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Nutritional Security: Carbohydrate Profile and Folk Remedies of Rare Edible Mushrooms to Diversifying Food and Diet: Thailand Case Study

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Abstract: The aimed of this study was to explore the current state of knowledge of agrobiodiversity to improve nutrition and health. The study focused on wild mushrooms commonly consumed in North-Eastern of Thailand in term of ecology, cooking and preservation method and folk remedies. The monosaccharide, sugar alcohol, glucans and carbohydrate polymer content and the glycemic index of these wild mushrooms was determined using Enzymatic method. The mushrooms collected were belong to three biological groups and mostly saprotrophic and symbiotic. The most abundant mushrooms are Amanita, Boletus and Russula and followed by *Calostoma sp*., *Astraeus asiaticus* C. Phosri and *Astraeus odoratus* C. Phosri. Wild edible mushrooms can be used include food and medicinal purpose. Cooking methods utilized in the area are steaming, boiling and grilling. The carbohydrate profiles results showed that glucose is the major monosaccharide detected in mushroom samples. Xylitol and inositol was found in all mushroom sample while mannitol and arabitol was detected in some samples. Glucan was presented in all mushroom samples ranged from 8.03-31.1 mg/g DW. All mushrooms are classified as low GI food. Therefore, this finding provide important information that may be adopted to enhance and promote the utilization of wild mushroom to improve nutrition security and sustainability.

Keywords: carbohydrate; glucan; mushroom;nutrition security; sustainability

1. Introduction

Edible Mushroom is nutrient rich and versatile and becoming the new popular nutritious, delicious new resources food in the world. It is rich in proteins, vitamins, fibers and minerals while the content of fat is low. Edible mushroom has been used in several cuisines in many forms of fresh, dried and processed due to their unique and delicate flavor. Recently, mushrooms were proved to have medicinal effect in animal study model and clinical trial. They have various health benefit properties, including anti-inflammation, anti-tumor, anti-cancer, anti-obesity, hepato-protective and even anti-depression [11]. Among the numerous health benefits associated with the consumption of mushroom, our attention in particular is towards the characterization of carbohydrate profiles. Carbohydrates are also the most abundant component of mushrooms, usually accounting for about 35-70%, including digestible and non-digestible carbohydrates. The digestible carbohydrates are including monosaccharide, disaccharide, and sugar alcohol. Among sugar alcohol, arabitol, mannitol and trehalose are naturally occurring and widely distributed in mushrooms [1]. Non-digestible carbohydrates, especially polysaccharides which are related to the prebiotic activity. Mushroom polysaccharides are structural components of cell walls which can be divided into two major types: rigid fibrillars of chitin and the more abundant glucans. Glucans include β-glucans with variable proportion of β-1,3 and β-1,6 linkages as well as α-1,3-glucans (Ruiz-Herrera, 1956).

Our research group has been interested in the carbohydrate profile, estimated glycemic index (eGI) and folk remedies of wild mushrooms commonly consumed in North-Eastern of Thailand, one of the Asian regions with higher wild mushrooms diversity: *Russula* sp., *Amanita aff.* sp., *Calostoma* sp., *Boletus griseipurpureus*, *Astraeus odoratus* [14]. Even among the already species the proportion of well investigated mushroom is very low. Some researchers have reported the nutritional composition of these wild mushrooms but there are no studied on their individual composition in monosaccharide, disaccharide, sugar alcohol and glucan and on their glycemic index which significant influence on blood glucose levels. Some type of mushroom is regions specific. It is due to different climate, soil type, pattern of rain and seasonal resulted in different growth which influence their fruiting body and mycelium [2]. Traditional eating of wild mushrooms is varied from countries to countries which also influenced by culture and beliefs. For example, African countries doesn't utilize *Agarigus* species while it does in Asian countries. In addition, cooking method and traditional medicine properties are varied. The knowledge of medicinal usage was based on trial and error experience and was not supported by either systematic research and well documented. While some of the plant knowledge has been lost due to the environment degradation and changes in modern social and economic system. Valuable information which has been passed more than 50 years such as traditional knowledge will also be emphasized including cooking method to reduce bitterness and preservation method. This knowledge could be applied and transformed into modern practical. Enzymatic method for carbohydrate analysis has been well established and there are several analysis kits commercially available. The usage of the kits enables the rapid analysis and reduce the usage of complicate instruments. Therefore, in off field work, the implementation of enzyme kit with proper calibration standard, will be simple and suitable for carbohydrate analysis in rural area. As it can be done near the site of mushroom harvest to reduce transportation time which influence mushroom compound degradations.

