm 0.31^{a}$	$13.08 \pm 0.08^{a}$	$13.37 \pm 0.15^{a}$
Expansion Ratio	$2.02\pm0.05^{\rm a}$	$1.98 \pm 0.10^{a}$	$1.90 \pm 0.11^{b}$
Bulk density $(g/cm^3)$	$0.25 \pm 0.01^{b}$	$0.27\pm0.01^{a}$	$0.28\pm0.01^{a}$
Hardness (g)	$603.45 \pm 183.28^{a}$	$615.28 \pm 166.89^{a}$	$640.57 \pm 224.72^{a}$
Crispiness (mm)	$0.85\pm0.20^{\rm a}$	$0.86\pm0.15^a$	$0.92\pm0.10^{\text{a}}$

Table 2 Physical properties of snacks with different levels of Sa-med mushroom powder<sup>1,2</sup>.

Legand: <sup>1</sup>All data were represented as mean  $\pm$  SD. <sup>2</sup>Different letters in same row indicate significant difference at p < 0.05 using one-way ANOVA followed by Duncan multiple range test.

#### Sensory attributes of Sa-med mushroom snacks

The sensory attribute scores are given in **Table 3**. There is no significant difference on appearance, color, texture, and overall liking score between the snacks with different levels of Sa-med mushroom powder. However, the flavor and taste scores of snacks with 14 % Sa-med mushroom powder showed to be higher than snacks with lower levels. This might be due to mushroom contain free amino acids and 5'-monophosphate nucleotides, which are responsible for the umami taste [41]. Moreover, the result of sensory evaluation on texture was in line with the results of hardness and crispiness of snacks. The mean overall acceptability scores of the snack made with 14 % Sa-med mushroom powder was the highest. Therefore, the formulation made with 14 % Sa-med mushroom powder was selected for future study.

**Table 3** Sensory attribute scores of liking of snack obtained from Sa-med mushroom powder substitution at different levels<sup>1,2,3</sup>.

Test	Sensory	Sa-med mushroom powder (%)		
	characteristics	10	12	14
Before test	Appearance	$6.80 \pm 1.16^{a}$	$7.03\pm1.54^{\text{a}}$	$6.90 \pm 1.56^{a}$
	Color	$6.73 \pm 1.34^{a}$	$6.87\pm1.43^{\rm a}$	$6.80 \pm 1.61^{a}$
After test	Flavor	$6.87 \pm 1.25^{b}$	$7.30 \pm 1.02^{a,b}$	$7.57 \pm 1.14^{a}$
	Taste	$7.20 \pm 0.85^{b}$	$7.40 \pm 1.04^{a}$	$7.47 \pm 1.22^{a}$
	Texture	$6.83 \pm 0.34^{a}$	$7.13 \pm 1.53^{a}$	$7.50 \pm 1.25^{a}$
	Overall liking	$7.00 \pm 1.23^{a}$	$7.33\pm1.27^{a}$	$7.60 \pm 1.25^{a}$

Legand: <sup>1</sup>All data were represented as mean  $\pm$  SD. <sup>2</sup>Different letters in same row indicate significant difference at p < 0.05 using one-way ANOVA followed by Duncan multiple range test. <sup>3</sup>9-points hedonic scale (1 = Dislike extremely, 5 = Neither Like nor Dislike, 9 = Like Extremely.

### Nutrition values of Sa-med mushroom snack

The nutrition values of the control formula and snack with 14 % Sa-med mushroom powder are shown in **Table 4**. The total energy, fat and carbohydrate decreased from 129 to 116 kcal, 5.04 to 3.78 g and 19.72 to 16.81 g per serving size (25 g) in the control formula and 14 % mushroom snack. Moreover, mushroom snack presented an increase in protein, fiber, and sodium content when mushroom was added. This due to mushroom is a good source of protein and fiber but low in fat and carbohydrate [5]. According to Thai FDA regulation on nutrition labelling, it observed that protein and fiber content was 14.14 and 36 % Thai RDI so this product could be claimed as source of protein and high fiber. For

sodium content, the mushroom snack contained lower sodium than 140 mg per serving size so it could be also claimed as low sodium product. Therefore, this product could be popularized as healthy food for different age groups.

