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Contributed Paper

Effect of Drying Process on the Physicochemical Properties and Biological Activities of Enzymatic Protein Hydrolysate from Shiitake Mushroom (*Lentinula edodes*)

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ABSTRACT

Protein hydrolysate has been used for many years as flavouring agents, food supplement, and several food applications. In this research, *Lentinula edodes* was hydrolysed by 15% (w/w mushroom powder) papain (4,600 NF/mg) at 65 °C, pH 7.0 for 18 h. Protein hydrolysate was dried with two different methods (spray drying and freeze drying). Physicochemical properties, antioxidant activity, angiotensin-I converting enzyme (ACE) inhibitory and sensory characteristics of mushroom protein hydrolysate dried with spray-drying (SD-MPH) and freeze-drying (FD-MPH) were evaluated. The results showed that SD-MPH had a higher dry weight of protein content and a* value than that of FD-MPH ($p<0.05$). In contrast, SD-MPH had a lower a_w and moisture content than that of FD-MPH ($p<0.05$). SD-MPH exhibited higher water solubility and pH value than FD-MPH ($p<0.05$). Most of amino acids loss was affected by drying process, except aspartic acid. The most abundant amino acids found in both MPHs powder were Glu, Phe, and Asp and ranged from 1.65 to 4.05 mg/g sample. According to 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, it was shown that both MPHs have high antioxidant activity. Whereas SD-MPH showed~10-fold higher ACE inhibitory activity comparing to FD-MPH. This was concomitant with the high amino acid contents. Sensory characteristics were evaluated by an electronic tongue using the Principle Component Analysis (PCA) and the result showed two separated clusters between SD-MPH and FD-MPH. Based on these results, spray-drying might be a suitable method to produce quality hydrolysates for functional food applications.

Keywords: *Lentinula edodes*, enzymatic mushroom protein hydrolysate, antioxidant properties, ACE inhibitory activity, drying methods

1. INTRODUCTION

Shiitake (*Lentinula edodes*), an edible mushroom, is cultivated and widely consumed in many Asian countries including, China, Japan, Korean, Taiwan, and Thailand. Generally, it is sold as dried form and traded all over the world. It has low fat and carbohydrate, but it is rich in dietary fiber, vitamins, and minerals [1]. It also has higher protein content than

most of vegetables as well as high essential amino acids, particularly glutamic acid and aspartic acid producing a unique flavor known as "umami" [2]. Shiitake has long been used as medicinal mushroom because of its biologically active compounds, particularly lentinan, lenthionine, ergosterol, and LEM [1, 13]. Animal research and human clinical work showed strongly effect on anti-cancer, anti-bacterial, anti-viral, and anti-tumor properties of shiitake mushroom [1]. The strong antioxidant activity and angiotensin-I converting enzyme (ACE) inhibitory activity were also found in mushroom extracts providing high phenolic contents [3-5].

Vegetable protein hydrolysate (VPH) is a savory flavouring agent used in various food applications such as soup, gravy, sauce, and snack. It can be produced by hydrolysis protein from different sources, such as soybean, wheat, rice, or algae using base solution, acid, or enzymes [6]. Commercially, enzymatic hydrolysis is a mild process produced by breaking protein down into peptide with protease enzymes at suitable conditions for 12-24 h [7]. At the final process, VPH was neutralized and dried. Nowadays, VPH has become an attractive food supplement because of its physicochemical properties and biological active substances. However, it is known that the compositions, properties, and biological activity of VPH are influenced by different drying techniques [8]. Spray drying is the most popular drying technology used within food, chemical, and pharmaceutical industries to prepare protein powder. During the spray-drying process, liquid feed is atomized and placed in contact with hot gas, which may change the conformation of proteins including the type and proportion of covalent or non-covalent interactions [9]. Freeze drying basically utilizes the mechanism of ice sublimation under low pressure and induces less heat denaturation than spay-drying [10]. Freeze drying is the most common method for drying heat-sensitive materials in the pharmaceutical and food industries. However, freeze-drying takes an extensive amount of time for the drying process, and it has high capital and process costs [11].

