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Phenolic content and free radical scavenging activity of herbal seasoning enriched with oyster mushroom (*Pleurotus sajor-caju*) powder

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Abstract
Mushrooms have received great attention for their health benefits due to their polyphenols content and the related antioxidant activity. Edible mushrooms are known as low calorie functional foods that suit to the design of healthy diet food patterns. The aim of this work is to evaluate the antioxidative activity of methanolic extracts of the herbal seasoning (HS) enriched with *Pleurotus sajor-caju* (PSC) powder in different assays, namely, 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity, ferric reducing scavenging power (FRAP) and total phenolic content (TPC). Chemical compositions were determined by standard AOAC methods. Six different formulations with different levels of PSC powder at 0% (A), 20% (B), 40% (C), 60% (D), 80% (E) and 100% (F) to substitute coconut milk powder were used in this study. The methanolic extracts of HS(F) showed the highest antioxidant activity in scavenging DPPH free radicals (54%) than the control treatment HS(A) (43%). Meanwhile, the reducing capacities of PSC powder incorporated at different levels were in the range of 23.57 – 60.52 mg ascorbic acid equivalents (AAEs)/g extract. Among all samples, HS(F) had the highest total phenolic content [1,823.84 ± 0.84 mg gallic acid equivalents (GAEs)/g]. Overall, the antioxidant activities in DPPH and FRAP increased with increasing concentration of PSC powder in the formulations. The high content of ash which was recorded in PSC-based HS might be contributed by some minerals present in PSC powder in the HS. This study indicated that PSC powder exerts some antioxidative capacities, thus it can be potentially used as a natural antioxidant in processed food products.

Keywords: antioxidant activity, herbal seasoning, nutritional composition, *Pleurotus sajor-caju* (PSC), total phenolic content

Introduction

Mushrooms belong to the fungi kingdom although in the past, they were classified as plants. Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates, proteins, vitamins, minerals, fat, fibres and various amino acids (Okwulehie and Odunze 2004). Mushrooms are extremely perishable. Freezing, drying and canning are popular techniques commonly used for long-term preservation. *Pleurotus* mushroom is

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commonly recognised as oyster mushroom, due to its oyster-like shape. Furthermore, *Pleurotus* species are a rich source of vitamin C, B-complex (thiamin, riboflavin, folic acid and niacin), minerals (Ca, P, Fe, K and Na) and protein (Heleno et al. 2010; Caglarirmak 2007: Manzi et al. 1999). *Pleurotus* species are consumed for their nutritive benefits as well as medicinal values (Jonathan and Esho 2010).

The antioxidants available in mushrooms are protective agents to reduce oxidative damage in the human body without any interference. Interestingly, these fungi are recognised as functional foods and as a source of pharma-nutritional components. They are reported to boost heart health, lower the risk of cancer, promote immune function, ward off viruses, bacteria and fungi, reduce inflammation, combat allergies and help to balance blood sugar levels and support the body's detoxification mechanism (Barros 2007a). According to Olajire and Azeez (2011), antioxidants exhibit the ability in scavenging free radicals by inhibiting the oxidation process of lipid and act as antioxidants by several actions to slow down the rate of oxidation. Although antioxidants defense and repair systems are already available in humans and other organisms to protect them against oxidative damage, they are insufficient totally to prevent the damage (Mau et al. 2002). Therefore, the introduction of food containing antioxidative properties are needed to fulfill the non-adequate protection to the body. More findings have suggested that some of the species contain significant amounts of therapeutic compounds with antimicrobial properties, anti-cholesterol, antioxidant and effective in lowering cholesterol levels (Barros et al. 2007a; Synytsa et al. 2009). Many findings reported that phenolic compounds, polyketides and terpenes are the examples that accumulate as secondary metabolites in mushrooms (Turkoglu et al. 2007).

