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Effect of Blanching on B-glucan Content of Native Mushrooms in Thailand

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β-Glucans are a group of polysaccharide which composed of glucose units linked with beta-glycosidic bonds. They have been found in several natural sources including mushrooms. From previous reported, this compound showed interesting properties such as immune response stimulation in human and animal, anti-tumor, reduction of cholesterol and others. Therefore, mushrooms have been used as medicinal food for a long time. Thailand are rich of several resources including the strains of local mushrooms. However, no studied on the affected of blanching on β-glucan content of those mushrooms were observed. So, the objective of this studied was to compare β-glucan content of eleven local mushrooms between blanching and un-blanching process. The result shown that three groups of β-glucan content were exhibited. The first group; β-glucan content of most local blanching mushrooms species (*Schizophyllum commune*: Hed Krang, *Pleurotus ostrearus*: Hed Pao Hea, *Auricularia fuscosuccinea:* Hed Hunu Dum , *Lentinus polychrous:* Ked Kon, *Flammulina velutipes* : Ked Kem Thong, *Hypsizygus marmoreus*: Hon-Shimeji: Hed Shimeji Dum and *Lentinus edodes*: Hed Hom) were found higher than un-blanching samples. However, only four mushrooms species (*Pleurotus ostrearus*: Hed Pao Hea, *Hypsizygus marmoreus*: Hon-Shimeji: Hed Shimeji Dum, *Flammulina velutipes:* Ked Kem Thong and *Lentinus edodes*: Hed Hom) were showed significantly higher than un-blanching samples. The percent increasing of β-glucan were 28.43, 40.44, 14.26 and 6.23 %, respectively ($p \leq 0.05$). The second group, β-glucan content of un-blanching of two local mushrooms species (*Hypsizygus tessellates* : Hed Nang Rom Hanggari *and Hypsizygus marmoreus:*Buna-Shimeji :Hed Shimeji Kaw) were found higher than blanching samples. The third group; the amount of β-glucan content were found similarity between blanching and un-blanching process. On the other hand, non significantly differences ($p \leq 0.05$) were demonstrated in the last two groups. In conclusion, in blanching process could be affecting to decrease amount of α-glucans and tend to be increasing the amount of β-glucan.

Keywords: β-glucan, blanching, polysaccharide, native mushrooms

Introduction

Nowaday, mushroom have been used as medicinal food in Asian region for long time ago especially in Japan, Chinal and Korea (Manzi and Pizzoferrato,

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2000). Thailand also found and cultivated various mushrooms. Previously researches reported that mushrooms have been an important source of novel bio-active compounds (Hawksworth, 1995). It also has great potential as a nutritionally functional food and a source of physiologically beneficial and 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 the drugs development and nutraceuticals. Furthermore, recent studies reported that medicinal mushrooms have been shown medicinal properties. For example, they are reported 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 have been reported to below in calories and in fat but they are rich in proteins, minerals and dietary fibre. In addition, Manzi *et al.* (1999), described that mushrooms are much more for their texture and flavor. Mushrooms also contain significantly amounts of vitamins that are thiamin, riboflavin and 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-glucans. β-Glucans are groups of polysaccharides that are composed of glucose units linked together with β-glycosidic bonds (Klis *et al*., 2002). β-Glucans also exhibit 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).

Blanching is used in the preparation of vegetables before freezing, dehydration and canning, depending the final preservation method. Blanching serves many functions: inactivation of enzyme causing discoloration, pre-shrinkage, removal of air to reduce oxidative change, reduction of microbial population, reduction cooking time and making more pliable to facilitate the filling operation (Sensoy and Sastry, 2003).

Asian countries are known to be rich source of medicinal mushrooms. However, no studied on the affected of blanching process on β-glucan content of those mushrooms were observed. Therefore , the objective of this studied was to compare total-glucan, α-glucan and β-glucan content of eleven local mushrooms between blanching and un-blanching process.

Materials and methods

Materials

Eleven local mushrooms such as *Schizophyllum commune* : Hed Krang, *Pleurotus ostrearus*: Hed Pao Hea, *Lentinus polychrous*: Hed Kon, *Hypsizygus tessellates*: 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 purchased from a local farm and Krating market in Amphur Kao Kitchakut, Chanthaburi province, Thailand and transported to the laboratory.

Sample Preparation

All of local mushrooms as mentioned above were sliced in the small size (1 cm^3) and divided in two groups. The first group, blanching samples; mushrooms sample (50 gram) were diped in the blanching fluid at 100 ºC for 5 min and then dip in cold water suddenly. Then, all of sample was dried by using hot air oven (Binder, FD115, Germany) at 70 ˚C for 24 hours. The dry samples were grinding by using blender.