Based on the nutritional and medicinal properties of shiitake with widely cultivated for economy produces in Thailand, this mushroom cultivar was selected for this research. Therefore, this study was aimed to investigate the physical properties, antioxidant activity, and ACE inhibitory activity of enzymatic MPHs powder from shiitake comparing spray drying to freeze drying. In addition, this research results will be provided for functional product applications.

2. Materials and methods

2.1 Chemicals

Hydrochloric acid solution (HCl), sodium chloride solution (NaCl), and monosodium glutamate solution (MSG) were used for calibrating, conditioning, and diagnostic of the electronic tongue and supplied by Alpha M.O.S., Tolouse, France. Papain (EC.3.4.22.2, 4,600 NF/mg) was ordered from Siam Victory Chemicals Co., Ltd, Thailand. Gallic acid (GA), Folin-Ciocalteau phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), ferrous sulphate ($FeSO_4$), hippuryl-histidyl-leucine (HHL), angiotensin converting enzyme from rabbit lung, and other chemicals were purchased from Sigma-Adlrich. Maltodextrin (DE = 10) was purchased from U&V holding Co. Ltd., Thailand. All reagent and chemical uses were analytical grade, except maltodextrin (food grade).

2.2 Sample Preparation

Dried shiitake were bought from a local market in Pathum Thani province, Thailand and ground into powder using a blender. Shiitake powder (100-300 μm, 9.54% of moisture content) was hydrolysed by 15% (w/w of mushroom powder) papain at 65 °C, pH 7.0

for 18 h [12]. After the hydrolysis reaction, the sample was heated at 95±5 °C for 15 min to terminate the enzyme activity, and then filtered through Whatman paper (no.4). Mushroom protein hydrolysate in liquid form (HVP) with a total soluble solid (TSS) of 3± 0.2 °Brix was then collected and kept at 4 °C for overnight. After that, HVP solution was mixed with maltodextrin (30% w/v) and heated to 80 °C for 10 min, following by thoroughly stirring for 1 h [13]. This mushroom hydrolysate protein solution (Pre-MPH) was used to produce spray dried-mushroom hydrolysate protein (SD-MPH) and freeze dried-mushroom hydrolysate protein (FD-MPH).

2.3 Drying Process

According to spray drying process, sample solution was spray-dried using a Niro atomizer spray drier (GEA Process Engineering Inc., Denmark) at inlet and outlet temperatures of 150-170 °C and 80-90 °C, respectively. The feed rate was controlled at 3 mL/min.

For freeze-dried process, sample solution was poured into a stainless tray with 10-mm-thick layer and then frozen at -20 °C for 24 h. After that, a tray with frozen sample was put into a freeze dryer (Lyolab ST, Lyophilization Systems Pvt Limited, India). Drying was performed at a pressure of 0.133 kPa. The shelf temperature was kept at -20 °C for 4 h, and then increased to 40 °C more than 20 h.

After drying from each process, dried sample was ground with a mortar and passed through a 100-mesh sieve to achieve uniform particle size. Both SD-MPH and FD-MPH were collected and kept in an aluminium foil bag at 4 °C before analysis.

2.4 Physicochemical Properties

Water activity (a_w) of samples was measured using a water activity analyser (AquaLab 4TEV, Decagon Devices Inc., USA).

The moisture content of dried powders was determined gravimetrically according to AOAC method 925.10 [14].

Protein content of samples was determined as crude protein by a generic combustion method using a protein/nitrogen determinator (FP-528, LECO, USA). The nitrogen content was measured by thermal conductivity detection and then converted to equivalent protein using the Windows®-based operating software with a default protein factor of 6.25.

The color of sample was measured with a color measurement spectrophotometer (Color Quest® XE, Color Global Co. Ltd., USA). The parameter L* value indicates lightness (0 = black, 100 = white), a* value indicates chromaticity on a green (-) to red (+) axis, and b* value indicates chromaticity on a blue (-) to yellow (+) axis.