To the best of our knowledge, several Thai wild mushrooms have never been studied. It is important to gather the information of mushroom diversified as to add the database or for comparison. For example, *Calostoma* spp. which is the sclerodermatoid fungi, despite its placement within the ectomycorrhizal sclerodermatoid fungi in evolutionary trees. Therefore, this work aimed to obtain the information of 8 different wild mushrooms commonly consumed in North-Eastern of Thailand in term of ecology, cooking and preservation method and folk remedies. The monosaccharide, disaccharide, sugar alcohol and glucans content were determined. Moreover, the glycemic index of these wild mushrooms was estimated. The data obtained will be added to the existing knowledge and documented as folk remedies. In addition, it can be used further to policy planning development and as the database for reference and find new species for mushroom consumption as well as to used food based approaches to improving nutrition.

2. Materials and Methods

2.1. Samples

Samples of *Russula delica* Fr. (Hed Kai-Khao), *Russula virescens* (Schaeff.) Fr. (Hed Kai-kiew), *Calostoma sp*. (Hed Ta-lo), *Russula cyanoxantha* (Schaeff.) Fr. (Hed Na-lae), *Boletus griseipurpureus* Cor. (Hed Sa-med), *Amanita princeps Corner & Bas*. (Hed Rangok), *Astraeus asiaticus* C. Phosri (Hed Por-fai) and *Astraeus odoratus* C. Phosri (Hed Por-nung) were collected from Dong Yai community forest, Hua Taphan district, Amnat-Charoen province Thailand during the wet seasons (June-September) of 2016. All samples were lyophilised ((lyophilizer from GEA freeze-drying equipment, Köln, NW, Germany) and grounded (Philips 600W from Philips Electronic Co., Ltd., Jakarta, Indonesia). Powder samples were kept in vacuum aluminum foil bag at -20°C until analysis.

2.2. Data collection

The professional pictures were used to make interviews in order to have reliable identifications because several fungi had very close morphology. To conduct this survey, a detailed questionnaire containing the main topics (local name, ecology, cooking and preservation method and folk remedies)

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to be addressed was elaborated [6]. The informative survey was conducted by interviewing. The questioner was asked 45 villagers (including male and female) ages between 31-90 years old including folk medicinal property, cooking and preservation method.

2.3. Monosaccharide analysis

In brief, sample was weighed 100 mg into a tube and mixed with 80% ethanol and shake slowly. The mixture was then placed in water bath at 80 °C for 15 min and centrifuged for 10 min at 10 °C (rpm 4600) subsequently. The supernatant was transferred to a new tube and mixed with 60% (v/v) sulphuric acid and stirred continuously with a magnetic bar for 1 h at 100 °C. The heat mixture was reacted with NADP+/ATP (nicotinamide-adenine dinucleotide phosphate + adenosine triphosphate), HK/G-6-PDH (hexokinase + glucose-6-phosphate dehydrogenase) and PGI (phosphoglucose isomerase), respectively and then measured at 340 nm wavelength.

2.4. Sugar alcohol analysis

Sample was weighed 100 mg into a tube and mixed with ice cold perchoric acid (1M) then centrifuged at 10 °C for 10 min (rpm 4600) to obtain only clear supernatant with pH adjusted to 7. Then pipette 0.01 mL of the supernatant into 96 well plate, mixed with NAD+/INT (nicotinamide-adenine dinucleotide + iodonitrotetrazolium chloride), diaphorose enzyme and SDH (sorbitol dehydrogenase), respectively and measured the mixture at 492 nm wavelength.