Herbs have a variety of uses including culinary, medicinal, and in some cases spiritual usage. Culinary herbs have a wide range of use in food preparation. Moreover, herbs and spices also contain natural antioxidants due to the presence of polar phenolic compounds and essential oils (Demo et al. 1998). On the other hand, due to their strong flavours, these food items are frequently used in small quantities to add pleasant flavour. Incorporation of mushroom powder with herbal ingredients will give some new good combination in another context to diversify the application of herbs and mushroom as well in ready to eat (RTE) food products. Aishah and Wan Rosli (2013) found that the *Pleurotus sajor caju* (PSC) powder exhibits 80.45% DPPH scavenging activities, with a reducing power of 0.72. A few studies have been conducted to incorporate PSC powder in beef products (Wan Rosli and Solihah 2012), bakeries (Wan Rosli et al. 2012), rice-based products (Aishah and Wan Rosli 2013) and chicken patties (Wan Rosli et al. 2011).The seasoning manufacturing process needs to be developed and promote products that are able to be marketed in domestic markets as well as compete at the international stage. The supplementations of food with mushrooms and herbs are much recommended due to their sources of bioactive compounds that are capable of deactivating free radicals.

The purpose of the present study was to investigate the nutritional content in different ratios of oyster mushroom powder used and the acceptance levels of the products. The findings from the present study are vital for embarkation of future investigations towards the investigation of antioxidant activities as well as the functional properties in the finished processed products. Hence, this study was conducted to investigate the nutritional content in different ratios of PSC powder emphasising on total polyphenol content and the scavenging activities of the developed products.

Materials and methods

Standards and reagents

All the chemicals and reagents were of analytical grade purity. Gallic acid, ascorbic acid and iron (III) chloride anhydrous were purchased from Fisher Scientific (UK). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany). The standard for DPPH was purchased from Sigma Aldrich (St. Louis, MO). Distilled and deionised water was used throughout the experiment.

Sample preparation and product development

There were six samples of herbal seasoning (HS) which were formulated with PSC powder at different percentages, namely, 0% (A), 20% (B), 40% (C), 60% (D), 80% (E) and 100% (F) to replace coconut milk powder in HS formulations. All local herbs were purchased from the local market. One part of the shredded culinary herbs and three parts of the blended spices and the rest of the ingredients were mixed together. The ingredients used are shown in *Table 1*. The pH of the herbs-spices mixture was adjusted to less than 4.5 and the mixture was then heated to boiling. This is followed by hot-filling into bottles of 230 g followed by processing in boiling water until the internal temperature of the product reaches 93 °C. The finished products were hot-filled into the glass bottle, kept at room temperature and analysed for nutritional composition and antioxidative properties.

Table 1. Raw ingredients used in HS enriched with PSC powder

Ingredients (%)	PSC powder level (%)				
	0	20	40	60	80
Pleurotus sajor caju (PSC) powder	0	4	8	12	16
Coconut milk powder	20	16	12	8	4
Dried chilli	1.6	1.6	1.6	1.6	1.6
Fresh turmeric	1.1	1.1	1.1	1.1	1.1
Ginger	1.7	1.7	1.7	1.7	1.7
Galangal	0.8	0.8	0.8	0.8	0.8
Onion	20.4	20.4	20.4	20.4	20.4
Shallot	9.4	9.4	9.4	9.4	9.4
Garlic	2.4	2.4	2.4	2.4	2.4
Cumin powder	0.8	0.8	0.8	0.8	0.8
Fennel powder	0.8	0.8	0.8	0.8	0.8
Coriander	1.6	1.6	1.6	1.6	1.6
Crude coconut	6.3	6.3	6.3	6.3	6.3
Dried lime	0.4	0.4	0.4	0.4	0.4
Salt	2.4	2.4	2.4	2.4	2.4
Black pepper	0.4	0.4	0.4	0.4	0.4
Lemon grass	1.6	1.6	1.6	1.6	1.6
Water	26.7	26.7	26.7	26.7	26.7
Citric acid	0.4	0.4	0.4	0.4	0.4
Sugar	1.6	1.6	1.6	1.6	1.6
'Bunga kantan' (*Etlingera elatior*)	10	10	10	10	10
Turmeric leaves (*Curcuma longa* L.)	20	20	20	20	20
'Daun limau purut' (*Citrus hystrix*)	10	10	10	10	10
'Pegaga' (*Centella asiatica* L.)	60	60	60	60	60