Water solubility index (WSI) was determined using shake-flask method. Sample (0.1 g) was suspended in distilled water (10 mL). The pH value was measured using a pH meter by AOAC method 943.02 [14]. The suspension was incubated at 60 °C for 1 h and then centrifuged at 4,000 rpm for 30 min. The insoluble residue was recovered and dried at 105 °C until constant weight. Water solubility index (WSI) was calculated as percentage of insoluble residue weight compare with sample weight [15].

2.5 Amino Acid Analysis

The amino acid content of HVP (5 mL) and MPHs (5 mg) was determined by a high performance liquid chromatography (HPLC) technique with UV detector. The procedure was managed by Industrial Metrology and Testing Service Centre using the following method of AOAC international [14].

2.6 Total Phenolic Content (TPC) Assay

Total phenolic content was measured using Folin-Ciocalteu method as described by Miliauskas *et al.* [16] whereas gallic acid was

used as standard. The 0.1 mL of extracted sample (0.1 mg/L) or gallic acid standard (0-100 mg/L) was added into a test tube with 2.5 mL of diluted 10-fold Folin-Ciocalteu's reagent was mixed thoroughly. The mixture was allowed to stand at room temperature for 5 min. Then, 2.0 mL of 7.5% (w/v) sodium carbonate was added to the mixture and mixed gently. After left sample at room temperature in darkness condition for 1 h, the absorbance was measured using the U2900 UV/Vis spectrophotometer (Hitachi, Japan) at 765 nm. The absorbance of standard was plotted as linear graph and calculated to obtain linear equation. TPC of sample, expressed as mg GA equivalent/g dried sample, was evaluated using obtained linear equation from standard curve.

2.7 DPPH Free Radical Scavenging Activity

The DPPH radical scavenging activity test of mushroom protein hydrolysate was conducted by the modified method of Cheung *et al.* [4]. DPPH radical solution (0.1 mM) in methanol was prepared. Samples were extracted with distilled water to make a concentration ranged from 0 to 20 mg/mL. After that, 1 mL of extracted solution was mixed with 0.5 mL of DPPH solution. The mixtures were left for 30 min at room temperature in the dark. Absorbance was measured at 515 nm with the U2900 UV/Vis spectrophotometer. Vitamin C was used as positive control. The IC_{50}, concentration of the sample required to produce 50% inhibition of DPPH activity, is expressed as milligrams of sample per millilitre of reaction mixture.

2.8 Ferric Reducing Antioxidant Power Assay (FRAP)

Reducing power was determined using ferric reducing antioxidant power assay [15]. 0.5 mL hydrolysate sample (20 mg/mL), in distilled water) and standard $FeSO_4$ (concentration 0-500 μM) was added to 2.5 mL FRAP reagent (10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$, 300 mM acetate buffer pH 3.6 in 1:1:10 v/v/v ratio). The reaction mixture was kept in dark at ambient temperature for 10 min. The absorbance was then measured using a spectrophotometer at 593 nm. The absorbance of standard $FeSO_4$ was plotted as linear graph and calculated to obtain linear equation. FRAP, expressed as mM Fe(II) equivalent/g dried sample, was evaluated using obtained linear equation from standard curve.

2.9 Angiotensin-converting Enzyme (ACE) Inhibitory Activity

The method of Jimsheena and Gowda [17] was assayed by monitoring the release of hippuric acid (HA) from the hydrolysis of the substrate hippuryl-histidyl-leucine (HHL). The assay mixtures consist of 0.125 mL of 0.05 M sodium borate buffer pH 8.2 containing 0.3 M NaCl, 0.05 mL of 5 mM HHL, and 0.025 mL of ACE enzyme. The extracted solution (20 mg/mL, 0.125 mL) was added to the above mixture. The reaction was arrested after incubation at 37 °C for 30 min by the addition of 0.2 mL of 1 M HCl. After stopping the reaction, 0.4 mL of pyridine was added followed by 0.2 mL of BSC, and the solution was mixed by inversion for 1 min and cooled on ice. The developed yellow color was measured at 410 nm using the U2900 UV/Vis spectrophotometer. One unit of ACE activity is defined as the amount of enzyme that releases 1 μmol of HA per min at 37 °C and pH 8.2. The ACE inhibitory activity was expressed as percentage of inhibition.