β-Glucan contents determination

β-Glucan contents in mushroom powder were analyzed using a Yeast Beta-Glucan Assay Kit (Megazyme, Ireland) as follows.

For total-glucan content, 100 mg of milled mushrooms were placed in test tube then 1.5 ml of 37 % hydrochloric acid was added. The solution was mixed and incubated at 30 °C for 45 min (mixed every 15 min). Then, 10 ml of distilled water was added, mixed and incubated at 100 °C for 2 h before added with 10 ml of 2 N KOH. The solution was taken, adjusted volume to 100 ml with sodium acetate buffer pH 5 and mixed. After that, the mixtures were centrifuged at 1,500 ×g for 10 min. Samples (100 µl) were taken to each test tube (in duplicates) before added with 100 µl of a mixture of exo-1,3-β-glucanase plus β-glucosidase and then incubated at 40 °C for 60 min. Finally, 3 ml of glucose oxidase/peroxidase were added and incubated at 40 °C for 20 min. The absorbance was measured at 510 nm with 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 were placed in test tubes and then 2 M KOH (2 ml) was added. The pellets were stirred with magnetic stirrer in ice bath for 20 min. 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 added, incubated at 40 °C for 30 min and mixed by vertex stirrer. After that, the mixture was centrifuged at 1,500 ×g for 10 min. Supernatant (100 μl) were taken to test tube (in duplicates). Glucose oxidase / peroxidase (3 ml) were added to each tube and incubated at 40 °C for 20 min. The absorbance was measured at 510 nm with spectrophotometer (Celli, CE1011, England). The concentration of glucose in the sample was calculated from the assay kit procedure. For the amount of β-glucan content, it was calculated by total-glucan substract α-glucan (Megazyme, Ireland; Mongkontanawat *et al*., 2011).

Data analysis

Glucan analysis was carried out in four replicates. The data were subjected to Analysis of Variance (ANOVA) ($p \leq 0.05$) (Steel *et al*., 1997). Mean with significant differences were separated by Duncan's Multiple Range Test (DMRT) using computer software. For percent of β-glucan increasing, mean with significant difference were compared by T-Test also using computer software.

Results and discussion

From glucans determination of eleven local mushrooms as mention previously, our result has been exhibited in Table1.

Table 1. Total-glucan, α-glucan and β-glucan content of native mushrooms species between blanching and un-blanching

Local name	Scientific name	Total-glucan(%w/w)		α-glucan(%w/w)		B-glucan(%w/w)	
		blanching	un-blanching	blanching	un-blanching	blanching	un-blanching
Hed Nang Nuan	*Pleurotus djamor*	33.66±2.93^{b}	34.00±7.58^{ab}	0.86±0.11^{cd}	0.57±0.16^{d}	32.80±2.90^{bc}	33.43±7.72^{ab}
Hed Shimeji Dum	*Hypsizygus marmoreus* :Hon-Shimeji	31.20±1.47^{bc}	19.01±0.55^{e}	0.43±0.18^{de}	0.68±0.05^{cd}	30.77±1.57^{cd}	18.32±0.57^{d}
Hed Shimeji Kaw	*Hypsizygus marmoreus:*Buna-Shimeji	27.10±3.86^{cd}	29.31±2.60^{bcd}	0.50±0.14^{de}	0.51±0.11^{d}	26.60±3.86^{ef}	28.81±2.64^{bc}
Hed Kem Thong	*Flammulina velutipes*	26.29±1.34^{d}	22.59±1.50^{de}	0.58±0.17^{de}	0.55±0.17^{d}	25.71±1.34^{f}	22.04±1.36^{cd}
Hed Kon	*Lentinus polychrous*	29.49±0.59^{cd}	25.95±1.67^{cde}	2.30±0.27^{b}	0.27±0.07^{d}	27.19±0.51^{ef}	25.68±1.74^{cd}
Hed Krang	*Schizophyllum commune*	39.67±2.77^{a}	35.91±7.36^{ab}	1.20±0.13^{c}	0.43±0.16^{d}	38.48±2.71^{a}	35.48±7.24^{ab}
Hed Hunu Dum	*Auricularia fuscosuccinea*	30.01±1.87^{cd}	28.64±3.20^{bcd}	0.23±0.03^{e}	0.12±0.03^{d}	29.78±1.84^{cde}	28.52±3.18^{bc}
Hed Hom	*Lentinus edodes*	21.94±0.53^{e}	20.83±0.56^{e}	0.46±0.09^{de}	0.69±0.15^{cd}	21.48±0.53^{g}	20.14±0.65^{d}
Hed Pao Hea	*Pleurotus ostrearus*	37.60±1.74^{a}	30.51±1.46^{bc}	2.58±0.30^{b}	5.44±1.46^{a}	35.02±1.54^{b}	25.06±0.46^{cd}
Hed Nang Rom Hanggari	*Hypsizygus tessellates*	33.94±4.12^{b}	39.51±2.72^{a}	3.09±0.79^{a}	1.36±0.22^{bc}	30.86±4.89^{cd}	38.15±2.79^{a}
Hed Nang Fa	*Pleurotus sajor-caju*	29.22±1.73^{cd}	30.83±10.59^{bc}	0.49±0.02^{de}	1.81±0.20^{b}	28.74±1.72^{ef}	29.02±10.76^{bc}