Proximate analyses
Samples of mushroom were analysed for chemical composition (moisture, fat, ash, protein and carbohydrate) by the Association of Official Analytical Chemists methodology (AOAC 2000) with slight modifications. The moisture content was determined by drying the samples in an oven at 105 °C for 24 h. Calculation of moisture was done by calculating the percentage by:

Weight = (loss of weight in grams of the sample/ weight in grams of the sample taken) x 100

The crude protein content (N x 6.25) of the samples was measured by macro Kjeldahl method. Calculation of percentage of protein was done using the formula:

{[(ml HCI-ml HCI blank) x 14.008 x 0.1N HCI x protein factor]/weight in grams of the sample} x 100

The crude fat was determined by extracting a known weight of samples with petroleum ether, using a Soxhlet apparatus. The ash content was determined by incineration at 600 ± 15 °C. Calculation of ash was also based on percentage by weight as follows:

[Weight of ash (grams)/weight of sample (grams)] x 100

The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fibre and protein from 100% of dry matter.

Preparation of HS extract added with PSC
Antioxidative capacity and total polyphenols were determined by following the method of Perez-Jimenez et al. (2008). 10g of HS was extracted with 40 ml of methanol:water (50:50, v/v; pH 2.0) at room temperature, using an ultra-speed homogeniser for 5 min. The homogenates were kept at 4 ºC for 1 h and then centrifuged at 4,000 rpm for 10 min. The supernatants were recovered and the residue was further washed with 40 ml of acetone:water (70:30, v/v) and centrifuged. The resulting supernatants were combined and stored at –20 °C.

Determination of total phenolic content
The total phenolic content in both PSC powder and HS containing PSC powder were determined with Folin and Ciocalteu's phenol reagent according to the method of Barros et al. (2007b). Briefly, 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm using the Perkin Elmer spectrophotometer (model Lambda EZ150, Middlesex, United Kingdom). Gallic acid was used to calculate the standard curve (5 – 25 ppm), R2 = 0.99. Estimation of the phenolic compounds was carried out in triplicate. The results were in mean values ± standard deviations and expressed as mg of gallic acid equivalents (GAEs) per gram of sample extract.

Determination of DPPH radical scavenging activity
Radical scavenging activity (RSA) in the HS extract was evaluated using DPPH radicals based on the method established by Xu and Chang (2007). The DPPH solution was prepared by dissolving 5.9 mg of DPPH in ethanol (100 ml). An accurate amount of 3.8 ml of ethanolic DPPH solution was added to 0.2 ml of HS extract. The mixture was shaken vigorously for 1 min and left to stand at room temperature in the dark for 30 min. Absorbance was measured against the blank reagent at 517 nm (Perkin Elmer spectrophotometer model Lambda EZ150, Middlesex, United Kingdom). All measures were carried out in triplicate. Radical scavenging activity was calculated according to the equation as follows:

% RSA = [(ADPPH – AS)/ADPPH] x 100,

where AS is the absorbance of the solution when the sample extract was added at a particular level, and ADPPH is the absorbance of the DPPH solution.

Determination of reducing power

The reducing power of the prepared extracts was determined according to the method of Oyaizu (1986). Briefly, each extract (20 mg/ml) immersed in water or ethanol (2.5 ml) was mixed with 2.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50 ºC for 20 min. Following this, 2.5 ml of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 1,200 rpm for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride. Absorbance of the reaction mixture was read spectrophotometrically at 700 nm using the Perkin Elmer spectrophotometer (model Lambda EZ150, Middlesex, United Kingdom); increase in absorbance of the reaction mixture indicated greater reducing power. Mean values from three independent samples were calculated for each extract.

Statistical analysis

All results were presented as mean (SD) values of three replicates. Analysis of variance (ANOVA) was performed using SPSS V. 20 (SPSS Inc., Chicago, IL, USA) and mean values were statistically different at $p < 0.05$. The significantly different results were further separated using the Duncan Multiple Range Test (SPSS Inc., Chicago, IL, USA).

Results and discussion

Nutritional compositions

Table 2 shows the proximate composition of PSC powder enriched HS with six different formulations. Herbal seasoning added with PSC powder had a moisture content in the range of 58.76 – 62.69%. Proximate analysis indicated that HS with more than 40% PSC powder substitution had significantly higher moisture content ($p < 0.05$) than the control sample. PSC powder exhibits high water content and water holding capacity that linked to the higher moisture content in HS added with PSC powder. The sugar and dietary fibre available in PSC powder could have contributed to the absorption of large amounts of water (Mohamed et al. 2010).