2.10 Sensory Characteristics

Principle component analysis (PCA) was evaluated using the α-Astree II electronic tongue (Alpha M.O.S., Toulouse, France). The electronic tongue consists of 7 field effect-transistor sensor arrays mounted around a central reference electrode (Ag/AgCl). Aqueous solutions of 0.1 mol/L of HCl, NaCl, and MSG were used for calibrating, conditioning, and diagnostic of

the e-tongue. A sensor set comprises 7 sensors which was developed for food applications. Each of sensors is denoted with a 2 letter code, namely ZZ, JE, BB, CA, GA, HA, and JB. Two grams of MHPs powder were rehydrated in 100 mL hot water. HVP and Pre-MHP were used as controlled sample. All samples were fixed TSS at 2±0.2 °Brix before testing. The analytical measurement of each sample was conducted by immersion of the sensor array in an aliquot for 120 sec. The average mV reading of the last 20 sec of analysis was used for statistical calculation. Each sample was analyzed 15 times, with the first 3 analysis disregarded (as per manufacturer's instructions) due to varied or unstable mV readings.

2.11 Statistical Analysis

The analysis of variance (ANOVA) was performed using the Microsoft EXCEL. Duncan's new multiple range was used for mean comparison at significant level $p<0.05$. In case of two treatments, t-test was employed for comparison between means ($p<0.05$). All experiments were conducted in triplicate, except for the electronic sensory test (n = 12). Principal component analysis (PCA) was carried out to summarize the effects of drying process on the response patterns of taste sensors, using the Alpha M.O.S. statistical software.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties

The physicochemical properties of both MPHs are presented in Table 1. The results showed that all properties of both MPHs were significantly different ($p<0.05$). It seems that product moisture content was related to the drying temperature [8]. Moisture content and a_w of FD-MPH (9.74% and 0.526) were higher than that of SD-MPH (7.14% and 0.378). It might be from a difference in molecular structure of particles produced from spray drying and freeze drying technique. In spray drying process, free water droplets are rather removed rapidly causing particle shrinkage and possibly lowered hydrogen-bonding sites which would decrease an absorption of water molecule bound in dried particles [11]. In contrast, the freeze-concentrated glass region in freeze-dry mechanism that free water molecules, which were hydrogen-bound to solute molecules, might be removed during the process and resulted in higher amount of hydrogen-bonding sites available for another free water molecule to bind with the solute molecule after the end process.

Table 1. Physicochemical property of MHP with the different processes.

Physicochemical property	SD-MPH	FD-MPH
Water activity	0.378 ± 0.001^{b}	0.526 ± 0.001^{a}
Moisture content (%)	7.14 ± 0.13^{b}	9.74 ± 0.12^{a}
Protein content (%dry weight)	2.30 ± 0.04^{a}	2.09 ± 0.03^{b}
pH value	3.81 ± 0.01^{b}	5.07 ± 0.01^{a}
WSI (%)	96.64 ± 0.32^{a}	94.41 ± 0.58^{b}
Color: L* value	29.66 ± 0.22^{b}	30.40 ± 0.03^{a}
a* value	0.13 ± 0.03^{a}	0.03 ± 0.02^{b}
b* value	-0.49 ± 0.08^{b}	-0.18 ± 0.07^{a}

$^{a-b}$ Values in the same row with different letters are significantly different ($p<0.05$).