Nutritional values	Snack Formulas		
Nutritional values	Control	Sa-med mushroom	
Energy (kcal)	$129.28 \pm 0.19^{*}$	$116.88 \pm 1.70$	
Total fat (g)	$5.04 \pm 0.07^{*}$	$3.78 \pm 0.20$	
Protein (g)	$1.26\pm0.00$	$3.54 \pm 0.03^{*}$	
Total carbohydrate (g)	$19.72 \pm 0.11^*$	$16.81 \pm 0.21$	
Dietary fiber (g)	$0.20\pm0.01$	$4.50 \pm 0.06^{*}$	
Sodium (mg)	91.15 ± 7.26	$124.59 \pm 6.17^*$	

**Table 4** Nutrition values of control snack and Sa-med mushroom snack (per 25 g (service size))<sup>1</sup>.

Legand: <sup>1</sup>All data were represented as mean  $\pm$  SD. \*Significantly different (p < 0.05) at p < 0.05 using t-test.

# Total phenolic contents, antioxidant activities and $\alpha$ -glucosidase and angiotensin-converting enzyme inhibitory activities

The total phenolic contents by Folin-Ciocalteu colorimetric method and antioxidant activities using the DPPH-radical scavenging assay, ORAC assay and FRAP assays of the control formula and snack with 14 % Sa-med mushroom powder were done (as shown in **Table 5**). Total phenolic contents and antioxidant activities of mushroom snack was significantly higher than the control formula. This might confirm that the addition of mushroom in the product could result in a healthier product with functional properties. Moreover, these data suggested that a total phenolic content was positively correlated with antioxidant activities which were found in many plants [42]. For the percentage of enzyme inhibition in snack made with 14 % Sa-med mushroom powder, the results exhibited that  $\alpha$ -glucosidase and ACE inhibitory activities were 48.25 and 88.56 %, respectively. The higher percentage of ACE inhibition in mushroom snack than the control formula might was related with the higher amount of protein content. As the researchers reported that the presence of peptides may promote the ACE-inhibition of food protein [43-45]. However,  $\alpha$ -glucosidase inhibitory activities in mushroom snack were lower than the control formula. This might be due to the composite flour had higher  $\alpha$ -glucosidase inhibitory activities. However, these enzyme inhibition activities were an *in-vitro* experiment. Therefore, *in vivo* bioavailability and absorption of bioactive components of the product is required for further experiment. Table 5 Total phenolic contents, antioxidant activities and  $\alpha$ -glucosidase and angiotensin-converting enzyme inhibitory activities<sup>1</sup>.

Healthy properties	Snack Formulas	
ficility properties	Control	Sa-med mushroom
Total phenolic contents (mg GAE/g DW)	$0.90\pm0.04$	$5.28 \pm 0.50*$
Antioxidant activities		
DPPH radical scavenging assay (umol TE/100g DW)	$0.07\pm0.01$	$0.28 \pm 0.03*$
FRAP assay (umol TE/g DW)	$4.29\pm0.35$	$16.38 \pm 0.68*$
ORAC assay (umol TE/g DW)	$5.21\pm0.45$	$31.90 \pm 2.68*$
$\alpha$ -glucosidase inhibitory activities (%)	$61.98 \pm 4.31^{*}$	$48.25\pm4.03$
ACE inhibitory activities (%)	$83.75\pm0.27$	$88.56 \pm 4.89^{*}$

Legand: <sup>1</sup>All data were represented as mean  $\pm$  SD. \*Significantly different (p < 0.05) at p < 0.05 using t-test.