HS had relatively medium fat content ranging from 8.16 – 13.82%, which was not surprising for all coconut based foods. Ash content was generally higher in all treatments ranging from 14.27 – 15.72%. Moreover, ash content basically gives a rough idea about the mineral content in the product. The highest ash content present in HS(F) recorded 15.72% which was in line with the highest amount of PSC powder in the sample and significantly different ($p < 0.05$) from other remaining samples. Control samples recorded the lowest content of ash (14.27%) and not significantly different from HS(B) and HS(C) which was 14.3% and 14.4% respectively. Changes in proximate composition of HS supplemented with PSC powder indicated significant

Table 2. Proximate analyses of herbal seasoning incorporated with PSC powder

PSC powder level (%)	A (0)	B (20)	C (40)	D (60)	E (80)	F (100)
Moisture	58.76 ± 2.25^{c}	$60.43 \pm 3.21b^{c}$	61.08 ± 2.48^{ab}	62.49 ± 2.36^{a}	62.69 ± 3.35^{a}	62.09 ± 3.65^{ab}
Fat	13.82 ± 0.84^{a}	13.2 ± 0.65^{b}	11.88 ± 0.65^{c}	10.03 ± 0.54^{d}	9.25 ± 0.95^{d}	8.16 ± 0.74e
Ash	14.27 ± 0.25^{c}	14.3 ± 0.28^{c}	14.40 ± 0.38^{c}	15.09 ± 0.45^{b}	15.11 ± 0.22^{b}	15.72 ± 0.18^{a}
Protein	8.62 ± 0.25^{f}	9.15 ± 0.15^{e}	9.37 ± 0.28^{d}	10.74 ± 0.87^{c}	11.14 ± 0.25^{b}	11.67 ± 0.45^{a}
Carbohydrate	45.36 ± 0.98^{a}	45.04 ± 0.88^{a}	45.03 ± 0.45^{a}	43.01 ± 0.25^{b}	41.71 ± 0.44^{c}	40.13 ± 0.56^{c}

[a-d]Mean values with different letters are statistically different ($p < 0.05$)

increase (p <0.05) in protein content. On the other hand, carbohydrate content ranging from 40.13 – 45.36% was reduced as a result of PSC powder. The highest and the lowest carbohydrate content recorded were 45.36% and 40.13% in the control sample and HS(F) respectively. FPSC powder incorporated HS contained higher protein and lower fat and was low in calories. Thus, these factors contributed to the higher carbohydrate content in the control sample.

Changes in proximate composition of HS supplemented with 100% PSC powder HS(F), indicated that the addition of PSC powder increased the protein content about 35% compared to the control sample. The lowest percentage of fat and high amount of protein in HS(F) may be due to the moderate amount of protein (23.3%) and fat content (3.0%) found in PSC powder generated originally from the previous research used in this study (Aishah and Wan Rosli 2013; Wan Rosli et. al. 2011). On the other hand, the ash content in HS(B) to HS(F) indicated a rough idea about the mineral content of PSC powder presented in the HS products. In fact, high ash content in products could come from the amount of fibre contained in the mushroom (Cheung 1998). Furthermore, other technological processes such as steaming, pasteurisation and hot-filling could have affected the nutritional composition of the HS (Manzi et al. 2001).

Total phenolic content (TPC)

Mushrooms produce a number of secondary metabolites, many of which are phenolic and polysaccharide compounds (Ferreira et al. 2007). This information is important as a guideline and selection criteria for further consumption in diet. On the other hand, free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity.

The total phenolic content for the HS added with PSC powder is shown in *Figure 1*. The calculation of TPC was carried out by using Folin and Ciocalteau's phenol reagent. The yellow colour of phosphotungstic acid reagent (Folin) was reduced to a blue colour in alkaline solution. The intensity of the blue complex increased as number of hydrogen donating groups in the phenolic compounds increased, thus indicating higher total phenol content (Kaur et al. 2008).

