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Effect of Freeze Drying and Spray Drying Processes to Amino Acids and Fatty Acids Contents in Haruan (*Channa striatus*) Extract

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Abstract

Haruan extract was dried using two methods of drying namely freeze drying and spray drying. The drying principles of these two methods are different where freeze drying is a dehydration process by sublimation of the frozen water in the materials while spray drying is a drying process that involves heat. Haruan extract contains amino acids and fatty acids that may be damaged during the aforemention drying processes. Therefore, the aim of this study was to determine the amino acids and fatty acids contents in the dried Haruan extract obtained from these two methods. The amino acids were determined using HPLC while fatty acids were analyzed using GC-MS. The study showed that the amount of amino acids in freeze dried Haruan extract (FDHE) was less than in spray dried Haruan extract (SDHE). Conclusively, some fatty acids were damaged by heat in spray drying process but SDHE still showed a higher total amount of fatty acids when compared to FDHE.

Keywords: Haruan, freeze drying, spray drying, amino acids and fatty acids

Introduction

Haruan (*Channa striatus*) is a freshwater fishes that native to South and Southeast Asia. It is rich in protein [1, 2], amino acids and fatty acids [3, 4, 5] and has been long used traditionally for wound healing especially in postpartum and post-operative wound [6, 7]. Haruan is also proven efficacious as antinociceptive, anti-inflammatory, anti-microbial, anti-cancer [4, 6, 8] and in treating pain for osteoarthritis [9]; giving its potential use as active pharmaceutical ingredient. Unfortunately, the liquid extract of Haruan was unstable when stored at room temperature where the changes in the odor and color of the extract could be seen within 24 hours. These changes were indicating the degradation of the contents. Therefore the present study was conducted to produce dried Haruan extract to address this instability issue of the extract.

Several methods are commonly used in the drying process of materials, namely sun-drying, air-drying, vacuum thermal drying, oven drying, freeze drying and spray drying [10, 11]. Freeze drying and spray drying are the two most widely used methods to preserve natural products and foods. Freeze drying, also known as lyophilisation, is a drying process involving freezing of the material following by reducing the surrounding pressure to allow the direct sublimation of the frozen water in the material from the solid to the gas phase. This method is suitable for heat sensitive and perishable materials as it uses low processing temperature [12]. On the other hand, spray drying is a drying method of producing a dry powder from a liquid or slurry by rapid drying using a hot air [10, 13, 14]. These two methods of drying may caused damage to the amino acids and fatty acids content in the Haruan extract. Therefore in this study, we evaluated the effect of drying methods on the amino acids and fatty acids contents in the Haruan extract and we also determine which amino acids and fatty acids that degraded by both the drying methods.

Materials and Methodology

Materials

Water extract *of Channa striatus* was obtained from Major Interest (M) Sdn. Bhd. (Malaysia).

Freeze Drying Method

Liquid Haruan extract was freezed at -20 ^{0}C prior to the freeze drying process. The sample was dried using Vacuum Freeze Dryer (Labconco, USA) and the dried Haruan extract was kept in desiccator until further analysis.

Spray Drying Method

Spray drying process was carried out in the spray dryer machine, manufactured by Yakin Gigih Sdn. Bhd. (Malaysia). Ultrasonic frequency was applied which was 40 kHz. The liquid sample was run at 70-80 ^{0}C with a flow rate of 100 ml/hour.