# **Consumer test**

The demographic information of consumers in Chon Buri province and Hua-Hin city showed that 21 % was male, while 79 % was female. Most consumers fell in the 20 - 30 years age range (72 %). The vast majority of consumers' education level was a bachelor's degree (73 %). The occupation of consumers included self-employed (21 %), employed (5 %), government officer (6 %) and students (68 %). The personal income of most respondents was 318 - 636 US dollars (50 %). For sensory acceptability test of healthy snack from Sa-med mushroom using 9-point hedonic scale, the result showed that the score in appearance, color, flavor, taste, texture and overall liking were at the level of "like moderately to like very much" (**Table 6**). Furthermore, 95 % percent of consumers found the healthy snack from Sa-med mushroom to be acceptable and 64 % were willing to buy this product.

**Table 6** Sensory acceptability test of healthy snack from Sa-med mushroom powder (n = 300).

Sensory attributes	Scores <sup>1</sup>
Appearance	7.27 ± 1.07
Color	$7.02 \pm 1.19$
Flavor	$7.21 \pm 1.16$
Taste	$7.33 \pm 1.29$
Texture	$7.94 \pm 1.15$
Overall liking	$7.51 \pm 1.08$

Legand: <sup>1</sup>9-point hedonic scale (1 = dislike extremely, 5 = neither like or nor like and <math>9 = like extremely)

#### Conclusions

Sa-med mushroom is not only a good source of protein and fiber but also has  $\alpha$ -glucosidase and ACE inhibitory activities. Therefore, it should be used as a natural source to develop functional food for controlling hypertension and/or oxidative stress, 2 major causes of cardiovascular diseases. Moreover, the results of this study showed the increase in protein has the potential to affect the physical properties of Sa-med mushroom snacks. However, healthy snack could be produced with 14 % Sa-med mushroom powder in line with consumer acceptability. The addition of Sa-med mushroom powder improved the nutritional content and health benefit of the snacks significantly. However, *in vivo* studies are also needed to verify their physiological effects.

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# References

- [1] A Aung-aud-chariya, P Bangrak, B Dell and N Kamlangdee. Genetic study of Boletus grisipurpureus from Melaleuca forest. *In*: Proceedings of the 22<sup>nd</sup> International Conference on Biotechnology and Healthy Living. Prince of Songkla University, Trang Campus, Thailand, 2010.
- [2] A Aung-Aud-Chariya, P Bangrak, B Dell, S Lumyong and N Kamlangde. Some species of wild boletes in Thailand. J. Agric. Technol. 2012; 8, 1991-8.
- [3] A Aung-Aud-Chariya, P Bangrak, S Lumyong, W Phupong, NS Aggangan and N Kamlangdee. RNA polymerase II second largest subunit molecular identification of *Boletus griseipurpureus* corner from Thailand and antibacterial activity of basidiocarp extracts. *Jundishapur J. Microbiol.* 2015; **8**, 1-4.
- [4] P Wongchalee and C Pukahute. Diversity of mushrooms in Dry Dipterocarp forest at Phuphan National Park, Sakon Nakhon Province. *Nat. Sci.* 2012; **4**, 1153-60.
- [5] U Suttisansanee, N On-nom, S Charoenkiatkul, P Thiyajai and W Srichamnong. *The Database Development of Nutritive Values, Bioactive Compounds and Functional Properties of Edible Mushroom in Amnat Charoen Province*. Final Report, Institute of nutrition, Mahidol University, Nakhon Pathom, Thailand, 2016.
- [6] S Seehanan and V Petcharat. Some species of wild Boletes in Thailand. J. Agric. Technol. 2008; 4, 109-18.
- [7] Y Sudjaroen and K Thongkao. Screening of nutritive values, in vitro antioxidant, anticancer and antimicrobial activities from *Boletus griseipurpureus* Corner. *Int. J.Green Pharm.* 2017; **11**, 174-81.
- [8] A Angajchariya, S Naranong, D Phichairat, L Kaewsongsang, W Phupong and N Mahae. Mycelial Growth, Antioxidant and Antibacterial Properties of *Boletus griseipurpureus* from South Thailand. *Int. J. Agric. Technol.* 2017; **13**, 521-9.
- [9] ICFR Ferreira, L Barros and RMV Abreu. Antioxidants in wild mushrooms. *Curr. Med. Chem.* 2009; 16, 1543-60.
- [10] M Rai, G Tidke and SP Wasser. Therapeutic potential of mushrooms. Nat. Prod. Rad. 2005; 4, 246-57.
- [11] N Balan and M Radhakrishnan. Microwave drying of edible mushroom. *Mushroom Res.* 2014; 23, 81-7.
- [12] KS Rhee, SH Cho and AM Pradahn. Composition, storage stability and sensory properties of expanded extrudates from blends of corn starch and goat meat, lamb, mutton, spent fowl meat, or beef. *Meat Sci.* 1999; **52**, 135-41.
- [13] P Chen, W Kuo, C Chiang, H Chiou, Y Hsieh and S Chu. Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. *Chem. Biol. Interact.* 2006; 163, 218-29.