Consequently, SD-MHP is likely to have a greater moisture contents and a_w than FD-MHP [11]. These findings showed that the critical a_w and water contents are affected by drying methods. However, the moisture content and a_w of samples were still less than 10% and 0.6, respectively, which are absolutely safe for powdery products [19].

Water solubility index (WSI) is the most important determinant in food product development [15]. According to these results, WSI and pH value of SD-MPH (96.64% and 3.81) was higher than that of FD-MPH (94.41% and 5.07) ($p<0.05$). It might be related to the increasing of net negative/positive charges residues at the different pH that associate the protein aggregates and protein solubility [27]. Regarding protein content, it was found that SD-MPH significantly contained higher protein (2.30% dry matter) than FD-MPH (2.09% dry matter) ($p<0.05$).

In comparison of the color value between two dried powder samples, FD-MPH exhibited significantly higher lightness (greater L* value) and yellowness (higher b* value) than that of SD-MPH ($p<0.05$). However, SD-MPH showed significantly higher redness (higher a* value) than FD-MPH ($p<0.05$). In general, the dry powders of FD-MPH and SD-MPH had a light yellow color and a light brown-yellow color, respectively. The slightly brown or yellow color of dried powder sample is possibly due to the compositions of the product containing peptides in hydrolysate and sugar that were generated in the Maillard reaction during the drying period, especially the high temperature in spray drying technique [28]. In general, the browning rate increases with increasing pH and maximum browning occurs at a_w between 0.6 and 0.85 [22]. The darker meat hydrolysate color is associated with higher its pH and the lighter meat hydrolysate color is associated with lower its pH [20]. Moreover, total phenolic content in hydrolysate can retard the enzymatic browning reaction and the oxidative degradation affected on minimal color deterioration [15]. In case of a Millard reaction, Maillard reaction products (MRPs) such as, melanoidins have been positively correlated with the level of radical scavenging activity and the antioxidant activity [23]. In this result, SD-MPH with lower pH (3.81), a_w (0.378), and 17.28 mg GAE/g dried sample of TPC showed a lighter color than that of FD-MPH with higher pH (5.07), a_w (0.526), and 19.71$\pm$0.42 mg GAE/g dried sample of TPC (as shown in Table 3).

3.2 Amino Acid Composition

Table 2 shows the amino acid profiles of three different hydrolysates. The total amino acid content of HVP, SD-MPH, and FD-MPH were 52.12, 19.48, and 17.27 mg/g of sample, respectively. Overall, the amino acid profile of both MPHs was similar, as they belong to the same enzymatic mushroom hydrolysis. In terms of individual amino acid, the most four abundant amino acids found in both MPHs powder were Glu, Phe, Asp, and Tyr. Regarding to HVP, it was found that Glu, Phe, Tyr, Leu, and Ala were the most abundant amino acid, which each amino acid content ranged from 5.12 to 6.62 mg/g sample. In this experiment, the result indicated that total free amino acids, especially essential amino acids were oxidized higher by freeze drying than spray drying process. Although the drying of protein hydrolysate could induce several stresses capable of denaturing peptides by modifying their structures, the drying process could not break down protein hydrolysate into smaller peptides [27]. Thus, drying method which has been popularly used in food processing reduces amounts of free amino acid composition resulting in lowering the health benefit of vegetable hydrolysates. Moreover, a result of drying, Asp, Pro, and Arg contents were detected higher in the dried powder than in the liquid hydrolysate form. This was possibly owing a

Table 2. Amino acid composition of MPHs powder compared with control (HVP).