2.5. Glucan and carbohydrate polymer analysis

Alpha-glucan, beta-glucan, cellulose and arabinose xylan content was determined using megazyme kit. In brief, sample was weighed 100 mg into a test tube. It was extracted with 2 mL of potassium hydroxide (2 M) solution by stirring the solution with magnetic stirrer for 20 min. Then 8 mL (1.2M, pH 3.8) of sodium acetate buffer solution and 0.2 mL enzyme (amyloglucosidase (1630 U/mL) + invertase (500 U/mL) was added and incubated at 40 °C for 30 min. 0.01 mL of extracted sample was pipette into 96 well plate. The absorbance was measured at the wavelength of 510 nm after mixed with GODPOD reagent, which determined the concentration by comparing the absorbance value to the standard glucose solution graph.

2.6. Estimated glycemic index analysis

The in vitro starch digestibility was assessed according to Chaipati et al.,2018 [4].

2.7. Statistical analysis

All of the values shown are the mean averages of triplicate determinations. The data were reported as the means and standard errors of the mean (mean±SEM). The area under the curve associated with a change in the glucose level was calculated using GraphPad Prism version 5.01 (GraphPad software, CA, USA). The glycemic index and starch fraction after hydrolysis were analyzed by one-way analysis of variance using SPSS version 19 at a 95% confidence interval.

3. Results

3.1. Ecology, cooking and preservation methods and folk remedies

According to the survey, 72% of female and 11% of male villagers which age between 31-90 years old were conducted in the interview. The interviewing allows to draw up a list of 8 wild edible mushroom in Amnat-Charoen province, Thailand. Table 1 presents the complete list of wild edible mushroom with their local name in Thai language, ecology, cooking and preservation method and folk remedies. The mushroom collected for this study belong to three biological groups and mostly saprotrophic and symbiotic. The mushrooms were collected on the dead wood and leaves, and litter mix soil as well as from their partner trees in forest prospected. Example Boletus griseipurpureus Cor. was collected under the eucalyptus tree. Moreover, the survey shows that the most abundant

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mushroom are Amanita, Boletus and Russula and followed by *Calostoma* sp. (stalked puffball shape), *Astraeus asiaticus* C. Phosri and *Astraeus odoratus* C. Phosri (barometer earth star shape). *Calostoma* spp. is the species that look like golf ball cover with jelly like fruiting body. It poses very interesting polysaccharide that is gel without gelation and no heat is required. *Astrause asiatius* and *Astraeus odourous* are puff ball mushroom similar to Pisolithus and Scleroderma. With the exception that, they are subterranean fungi as they can be found underground.

3.2. Monosaccharide content

The content of glucose (Table 2) in these mushrooms are in range of 2-5 g which is higher than Sanmee et al., 2003 [19] who reported that the content of glucose was 1-2 g. The glucose content was found in fallowing order: Russula delica Fr. > *Astraeus asiaticus* C. Phosri > Russula virescens (Schaeff.) Fr. > Boletus griseipurpureus Cor. > Russula cyanoxantha (Schaeff.) Fr. However, there was no glucose found in *Calostoma* sp., Amanita princeps Corner & Bas. and *Astraeus odoratus* C. Phosri.

3.3. Sugar alcohol content

The results indicated that sugar alcohol content was different in all mushroom samples (Table 2). Sorbitol, xylitol and inositol were detected in all mushrooms. However, the detection amount was low. The content of sorbitol, xylitol and inositol was in range between 0.03-0.30, 0.02-0.25, 0.71-1.17 mg/g DW, respectively. Mannitol in this study was in range between 26.20-62.0 mg/g DW. *Astraeus odoratus* C. Phosri showed the highest mannitol content (62 mg/g DW) while Russula delica Fr. had lowest mannitol content (26.20 mg/g DW). Drying and freezing during sample preparation can cause of the decreasing in the mannitol. Arabitol was not presented in *Calostoma* sp., Amanita princeps Corner & Bas. and *Astraeus odoratus* C. Phosri. This was similar with the report of Sanmee et al., 2003 [19] which was not detected arabitol in *Astraeus* .Regarding the differences between same species, *Astraeus odoratus* C. Phosri has the highest mannitol content while *Astraeus asiaticus* C. Phosri has the highest content of arabitol. In overall, *Astraeus asiaticus* C. Phosri contains the highest total sugar alcohol followed by Russula virescens (Schaeff.) Fr.