- [14] A Nath and PK Chattopadhyay. Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *J. Food Eng.* 2007; **80**, 1282-92.
- [15] MK Reddy, A Kuna, NL Devi, N Krishnaiah, C Kaur and Y Nagamalleswari. Development of extruded Ready-To-Eat (RTE) snacks using corn, black gram, roots and tuber flour blends. J. Food Sci. Technol. 2014; 51, 1929-37.
- [16] A Nemś, A Pęksa, AZ Kucharska, A Sokół-Łętowska, A Kita, W Drożdż and K Hamouz. Anthocyanin and antioxidant activity of snacks with coloured potato. *Food Chem.* 2015; **172**, 175-82.
- [17] A Pornpitakdamrong, A Aung-aud-chariya and Y Sudjaroen. Development of pickled "Hed sa med" mushroom (Boletus griseipurpureus Corner). *In*: Proceedings of the 5<sup>th</sup> Suan Sunandha Academic National Conference on Research for Sustainable Development, Bangkok, Thailand. 2016, p. 161-72.
- [18] S Segnini, F Pedreschi and P Dejmek. Volume measurement method of potato chips. *Int. J. Food Prop.* 2004; **7**, 37-44.
- [19] S Sahin and SG Sumna. Physical Properties of Foods. Springer Publications, USA, 2006, p. 1-36.
- [20] AOAC. Official Methods of Analysis of AOAC International. 20th eds. Gaithersburg, USA, 2016.
- [21] EA Ainsworth and KM Gillespie. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2007; 2, 875-7.
- [22] LR Fukumoto and G Mazza. Assessing antioxidant and provident activities of phenolic compounds. *J. Agr. Food Chem.* 2000; **48**, 3597-604.
- [23] B Ou, M Hampsch-Woodill and RL Prior. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agr. Food Chem. 2001; **49**, 4619-26.
- [24] IFF Benzie and JJ Strain. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 1996; 239, 70-6.
- [25] Q You, F Chen, X Wang, PG Luo and Y Jiang. Inhibitory effects of muscadine anthocyanins on alpha-glucosidase and pancreatic lipase activities. J. Agric. Food Chem. 2011; 59, 9506-11.
- [26] SL Schwager, AK Carmona and ED Sturrock. A high-throughput fluorimetric assay for angiotensin I-converting enzyme. *Nat. Protoc.* 2006; **1**, 1961-4.
- [27] M Meilgaard, GV Civille and BT Carr. Sensory Evaluation Techniques. 3<sup>rd</sup> eds. CRC Press, Boca, 1999.
- [28] P Kotler. Marketing Management: Analysis, Planning, Implementation, and Control. 9<sup>th</sup> eds. Prentice-Hall, New Jersey, 1997, p. 789.
- [29] H Matsuur, C Asakawa, M Kurimoto and J Mizutani. Alpha-glucosidase inhibitor from the seeds of balsam pear (Momordica charantia) and the fruit bodies of Grifola frondosa. *Biosci. Biotechnol. Biochem.* 2002; 66, 1576-8.
- [30] RL Soffer. Angiotensin-converting enzyme and the regulation of vasoactive peptides. *Ann. Rev. Biochem.* 1976; **45**, 73-94.
- [31] Y Zhang, ET Lee, RB Devereux, J Yeh, LG Best, RR Fabsitz and BV Howard. Prehypertension, diabetes, and cardiovascular disease risk in a population-based sample: The strong heart study. *Hypertension* 2006; **47**, 410-4.
- [32] PR Andrews, JM Carson, A Caselli, MJ Spark and R Woods. Conformational analysis and active site modelling of angiotensin-converting enzyme inhibitors. J. Med. Chem. 1985; 28, 393-9.
- [33] MZ Islam, MLJ Taneya, M Shams-Ud-Din, M Syduzzaman and MM Hoque. Physicochemical and functional properties of Brown rice (Oryza sativa) and Wheat (Triticum aestivum) flour and quality of composite biscuit made thereof. *Sci. J. Krishi Found* 2012; 10, 20-8.
- [34] M Nelson. Protein rich extruded snack foods using hydrolyzed proteins. *Am. Pshych. Assoc.* 2003; 1, 55.
- [35] JJ Berrios, P Morales, M Cámara and MC Sánchez-Mata. Carbohydrate composition of raw and extruded pulse flours. *Food Res. Int.* 2010; **43**, 531-6.