Amino acid composition (mg/g sample)	HVP	SD-MPH	FD-MPH
Aspartic acid, Asp(1)	1.32	1.91	1.65
Glutamic acid, Glu(1)	6.51	3.64	3.33
Threonine, Thr(2),*	3.31	0.93	0.76
Serine, Ser(2)	0.84	0.74	0.78
Proline, Pro(2)	ND	0.75	0.66
Glycine, Gly(2)	1.70	0.85	0.79
Alanine, Ala(2)	5.12	1.09	0.90
Valine, Val(3),*	3.77	0.80	0.60
Methionine, Met(3),*	0.84	0.14	0.10
Leucine, Leu(3),*	5.82	1.03	0.90
Isoleucine, Iso(3),*	3.31	0.61	0.43
Phenylalanine, Phe(3),*	6.62	4.05	2.54
Tryptophan, Try(3),*	0.91	0.15	0.17
Histidine, His(3),*	1.38	0.41	0.46
Cystine, Cys	ND	ND	0.07
Tyrosine, Tyr	6.46	1.49	2.26
Lysine, Lys*	4.21	0.63	0.55
Arginine, Arg	ND	0.26	0.32
TAA scores	52.12	19.48	17.27
EAA:TAA ratio	0.53	0.43	0.35

ND: none detected, *EAA: essential amino acid, TAA: total amino acid, (1)umami taste amino acid (UA), (2) sweet taste amino acid (SA), and (3)bitter taste amino acid (BA).

Table 3. TPC, DPPH scavenging activity, and ACE inhibitory activity of MPH samples.

Biological activity	HVP	SD-MHP	FD-MHP
TPC (mg GAE/g dried sample)	3.70 ± 0.04^b	17.28 ± 0.11^a	19.71 ± 0.42^a
IC_{50}, DPPH assay (mg/g dried sample)	19.41 ± 0.13^a	6.44 ± 0.02^b	6.45 ± 0.02^b
FRAP (mM Fe(II)/g dried sample)	1.44 ± 0.01^c	12.02 ± 0.26^a	9.71 ± 0.02^b
%ACE inhibitory activity	100 ± 0.00^a	64.02 ± 0.02^b	6.82 ± 0.01^c

$^{a\text{-}c}$Values in the same row with different letters are significantly different ($p<0.05$).

Maillard reaction between reducing sugars (i.e., maltodextrin) and free amino acids including peptides in the hydrolysate providing MRPs during drying [8]. The considerable quantities of Maillard compounds, heat sterilized glucose-protein hydrolysate mixtures are formed depending on a long time and high temperature produced. The rate of individual amino acid reacts with glucose to form Maillard products obviously depends on its concentration in the solution and the concentration of glucose [21]. However, the formation rate of each amino acid is not clearly know since it was found that the reaction of Pro and Arg, a secondary amine, is rather slow [21].

3.3 Total Phenolic Content

Phenolic compounds have been reported to be the major antioxidant components found in mushrooms, whereas other potential antioxidants such as ascorbic acid, β-carotene, lycopene, and γ-tocopherol have been found only in very small amounts [4]. The total phenolic content (TPC) of HVP, SD-MPH, and FD-MPH were 3.70$\pm$0.04, 17.28$\pm$0.11, and 19.71$\pm$0.42 mg GAE/g dried sample, respectively (Table 3). The results showed that TPC found in FD-MPH is significantly greater ($p<0.05$) than in SD-MPH. However, the powdery form of both dried MPHs had a considerably higher TPC than the liquid form ($p<0.05$).

3.4 DPPH Free Radical Scavenging Activity

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The DPPH radical scavenging activity of HVP, SD-MHP, and FD-MHP were shown in Table 3. The HVP (IC_{50} value = 19.41$\pm$0.13 mg/mL sample) expressed the lowest scavenging activity of DPPH radical ($p<0.05$). While, IC_{50} values of FD-MPH (6.44 mg/g dried sample) and SD-MPH (6.45 mg/g dried sample) were not significantly different. These results indicated that both MPHs powder had strong radical scavenging activity. It can be explained from the possible mechanism of DPPH free radical scavenging that either SD-MPH or FD-MPH had a high phenolic content providing a high hydrogen-donating ability to scavenge the free radical [4]. This finding agrees with the results obtained by Chen *et al.* [9]. They reported that no significant differences of DPPH radical scavenging activity between freeze-dried and spray-dried of egg white protein hydrolysates were found. There is probably that spray drying process has a short residence time compared to freeze drying process. Hence, it seems to have no any detrimentally different effects on the antioxidant activity of product from two different processes. Heat treatment is known to induce reaction between peptides or protein and reducing sugar resulting in MRPs by thermal process and can change the formation of peptides in hydrolysate with pronounced free radical scavenging activity [23, 24]. However, a heat sensitive amino acids such as Try, which acted as strong radical scavengers, might be lost during excessive heating of spray drying [25].