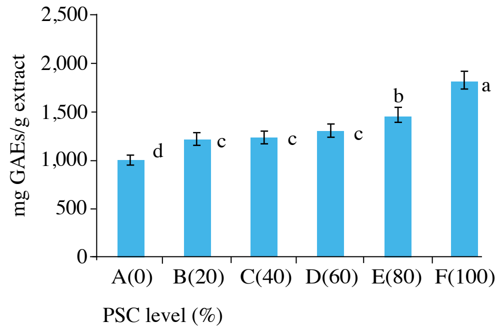

Figure 1. Total phenolic content of HS added with PSC powder. a-c Mean values with different letters are statistically different (p <0.05)

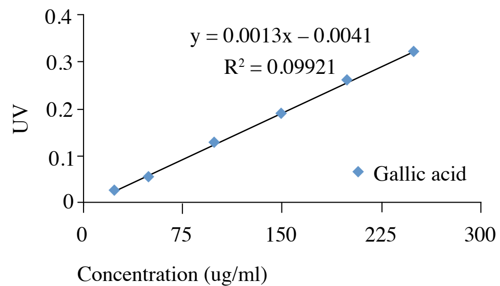

Figure 2. Standard curve of gallic acid for total phenolic content

Results are expressed as mg of gallic acid equivalents per gram of dried extract. From the gallic acid standard curve (*Figure 2*), total phenolic content in the methanolic extracts of the six treatments of HS ranged from 1,000.36 ± 0.32 – 1,823.84 ± 0.84 mg GAEs/g extract. Basically, the total phenolic content increased proportionally to the level of PSC powder added to HS. The highest amount of phenolic content was found in HS(F) (1,823.84 ± 0.84 mg GAEs/g extract). On the other hand, the lowest content of TPC was recorded in the control treatment HS(A) (1,000.36 ± 0.32 mg GAEs/g extract).

Meanwhile, HS(E) showed the second highest amount of TPC (1,462.30 ± 0.75 mg GAEs/g extract). It is known that total phenol content is responsible for the free-radical scavenging activities in many plants (Pourmorad et al. 2006).

Phenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds (Tepe et al. 2006). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang 2001). However, leaching of phenolic compounds into the cooking water would cause the decrease in total phenolics (Zhang and Hamauzu 2004)

Numerous studies have conclusively showed that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis because they act as antioxidants. Therefore, edible mushrooms may have potential as natural antioxidants in food. The assays were performed in the whole extract since it could be more beneficial than isolated constituents; a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Barros et al. 2008).

DPPH free radical scavenging activities of herbal seasoning extracts

Antioxidant activity of HS added with PSC powder was investigated by using the DPPH free radical method. This DPPH assay has been widely used and accepted world-wide to provide basic information on the antioxidant ability of extracts from plants, food materials and single compounds (Crozier et al. 2007). 2,2-diphenyl-1-picryhydrazyl is a free radical which gives a strong absorption band at 517 nm in the visible region of electromagnetic radiation. In this assay, the ability of extracts to donate a hydrogen atom or an electron to the unpaired DPPH radical was determined by the reduction of DPPH radical into the reduced form DPPH-H. The DPPH solution which could be seen as purple in colour will change to a pale colour or yellowish when reacted with a radical scavenger in the appropriate time given.

The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reaction of DPPH with HS was also fast but slower compared to that with ascorbic acid. It is usually noticeable as discolouration of methanolic extract of plant samples from purple to yellow and the reduction capacity of DPPH is determined by the decrease in its absorbance at 517 nm. Hence, DPPH is widely used to evaluate the free radical scavenging capacity of antioxidants (Molyneux 2003).The present study evaluated the DPPH scavenging ability of the aqueous extract of HS as relative activities against ascorbic acid.