Each data represents mean of four replications with standard errer.
Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's Multiple Range test.

From table 1, high level of total-glucan of blanching mushroom samples were exhibited in *Schizophyllum commune* : Hed Krang and *Pleurotus ostrearus* : Hed Pao Hea. The levels of total-glucans content were found 39.67±2.77 and 37.60±1.74 % w/w, respectively and significantly different ($p \leq 0.05$) from other mushrooms. Low level amount of total-glucan of blanching mushroom samples were demonstrated in *Lentinus edodes* : Hed Hom (21.94±0.53 % w/w) and significantly different ($p \leq 0.05$) from other treatments. High level of total-glucan of un-blanching mushroom samples were exhibited in *Hypsizygus tessellates* : Hed Nang Rom Hanggari, *Schizophyllum commune* : Hed Krang and *Pleurotus djamor* : Hed Nang Nuan. The levels of total-glucans content were found 39.51±2.72, 35.91±7.36 and 34.00±7.58 %w/w, respectively. However, only *Hypsizygus tessellates*: Hed Nang Rom Hanggari had significantly different ($p \leq 0.05$) from other mushrooms. Low level of total-glucan content of un-blanching mushroom samples were also demonstrated in *Lentinus edodes* : Hed Hom (20.83±0.56 % w/w) and significantly different ($p \leq 0.05$) from other treatments.

High level of α-glucan of blanching mushroom samples were exhibited in *Hypsizygus tessellates*: Hed Nang Rom Hanggari (3.09±0.79 % w/w) and significantly different ($p \leq 0.05$) from other mushrooms. Low level of α-glucan content of blanching mushroom samples were demonstrated in *Auricularia fuscosuccinea* : Hed Hunu Dum (0.23±0.03 % w/w) and significantly different ($p \leq 0.05$) from other treatments. High level of α-glucan of un-blanching mushroom samples were exhibited in *Pleurotus ostrearus* : Hed Pao Hea (5.44±1.46 % w/w) and significantly different ($p \leq 0.05$) from other mushrooms. Low level of α-glucan content of un-blanching mushroom samples were found in several samples such as *Pleurotus djamor* : Hed Nang Nuan, *Hypsizygus marmoreus:*Buna-Shimeji : Hed Shimeji Kaw, *Flammulina velutipes* : Hed Kem Thong, *Lentinus polychrous* : Hed Kon, *Schizophyllum commune* : Hed Krang and *Auricularia fuscosuccinea* : Hed Hunu Dum . The level of α-glucan content were found 0.57 ±0.16, 0.51±0.11, 0.55±0.17, 0.27±0.07, 0.43±0.16 and 0.12±0.03 % w/w, respectively.

According to β-glucan was calculated by total-glucan minus α-glucan as mention previously. Our result was indicated that high level of β-glucan of blanching mushroom samples were exhibited in *Schizophyllum commune*: Hed Krang (38.48±2.71 % w/w) and significantly different ($p \leq 0.05$) from other mushrooms. Low level of β-glucan content of blanching mushroom samples were also demonstrated in *Flammulina velutipes* : Hed Kem Thong (25.71±1.34 % w/w),in contrast non significantly different ($p \leq 0.05$) from *Hypsizygus marmoreus:*Buna-Shimeji : Hed Hunu Dum, *Lentinus polychrous* : Hed Kon and *Pleurotus sajor-caju* : Hed Nang Fa. The level of β-glucan content were found