- [36] B Pracha and C Chulaluk. Development of corn grit-broken rice based snack food by extrusion cooking. *Kasetsart J. Nat. Sci.* 2000; **34**, 279-88.
- [37] VD Pawar, GM Machewad, AV Durge and AS Maitre. Processing and characteristics of snacks extruded from rice and corn grits and two malted legumes. *J. Food Sci. Technol.* 2009; **46**, 494-6.
- [38] ML Sudha, R Vetrimani and K Leelavathi. Influence of fiber from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chem.* 2007; **100**, 1365-70.
- [39] ML Sudha, R Vetrimani and K Leelavathi. Fat replacement in soft dough biscuits: Its implications on dough rheology and biscuit quality. J. Food Eng. 2007; **80**, 922-30.
- [40] AA Anton, RG Fulcher and SD Arntfield. Physical and nutritional impact of fortification of cornstarch based extruded snacks with common bean (Phaseolus vulgaris L.) flour: Effects of bean addition and extrusion cooking. *Food Chem.* 2009; **113**, 989-96.
- [41] A Ranogajec, S Beluhan and Z Smit. Analysis of nucleosides and monophosphate nucleotides from mushrooms with reversed phase HPLC. J. Sep. Sci. 2010; 33, 1024-33.
- [42] A Ghasemzadeh, HZEJ aafar and A Rahmat. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules* 2010; 15, 4324-33.
- [43] RE Cian, M Alaiz, J Vioque and SR Drago. Enzyme proteolysis enhanced extraction of ACE inhibitory and antioxidant compounds (peptides and polyphenols) from Porphyra columbina residual cake. J. Appl. Phycol. 2013; 25, 1197-206.
- [44] MR Segura-Campos, F Peralta-González, A Castellanos-Ruelas, LA Chel-Guerrero and DA Betancur-Ancona. Effect of Jatropha curcas peptide fractions on the angiotensin I-converting enzyme inhibitory activity. *Biomed Res. Int.* 2013; **2013**, 541947.
- [45] R Ghanbari, M Zarei, A Ebrahimpour, A Abdul-Hamid, A Ismail and N Saari. Angiotensin-I converting enzyme (ACE) inhibitory and anti-oxidant activities of sea cucumber (Actinopyga lecanora) hydrolysates. *Int. J. Mol. Sci.* 2015; **16**, 28870-85.