3.5 Ferric Reducing Antioxidant Power

The higher FRAP was found in SD-MPH (12.02 mM Fe(II)/g dried sample), in comparison with that of FD-MPH (9.71 mM Fe(II)/ g dried sample) ($p<0.05$). This reaction indicated that antioxidant peptides had the capacity in reducing TPTZ–Fe(III) complex to TPTZ–Fe(II) complex. The low metal-chelating ability of FD-MPH indicated a poorer ability in chelating proxidative metals [25]. In this result, freeze drying was a major factor in damaging some antioxidative peptides. The conformational changing of mushroom hydrolysate peptides or free amino acids might occur to a large extent during the freeze drying process with a long time resulting in affect on the antioxidant

activity. Amino acid residues containing carboxyl groups (Asp, Glu, and Lys) and phosphorylated hydroxyl side chain (Thr) are metal-ion binders [26]. In this result, the FRAP of SD-MPH was the highest as a function of heating temperature, followed by those of FD-MPH and HVP, respectively. These results are in agreement with those obtained from the antioxidant activity determined by the DPPH radical scavenging assay.

3.6 ACE Inhibitory Activity

The ACE inhibitory activity has been evaluated in HVP, SD-MPH, and FD-MPH samples. As shown in Table 3, SD-MPH exhibited higher ACE inhibitory activity than that of FD-MPH ($p<0.05$). However, the ACE inhibitory activity of both SD-MPH and FD-MPH are likely to be lower than that of HVP ($p<0.05$). This result was closed to the study of Ching *et al.* [28] who reported that ACE inhibitory activity of the water extracts of shiitake, at a concentration of 10 mg/mL, was 95.2$\pm$0.03%. In addition, this result seems that the ACE inhibitory activities of HVP and MPH, possibly involved with high small peptides (di- or tri-peptides) like therapeutic ACE inhibitory drugs. The hypotensive action of autolyzed-shiitake was due to concomitant ACE inhibitory activities of peptides contained in higher amounts during the autolysis [3]. In general, the ACE generates the powerful vasoconstrictor angiotensin II that removes the C-terminal dipeptide from the lysate of the precursor decapeptide angiotensin I [29, 30]. In this system, ACE has four functional amino acid residues of Tyr, Arg, Glu, and Lys at the active site and three hydrolphobic binding subsites, favorable blockade of ACE action would be achieved by small peptides or peptidic inhibitors having high affinity with active sites; as a therapeutic ACE inhibitory drug, captopril, and enalapril, was designed on the basis structure of Ala-Pro or Phe-Ala-Pro [32, 29]. The structure-activity relationship of naturally occurring ACE inhibitory peptides have indicated that small peptides with aromatic amino acid residues such as Phe, Try, His, and Tyr at the C-terminal have a potent ability to inhibit ACE activity with a high potency inhibition (IC_{50} value of <100 μmol/L) [32]. N-terminal amino acids with long-chain or hydrophobic can provide peptides strong inhibitory activity, while Phe, Asp, Ser, or Gly at the N-terminus can mitigate the activity [30, 31]. In this result, the highest of ACE inhibitory power was observed in HVP (100%) comparison with SD-MPH (64.05%) and FD-MPH (6.82%). This activity may be relative to the highest of Phe, Tyr, and Thr contents in HVP (6.62, 6.46, and 3.31 mg/g sample), followed by those of SD-MPH (4.05, 1.49, anf 0.93 mg/g sample) and FD-MPH (2.54, 2.26, and 0.76 mg/g sample), respectively.