3.4. Glucan and carbohydrate polymer content

Beta and alpha glucan were found in all mushroom samples (Table 2). The mushroom with the highest alpha-glucan is Amanita princeps Corner & Bas. which results in the strong structure and easily dissolved in water. This was due to alpha-glucan is soluble in water. *Astraeus asiaticus* C. Phosri has the highest β-glucan content (29.6 mg/g DW) followed by Russula cyanoxantha (Schaeff.) Fr., *Astraeus odoratus* C. Phosri and Russula delica Fr. (27.1, 27.0 and 27.0 mg/g DW), respectively

3.5. Estimated GI in mushroom

All mushrooms are low glycemic index (GI) ranged from 25–37 (Table 2). Therefore, these mushrooms should be suitable for people who want to control their blood sugar because their sugar hydrolysis rate is very low. Moreover, the GI showed similar trend as total sugar content in mushrooms. The trend of total glucose content was Russula delica Fr. > *Astraeus asiaticus* C. Phosri. and GI range was Russula delica Fr. (30.3) > *Astraeus asiaticus* C. Phosri. (25.6). This indicated that the higher total monosaccharide content the higher GI index. Carbohydrate matrix in each mushroom plays important role in degree of sugar hydrolysis. High glucan content can slow down sugar hydrolysis. Therefore, mushroom is a suitable food that provide sufficient sugar but low GI

Table 1. Local name, ecology, cooking and preserving method and folk remedies of wild edible mushrooms in Amnat-Charoen province, Thailand.

Scientific name	Local name	Ecology/Host	Cooking method	Preservation method	Folk remedies
Russula delica Fr.	Hed Kai-khao	Near big tree and on the sand	Boiling, Steaming, Salad, Mixed with Chili paste	Either grill, steam or boil and keep in the fridge	Cancer prevention
Russula virescens (Schaeff.) Fr.	Hed Kai-kiew	On the ground and the furrow	Boiling, Steaming, Salad, Mixed with Chili paste	Either grill, steam or boil and keep in the fridge	Cancer prevention
Calostoma sp.	Hed Ta-lo	On the ground, near rotten leaves and wet land	Eating raw, Boiling, Mixing with Chili paste	Either wash or boil and keep in the fridge	Hypertension and cancer prevention, Reduce Aphthous Ulcers
Russula cyanoxantha (Schaeff.) Fr.	Hed Na-lae	Along the ground beside the brook, lowland, under shade	Boiling, Steaming, Salad, Mixed with Chili paste	Either grill, steam or boil and keep in the fridge	Cancer prevention, Used as laxative
Boletus griseipurpureus Cor.	Hed Sa-med	Under the shade Especially under the eucalyptus tree or the clay area	Need to boil with Guava leaf or tamarind leaf then cook similar with *Russula delica* Fr.	Boil and keep in the fridge	Diabetes and cancer prevention, used as laxative
Amanita princeps Corner & Bas.	Hed Ra-ngok khao	On the ground under big tree and under the shade	Boiling, Steaming, Salad, Mixed with Chili paste	Either grill, steam or boil and keep in the fridge	Used as laxative
Astraeus asiaticus C. Phosri	Hed Por-fai	Sandy land, clay or burnt soil Growing along the base of the tree	Eating raw, Boiling, Mixing with Chili paste	Either steam or boil and keep in the fridge	Prevent eye muscle degeneration, used as laxative
Astraeus odoratus C. Phosri	Hed Por-nung	The loam soils that used to have a fire under the shade of rice paddies	Boiling, Steaming, Salad, Mixed with Chili paste	Either steam or boil and keep in the fridge	Prevent eye muscle degeneration, used as laxative

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Table 2. Monosaccharide, sugar alcohol, content of wild edible mushrooms in Amnat-Charoen province, Thailand.