Amino Acids Analysis

Amino acids in dried Haruan extract were determined using a method by Henderson et al. [15] using HPLC Agilent Technologies

1200 series containing Diode Array Detector (DAD) UV 338 nm (for OPA-amino acids), 262 nm (for FMOC-amino acids) and Zorbax Eclipse-AAA 4.6x150 mm (3.5 µm) column. The Agilent 1313A auto sampler was used to automate the precolumn derivatization procedure resulting in a speedy and reproducible reaction with minimal operator intervention. Phosphate buffer pH 7.8 and acetonitrile : methanol : water (45:45:10 v/v) were used as eluents. Derivatization was carried out using reagents Borat buffer Agilent PN 5061-3339, OPA reagent Agilent PN 5061-3335, FMOC reagent Agilent PN 5061-3337 and deionized water HPLC grade. Two hundred milligram of the sample (FDHE and SDHE) were weighed accurately and filled into a hydrolysis tube followed by an addition of 12 ml HCl 6 M. The tube was then aerated for 1 minute with nitrogen gas and immediately sealed with a Teflon-lined cap. The tube was placed in an electric oven for 24 hours at 110 ^{0}C for sample hydrolysis and was then cooled to room temperature. The content of the tube was quantitatively transferred to a 50 ml clean volumetric flask and diluted to 50 ml with distilled water. After thorough mixing, 1 ml of diluted sample was filtered and 100 µl filtrate was placed in a limited volume insert (LVI) of an auto-sampler vial.

Fatty Acids Analysis

Fatty acids in dried Haruan extract were determined using a method by Febriyenti et al. [3] with slight modification. Analysis was run using GC-MS 6890.

Table 1. Experimental condition of GC-MS

Column	Capillary Column Agilent 19091S-433 HP-5MS, 60 meters, ID 0.250 mm, film 0.25 µm Initial flow 1.2 mL/min Initial pressure 10.36 psi Average velocity 40 cm/sec
Inlet temperature	280 C
Injection volume	1 µl
Carrier gas	Helium
Oven temperature	60 C for 2 min, 12 C /min to 225 C hold on 2 min, 10 C /min to 300 C hold on 0.5 min
Detector temperature	250 C
Detector gases	Hydrogen, flow 2.0 mL/min Air, flow 60.0 mL/min
Makeup gas type	Helium 30.0 mL/min

Results and Discussion

The amounts of amino acids in FDHE and SDHE from 6 batches were tabulated in Table 2.

Table 2. Amount of amino acid in FDHE and SDHE, mean ± SD, n = 6

Amino Acid		Amino acid (mg) / 1 g of dried Haruan extract					
		FDHE	±	SD	SDHE	±	SD
ASP	Aspartate*	3.59	±	0.36	6.02	±	0.69
GLU	Glutamate*	11.39	±	1.07	18.93	±	1.92
SER	Serine*	4.76	±	0.47	8.07	±	0.88
HIS	Histidine*	4.12	±	0.35	6.13	±	0.65
GLY	Glycine*	64.13	±	5.20	95.67	±	5.77
THR	Threonine	2.61	±	0.23	1.51	±	1.95
ARG	Arginine*	17.86	±	1.46	28.21	±	2.37
ALA	Alanine*	11.17	±	1.04	16.68	±	0.93
TYR	Tyrosine*	0.82	±	0.35	1.57	±	0.38
VAL	Valine*	3.36	±	0.32	5.50	±	1.04
MET	Methionine*	3.13	±	0.35	5.47	±	0.84
PHE	Phenylalanine*	3.54	±	0.27	5.14	±	0.86
ISO	Isoleucine*	1.67	±	0.21	2.97	±	0.90
LEU	Leucine*	5.02	±	0.53	9.64	±	1.97
LYS	Lysine*	8.15	±	0.82	13.77	±	2.35
PRO	Proline	0.00	±	0.00	0.61	±	0.86
SUM		145.22			225.91		

*Amount values of amino acid are significantly different ($p<0.05$) between FDHE and SDHE

The 16 quantified amino acids in the extract showed a significant different amount of amino acids between FDHE and SDHE except for threonine and proline. The SDHE showed a higher total amount of amino acids when compared to FDHE indicating the degradation of some amino acids in freeze drying method [16, 17]. Protective agents (lyoprotectants) were usually added to the sample before the freezing process. Typically polyhydroxy compounds such as sugars (trehalose and sucrose), and surfactants are usually used to overcome this problem [18]. Heat also affects and degrades the amino acids but the effect is not as severe as the freezing process.