26.60 ±3.86, 27.19±0.51 and 28.74±1.72 % w/w, respectively. High level of β-glucan of un-blanching mushroom samples were exhibited in *Hypsizygus tessellates*: Hed Nang Rom Hanggari (38.15 ±2.79 % w/w). However, non significantly different ($p \leq 0.05$) from *Pleurotus djamor* : Hed Nang Nuan and *Schizophyllum commune*: Hed Krang (33.43±7.72 and 35.48 ±7.24 % w/w**,** respectively**)**. Low level of β-glucan content of un-blanching mushroom samples were demonstrated in *Hypsizygus marmoreus :*Hon-Shimeji : Hed Shimeji Dum(18.32±0.57 % w/w), however non significantly different ($p \leq 0.05$) from *Flammulina velutipes* : Hed Kem Thong, *Lentinus polychrous* : Hed Kon, *Lentinus edodes* : Hed Hom and *Pleurotus ostrearus* : Hed Pao Hea (22.04±1.36, 25.68 ±1.74, 20.14 ±0.65 and 25.06 ±0.46 % w/w, respectively).

Our result could be explained that α-glucan (water soluble glucan) from mushrooms tissue were loss by the heat treatment, therefore β-glucan in most of mushroom were increasing. Moreover, Manzi *et al*. (2004) also reported that β-glucan and total phenol seem tobe more affected by the heat treatment. In addition, β-glucans in mushrooms show different water affinities. This particular behavior is probably due to different molecular structure, the molecules of β-glucan and different molecular weight (Manzi and Pizzoferrato. 2000). When the glucans of mushrooms were compared between blanching and un-blanching. Our result showed that most of blanching mushrooms had total-glucan, α-glucan and β-glucan content higher than un-blanching samples. Based on percent increasing of total-glucan, the amount of total-glucan of seven blanching mushrooms species exhibited higher than un-blanching samples (figure 1). However, only four mushroom samples such as *Pleurotus ostrearus* : Hed Pao Hea, *Hypsizygus marmoreus*:Hon-Shimeji: Hed Shimeji Dum, *Lentinus polychrous* : Hed Kon and *Flammulina velutipes* : Hed Kem Thong shown significantly from un-blanching sample ($p \leq 0.05$). The numbers of percent total-glucan increasing were 18.87, 39.08, 11.98 and 14.08 %, respectively.

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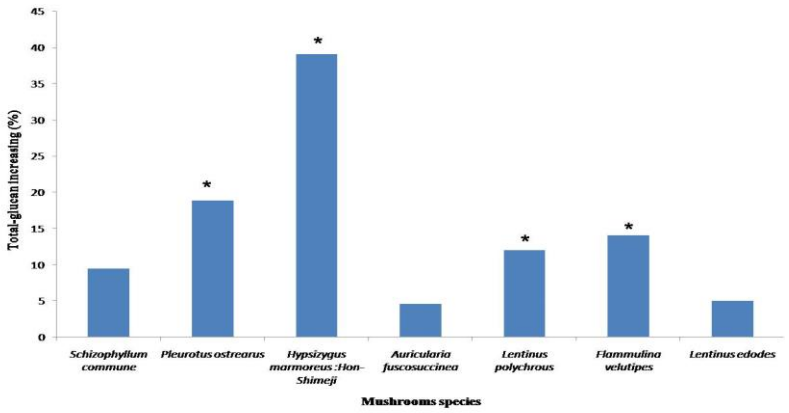

Figure 1. Percent increasing of total-glucans of blanching native mushroom species. Data with * are statistically different ($p \leq 0.05$) according to t-test

For the percent increasing of α-glucan, the amount of α-glucan of six blanching mushrooms species exhibited higher than un-blanching samples (figure 2). However, only five mushroom sample such as *Schizophyllum commune*: Hed Krang, *Pleurotus djamor* : Hed Nang Nuan, *Lentinus polychrous* : Hed Kon, *Auricularia fuscosuccinea* : Hed Hunu Dum *and Hypsizygus tessellates* : Hed Nang Rom Hanggari shown significantly from un-blanching samples ($p \leq 0.05$). The numbers of percent α-glucan increasing were 64.19, 33.77, 88.08, 44.75 and 55.85 %, respectively.