Mushrooms	Content mg/g dry basis										
	Glucose	Fructose	Mannose	Mannitol	Sorbitol	Xylitol	Arabitol	Inositol	α-glucan	β-glucan	Estimated GI
Russula delica Fr.	56.4±1.5	nd	2.80±0.4	26.2±0.42	0.09±0.00	0.08±0.00	21.9±0.35	1.01±0.02	1.76±0.04	27.0±0.08	29
Russula virescens (Schaeff.) Fr.	32.0±0.24	nd	4.3±0.0	38.3±0.75	0.17±0.01	0.14±0.00	32.0±0.62	1.17±0.01	2.08±0.02	20.0±0.02	37
Calostoma sp.	nd	3.90±0.3	49.1±0.47	nd	0.03±0.00	0.02±0.00	nd	0.71±0.00	1.03±0.03	7.0±0.04	29
Russula cyanoxantha (Schaeff.) Fr.	23.6±0.6	1.22±0.12	6.76±0.3	28.3±1.14	0.30±0.00	0.25±0.00	23.6±1.17	0.92±0.01	2.38±0.01	27.1±0.10	31
Boletus griseipurpureus Cor.	30.0±1.8	nd	8.82±1.14	36.0±0.50	0.15±0.00	0.12±0.00	30.0±0.35	0.97±0.01	1.72±0.01	17.2±0.13	33
Amanita princeps Corner & Bas.	nd	nd	4.44±0.15	nd	0.10±0.00	0.08±0.00	nd	0.81±0.01	4.42±0.05	19.2±0.17	33
Astraeus asiaticus C. Phosri	35.6±1.9	2.93±0.3	nd	42.6±0.32	0.16±0.00	0.13±0.00	35.6±0.33	0.88±0.00	1.53±0.01	29.6±0.14	26
Astraeus odoratus C. Phosri	nd	nd	8.96±0.15	62.0±0.81	0.05±0.00	0.04±0.00	nd	0.80±0.00	2.37±0.02	27.0±0.03	27

Detection limit is lower than 0.01 mg/g, nd = not detected. The data were reported as the means and standard errors of the mean (mean ±SEM).

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4. Discussion

4.1. Ecology, cooking and preservation methods and folk remedies

Most of the edible mushroom part are cap, gill and stem. The collection of wild edible mushroom could be done by any person without distinction of sex or age. The level of knowledge of these collectors varies from one respondent to another. In fact, the older age group is associated with more knowledge wild edible mushroom. The information of the respondents shows that wild edible mushroom can be used include food and medicinal purpose. Some mushroom (*Calostoma* sp.) can eat raw but some should not eat raw such as Boletus griseipurpureus Cor. Because it might cause nausea, vomiting, cramps and diarrhoea. Therefore, the most popular cooking method of these mushroom are boiling, steaming, salad and mixed with chili paste. Boletus griseipurpureus Cor. need to boil together with guava or tamarind leave to reduce the bitter and astringent taste. This could be due to the alkaloid content that present (Ribeiro et al., 2018). Mushrooms are highly perishable. Therefore, the preserve method is needed to extend shelf life of the mushroom. Most of mushrooms will be passed the heating process either steam or boil then kept in the fridge or freezer except *Calostoma* sp. That can be kept immediately after washing. Regarding medicinal properties, it is believed that mushrooms exhibit anti-cancer and anti-hypertension property which was passed on by ancestors. This was in agreement with Nandi et al., 2013 [12] who reported that *Russula* spp. exhibit immunomodulation activity which could link to cancer prevention. Moreover, some mushrooms show the health benefits on laxative and eye muscle degeneration. This might be due to terpene or terpenoid compounds contained in mushrooms such as Pleurotus cornucopiae, Flammulina velutipes [16]. Russula spp. is known for the ability to produce color pigment (red, yellow, purple, brown and blue or mixture of all). The pigments are from the laccase and ligninase enzyme that can degrade complex polyphenolic compounds such as lignin. Hence *Russula* spp. can absorb the digested phenolic nutrient from host debris [2] which also include pigments compounds.