The DPPH radical scavenging capacity of HS added with PSC powder is shown in *Figure 3*. Results indicated that PSC powder exhibited stronger antioxidant scavenging capacity as parallel with their increasing substitution of coconut powder in DPPH assay. In this study, at 1mg/ml, extracts showed DPPH percentage scavenging activity ranging from 43 – 54%. Control samples showed the weakest scavenging

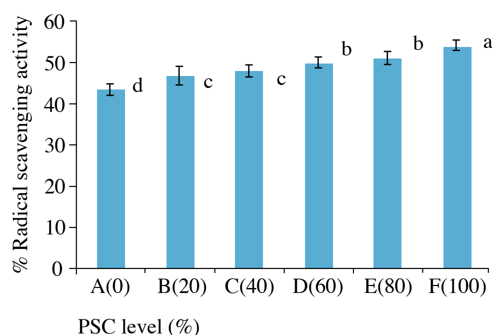

Figure 3. DPPH radical scavenging capacity of HS added with PSC powder. [a-c]*Mean values with different letters are statistically different (p <0.05)*

activity (43%) while HS(F) treatment showed the highest scavenging activity (54%). Herbal seasoning (F) was the most effective compared to other formulations as scavenging compounds or extracts.

The present study revealed that the difference in the values may be due to the presence of some potent molecules in the formulation which is more capable of quenching DPPH free radicals. Edible mushrooms are known as low calorie functional foods that suit to the design of healthy diet food patterns. Furthermore, there are significant amounts of bioactive substances present in mushrooms such as vitamins and vitamin precursors, minerals and trace elements (Ranogajec et al. 2010). The formulation of HS with added PSC powder formulation exerted some potential antioxidant properties. This result suggested that the HS sample contained pharma-nutritional compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron, which is responsible for the radical's reactivity (Olayinka and Anthony 2010).

The free radical scavenging activity of methanolic extracts of HS and also that of ascorbic acid was evaluated through its ability to quench the synthetic DPPH radicals. The DPPH assay enables a rapid and low cost method that has frequently been used for evaluation of the antioxidative potential of various natural products.

The antioxidative activities of ethanolic extracts of paste in all assays were concentration dependent and increased with increasing concentration. This observation concurs with that of Banerjee et al. (2012) who also noted a similar trend of antioxidative activities dose dependency and associated it with the presence of reductones that are reported to be the terminators of free radical chain reactions. Upon reduction, the colour of the solution fades from purple to yellow and the reaction progress is conveniently monitored by a spectrophotometer (Huang et al. 2005).

The presence of hydroxyl groups in the HS extracts may contribute to the radical scavenging activity. Plant phenolics and flavanoids in the extract reduced DPPH radicals by their ability to donate hydrogen. Plant flavanoids are believed to reside in their free radical scavenging capacity and their antioxidant activity increases with an increase in the number of hydroxyl groups that they bear and a decrease in their glycosylation (Rice evans et al. 1996).

Liu (2003) reported that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent bioactive properties and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This can be explained by the fact that no such single antioxidant can replace the combination of natural phytochemicals to achieve health benefits.

The DPPH scavenging activity in this study indicated that the formulation was a potent antioxidant. This also suggested that the formulation contained compounds that were capable of donating hydrogen to a free radical in order to remove an odd electron, which is responsible for the radical's reactivity (Olayinka and Anthony 2010).

Ferric reducing scavenging power

The results of the reducing power assay of HS added with PSC are summarised in *Figure 4*. High absorbance indicates high reducing power. The reducing power of the methanolic extract of HS was found to steadily increase in direct proportion to the increasing concentration of the extract. *Figure 5* shows the standard curve of ascorbic acid for reducing power analysis. Among the tested extracts, the 100% substitution of PSC powder showed highest reducing power (60.52 mg AAE/g extract), followed by 80% substitution of PSC powder (50.96 mg AAE/g extract). Meanwhile, HS(D), HS(C) and HS(B) showed reducing power of 46.28, 46.19 and 44.73 mg AAE/g extract respectively. On

the other hand, the control treatment without PSC powder (23.57 mg AAE/g extract) had the lowest reducing power and was significantly different (p <0.05) among all 6 HS formulations.

Generally, the reducing properties are associated with the presence of polyphenolic compounds, which exert their action by breaking the free radical chain by donating a hydrogen atom. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability (Olayinka and Anthony 2010).

In FRAP, ferric-ferric cyanide complex is reduced to the ferrous form depending on the presence of antioxidants (Amarowicz et al. 2004). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity; higher absorbance indicates a higher ferric reducing power (Yim et al. 2010).We have shown that HS had a comparable ferric reducing power to that of ascorbic acid.