3.7 Sensory Characteristic

The PCA result was mapped using the relative voltage response of the electronic tongues sensors to the samples (Figure 1). The sample was distributed on the two-dimensional plane of the first and second principal components. All samples are well discriminated (discrimination index of 78%). The PCA analysis was also obviously separated into 3 distinct clusters. Based on the cluster formations on the PCA, the electronic tongue can distinguish between SD-MPH and HVP on PC1, 67.27% of the PCA total variation. In addition, the electronic tongue can distinguish between FD-MPH and Pre-MPH on PC2, 25.00% of the PCA total variation. This result indicated that the taste sensor might be used to distinguish MPH with different drying process. The taste of MPH sample is enhanced by adding 30% maltodexdrin and heating (Pre-MPH) is similar to HVP taste (strong taste). Moreover, it showed that spray drying has a stronger negative effect on taste of MPH than that of freeze drying.

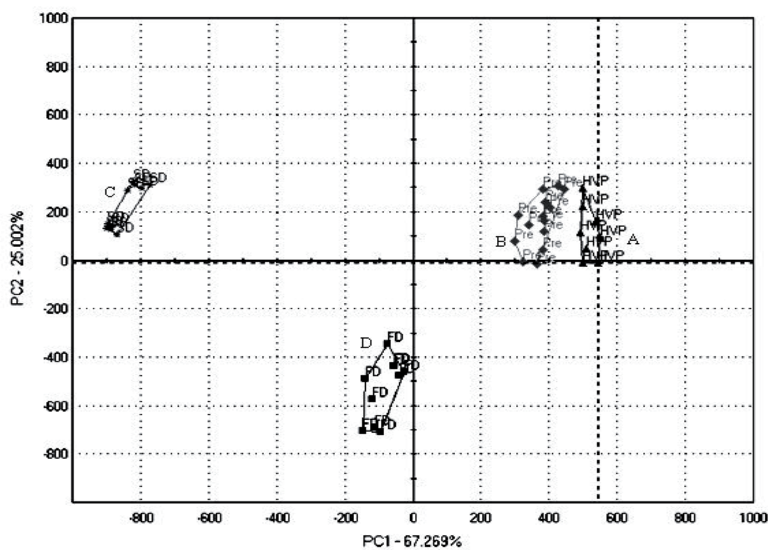

Figure 1. Principal profile of MPH powders (SD-MPH (C) and FD-MPH (D)) compared with control (HVP (A) and Pre-MPH (B)).

From Table 2, the amino acid compositions of SD-MPH and FD-MPH samples were considerably different from those of HVP and Pre-MPH. HVP had the highest bitter taste amino acid with a ratio of BA:SA:UA (43:21:15 %w/w) but the different ratios were found in SD-MPH (36:22:28 %w/w) and FD-MPH (30:22:28 %w/w). Thus, free amino acids, especially Asp, Glu, Ala, Leu, Lys, Try, and Phe, may be the main components, which affect the tastes of HVP and MPHs, and probably also result in the response pattern of the sensor [33]. In this case, the drying process influences the concentrations of free amino acids and amino acids producing specific taste.

4. Conclusions

Based on this research, it can be concluded that the mushroom hydrolysed protein using protease hydrolysis was effective in antioxidant and ACE inhibitory activities. Spray-dried and freeze-dried mushroom protein hydrolysates have different physicochemical properties, antioxidant activity, ACE inhibitory activity, and sensory characteristics. Generally, natural components might be lost during the thermal process since most bioactive compounds are relatively unstable to heat. Spray drying and freeze drying processes may affect on the contents, characteristics, and activities of naturally agents. Moreover, the formation of novel compounds having functional property, such as Maillard's reaction products, can be formed as a result of drying process. Spray drying would be an effective process to produce mushroom protein hydrolysate powder with the high quality in physicochemical property, antioxidant activity and ACE inhibition. Moreover, further work should be studied on the flavor profile, consumer acceptability, and food product applications.

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