4.2. Monosaccharide content

In general, monosaccharide compositions found in mushroom including glucose, mannose, galactose, xylose, arabinose, rhamnose, and fructose. However, the content of monosaccharide in mushroom was lower than other fruits and vegetables. This study found that *Calostoma* sp. contained highest amount of fructose and followed by *Astraeus asiaticus* C. Phosri and Russula cyanoxantha (Schaeff.) Fr. While mannose was detected in all species except *Astraeus asiaticus* C. Phosri. Mannose is a sugar that has a good water solubility properties. It is part of the glucan structure which acts as a structure of mushrooms. Therefore, the mushrooms which are contained mannose, glucan will subsequently be detected. However, mushroom which has glucan, may not have mannose as the sub unit molecules such as *Astraeus asiaticus* C. Phosri. Singdevsachan et al., 2016 [18] reported that galactose and arabinose might be the glucan structure of *Astraeus asiaticus* C. Phosri. This study found that there is no detected of xylose in all mushrooms which was similar with Wang et al (2017). However, there were some researchers found xylose in some mushrooms such as Lentinula edodes and some species of Ganoderma. The undetected of monosaccharide might be because it presents as hetero polysaccharide as well as medium variation and site of fruiting body.

4.3. Sugar alcohol content

The xylitol detection in mushrooms of this study was contradict to the finding of Carvalho et al. (2014) who found that xylitol is only specific marker for edible Suillus bovinus mushroom. However, this could be due the environmental growth of the mushroom. Moreover, the amount of inositol detected in *Astraeus asiaticus* C. Phosri and *Astraeus odoratus* C. Phosri in this study was higher than that reported by Sanmee et al., 2003 [19]. This might be due to the hydrolysis of xylose causes the increasing of inositol. Mannitol and arabitol were detected at higher concentration all mushrooms except *Calostoma* sp. and Amanita princeps Corner & Bas. Mannitol is major sugar alcohol identified

in mushrooms. Mannitol participates in growth and firmness of fruiting body. The variation depended on the environmental factors such as pH, carbon, and nitrogen availability [10].

4.4. Beta and alpha glucan is complex

Carbohydrates which acts as a shield and structure for mushrooms. Alpha-glucan is a mushroom structure, especially at the mushroom base. Polysaccharides present a diverse range of cellular functions as energy storage, cell wall structure, cell–cell interactions and signaling, host–pathogen interaction, and protein glycosylation. Hence, their contents are varied. Most of glucan represents in mushroom are located at cell wall. A middle layer of mushroom cell wall composed of the beta glucan network. However, the variations in layer composition are dependent upon species, growth conditions and stage of maturity [5]. It can be seen that beta-glucan can be accumulated in different species mushroom and express as different structure as *Astraeus asiaticus* C. Phosri has hard structure while in the cell wall of Russula delica Fr. is softer and smoother. The higher amounts of beta glucan results in difficult to dissolve in water and prevention of the spread of mushroom spores. The total glucan content was related with the content of total carbohydrate and fiber. *Astraeus asiaticus* C. Phosri seemed to have the highest amount of total glucan

5. Conclusions

All mushrooms which were collected from Dong Yai community forest, Amnat-Charoen province, Thailand exhibited various ranges of monosaccharides, sugar alcohol and glucans. The chemical characteristic of mushroom displays the important of the inclusion of mushroom in the diet. The cooking and preservative method could be used to recommend people who are unfamiliar with wild mushroom. In addition, these wild edible mushrooms are excellent source of beta-glucan especially *Astraeus asiaticus* C. Phosri. Further studies of these wild mushroom are needed to explore the potential as functional foods. Moreover, these findings lead to diversifying food and diet as well as help preserve the forest which are the growing area of the mushrooms.

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