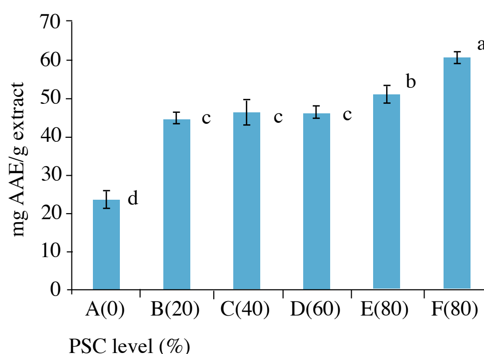

Figure 4. Reducing power of HS added with PSC powder. a-c Mean values with different letters are statistically different (p <0.05)

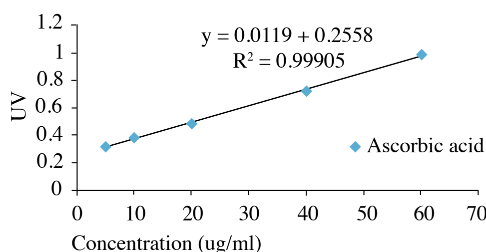

Figure 5. Standard curve of ascorbic acid for FRAP

Conclusion

All herbal seasoning containing PSC powder extracts possessed scavenging capacities against various antioxidant systems *in vitro*. Phenolic compounds seem to be the main components contributing to the antioxidant activity of all herbal seasoning enriched with PSC powder. The antioxidant activities in DPPH and FRAP increased with increasing concentration of PSC powder in the formulations. The associations of different levels of PSC powder found in this study can give consumers a choice in selecting a more nutritional and healthier herbal seasoning. The study also offers a better understanding of PSC powder as a potential food ingredient in product development in the future.The HS is the ethnic food and PSC powder is a nutritionally rich food ingredient. Future studies on the estimation of anti-diabetic activities of the PSC powder and the final product will give added value components to the HS. Thus, it is recommended that PSC powder can be used in foods as natural antioxidants or extracts, especially to enhance the nutritional compositions of the existing products.

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Abstrak
Cendawan telah mendapat perhatian besar kerana faedah kesihatan iaitu kandungan polifenol dan aktiviti antioksida. Cendawan juga dikenali sebagai makanan berfungsi rendah kalori dan sesuai ke arah corak pemakanan yang sihat. Tujuan kajian ini adalah untuk menilai aktiviti antioksidan ekstrak metanol perencah herba (PH) yang diperkaya dengan serbuk *Pleurotus sajor-caju* (PSC) di dalam pelbagai teknik iaitu aktiviti perencatan radikal bebas DPPH, kuasa perencatan penurunan ferric (FRAP) dan kandungan jumlah fenol (TPC). Komposisi kimia ditentukan menggunakan kaedah AOAC. Enam jenis formulasi dengan berbeza kandungan serbuk PSC iaitu 0% (A), 20% (B), 40% (C), 60% (D), 80% (E) dan 100% (F) menggantikan serbuk kelapa telah dikaji. Ekstrak metanol HS (F) menunjukkan aktiviti antioksidan yang paling tinggi untuk merencatkan radikal bebas DPPH (54%) berbanding sampel kawalan (43%). Sementara itu, kuasa penurunan serbuk PSC yang ditambah pada tahap berbeza adalah dalam julat 23.57 – 60.52 mg AAE/g ekstrak). Antara semua sampel, HS (F) mempunyai kandungan fenol paling tinggi iaitu (1,823.84 ± 0.84 mg GAE/g ekstrak). Secara keseluruhan, aktiviti antioksidan didalam DPPH dan FRAP meningkat dengan pertambahan serbuk PSC didalam formulasi. Kandungan abu yang tinggi yang direkodkan dalam HS berasaskan PSC mungkin disumbangkan oleh jumlah mineral yang hadir didalam serbuk PSC. Kajian ini menunjukkan bahawa serbuk PSC menunjukkan ciri-ciri kapasiti antioksidatif dan berpotensi sebagai antioksidan semulajadi dalam produk makanan yang diproses.