Table 3 shows the amount of fatty acids in FDHE and SDHE from 6 batches. The total amount of fatty acids in SDHE is more than in FDHE as previously reported by another researcher [19]. Many long-chain fatty acids in the Haruan extract were undetectable when using spray drying method. These fatty acids were likely to turn into short-chain fatty acids that are more stable such as palmitic acid and stearic acid. It can be confirmed by the increment in the concentration of both fatty acids in the SDHE. Some fatty acids showed the amount values that were significantly different between FDHE and SDHE sample. Fatty acids in Haruan extract could be damage by heat in the spray drying process.

Table 3. Amount of fatty acid in FDHE and SDHE, mean ± SD, n = 6

FATTY ACID		Fatty Acid (µg) / 1 g dried Haruan extract					
		FDHE	±	SD	SDHE	±	SD
C12:0	Lauric Acid	0.025	±	0.062	0.000	±	0.000
C13:0	Tridecanoic Acid	0.009	±	0.022	0.000	±	0.000
C14:0	Myristic Acid	1.003	±	0.490	0.890	±	0.251
C14:1	Myristoleic Acid	0.007	±	0.016	0.000	±	0.000
C15:0	Pentadecanoic Acid	0.472	±	0.149	0.483	±	0.216
C16:0	Palmitic Acid*	10.497	±	6.027	19.214	±	1.448
C16:1	Palmitoleic Acid*	2.168	±	0.767	0.096	±	0.179
C17:0	Heptadecanoic Acid	0.790	±	0.194	1.000	±	0.335
C17:1	Heptadecenoic Acid*	0.468	±	0.130	0.114	±	0.200
C18:0	Stearic Acid*	4.608	±	1.122	10.499	±	0.845
C18:1n-9	Elaidic Acid (trans)*	0.000	±	0.000	10.468	±	1.413
C18:1n-9	Oleic Acid (cis)*	10.694	±	2.449	0.000	±	0.000
C18:2n-6t	Linolelaidic Acid (trans)	0.013	±	0.031	0.000	±	0.000
C18:2n-6c	Linoleic Acid (cis)*	1.459	±	1.600	0.000	±	0.000
C18:3n-6	gamma-Linolenic Acid *	0.231	±	0.211	0.000	±	0.000
C20:0	Arachidic Acid*	0.244	±	0.057	0.000	±	0.000
C20:1	Eicocenoic Acid*	0.564	±	0.350	0.000	±	0.000
C20:3n-3	Eicosatrienoic Acid*	0.467	±	0.299	0.000	±	0.000
C20:3n-6	Eicosatrienoic Acid*	0.397	±	0.219	0.000	±	0.000
C20:4n-6	Arachidonic Acid*	1.729	±	0.360	0.895	±	0.251
C20:5n-3	Eicosapentaenoic Acid*	0.965	±	0.259	0.000	±	0.000
C21:0	Heneicosanoic Acid*	0.023	±	0.019	0.000	±	0.000
C22:0	Behenic Acid*	0.103	±	0.060	0.000	±	0.000
C22:1n-9	Erucic Acid	0.013	±	0.031	0.000	±	0.000
C22:6n-3	Docosahexanoic Acid	0.578	±	0.648	0.209	±	0.309
C23:0	Tricosanoic Acid	0.019	±	0.022	0.000	±	0.000
C24:0	Lignoceric Acid*	0.051	±	0.040	0.000	±	0.000
C24:1	Nervonic Acid	0.005	±	0.012	0.000	±	0.000
SUM		37.60			43.87		

*Amount values of fatty acid are significantly different ($p<0.05$) between FDHE and SDHE

Conclusion

Freeze drying method could degrade the amino acids in Haruan extract except threonine and proline while spray drying method could damage the fatty acids in Haruan extract. Long-chain fatty acids were likely to turn into shorter-chain fatty acids (palmitic acid and stearic acid) that were indicated by the increased in the amount of these two fatty acids.

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