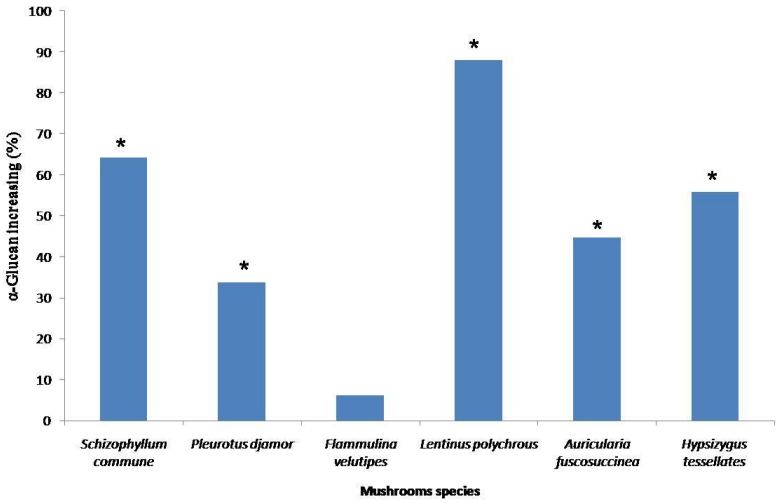

Figure 2. Percent increasing of α-glucan in blanching native mushroom species Data with * are statistically different ($p \leq 0.05$) according to t-test.

The amount of β-glucan content of several mushrooms comparing between blanching and un-blanching, the result shown that three groups of β-glucan content were exhibited. The first group; the β-glucan content of seven blanching mushrooms species exhibited higher than un-blanching samples (figure 3). However, only four mushroom species such as *Pleurotus ostrearus*: Hed Pao Hea, *Hypsizygus marmoreus* :Hon-Shimeji : Hed Shimeji Dum, *Flammulina velutipes*: Hed Kem Thong and *Lentinus edodes* : Hed Hom shown significantly from un-blanching sample ($p \leq 0.05$). The numbers of percent β-glucan increasing were 28.43, 40.44, 14.26 and 6.23 %, respectively.

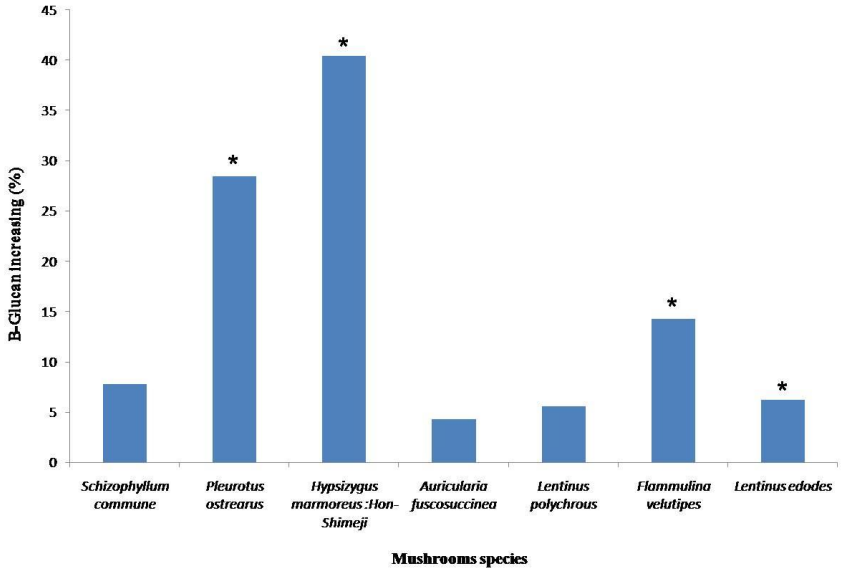

Figure 3. Percent increasing of β-glucan in blanching native mushroom species
Data with * are statistically different ($p \leq 0.05$) according to t-test.

The second group, β-glucan content of un-blanching of a few local mushrooms species (*Hypsizygus tessellates* : Hed Nang Rom Hanggari *and Hypsizygus marmoreus:*Buna-Shimeji :Hed Shimeji Kaw) were found higher than blanching samples. The numbers of percent β-glucan increasing were 19.12 and 7.66 %, respectively. The last group; the amount of β-glucan content were found similarity between blanching and un-blanching process (*Pleurotus djamor* : Hed Nang Nuan . *Pleurotus sajor-caju*: Hed Nang Fa). On the other hand, non significantly differences ($p \leq 0.05$) were demonstrated in the last two groups.

Conclusion

The results showed that the amount of total-glucan, α-glucan and β-glucan of most blanching native mushrooms species were higher than un-blanching samples. In blanching process could be affecting to decrease amount of water soluble compound (α-glucan) and tend to be increasing the amount of β-glucan. However, the results also depended on the species of the mushrooms. In conclusion, this research is good preliminary study on the effect of blanching on β-glucans content in native mushrooms in Thailand. However, the nutritional component determination could be required to compare between blanching and un-blanching mushrooms in the further research.

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