Effects of aerosol formulation to amino acids and fatty acids contents in Haruan extract

Febriyenti[1,2]*, Saringat Bin Bai@Baie[2] and Lia Laila[2,3]
[1]Faculty of Pharmacy, Andalas University, Padang, Indonesia
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia
[3]Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia

Abstract: Haruan (*Channa striatus*) extract was formulated to aerosol for wound and burn treatment. Haruan extract is containing amino acids and fatty acids that important for wound healing process. The purpose of this study is to observe the effect of formulation and other excipients in the formula to amino acids and fatty acids content in Haruan extract before and after formulated into aerosol. Precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method is used for amino acids analysis. Fatty acids in Haruan extract were esterified using transesterification method to form FAMEs before analyzed using GC. Boron trifluoride-methanol reagent is used for transesterification. Tyrosine and methionine concentrations were different after formulated. The concentrations were decrease. There are six fatty acids have amount that significantly different after formulated into concentrate and aerosol. Contents of these fatty acids were increase. Generally, fatty acids which had content increased after formulated were the long-chain fatty acids. This might be happen because of chain extension process. Saponification and decarboxylation would give the chain extended product. Therefore contents of long-chain fatty acids were increase. Generally, the aerosol formulation did not affect the amino acids concentrations in Haruan extract while some long-chain fatty acids concentrations were increase after formulated into concentrate and aerosol.

Keywords: Aerosol, Haruan, *Channa striatus,* amino acids, fatty acids.

INTRODUCTION

According to Mat Jais (2007) and Zuraini (2006) Haruan contained protein (78.32 ± 0.23%), lipids (2.08 ± 0.08%) and 0.265 ± 0.013 mg vitamin A (an essential factor for wound healing) per total lipid. The major amino acids in Haruan were glutamic acid, aspartic acid and lysine, ranging from 9.7% to 21.7%. The lipids were categorised into phospholipids, partial glyceride, cholesterol, fatty alcohol, triglyceride and cholesterol ester. It also has a high content of arachidonic acid (AA) 20:4n-6, and docosahexaenoic acid (DHA) 22:6n-3. Flesh of Haruan contain high arachidonic acids (AA) but almost no eicosapentaenoic acids (EPA), which were hypothesised involved in initiating wound repair of tissue (Mat Jais *et al*., 1994). Haruan contained 16.2 ± 0.1 % lipid, 1.2% ω-3, 16% ω-6 (Salimon and Rahman, 2008). Omega-3 and omega-6 were required in wound healing process. Generally, the fatty acids of the Malaysian Freshwater fish (included Haruan) showed a higher unsaturated, rather than saturated acid content. The ratio of unsaturated/saturated ranged from 1.2 to 2.3. Haruan has lipid content 2.10g/100g fillet, ratio unsaturated: saturated 1.28 (Endinkeau and Kiew, 1993).

Amino acids in Haruan extract were analysed in this study using high performance liquid chromatography (HPLC) method for separation techniques. There are two derivatization techniques for spectroscopic detection i.e. postcolumn and precolumn derivatization. Postcolumn techniques should be run on line for maximum accuracy while precolumn techniques can be run either off line or on line. Numerous derivatizing agents have been developed for both techniques (Callejón *et al*., 2010). Some of them are phenylisothiocyanate (PITC) (Marrubini et al., 2008; Ponce-Soto et al., 2007; Mat Jais et al., 1994; Heinrikson and Meredith, 1984), 9-fluorenylmethyl-chloroformate (FMOC-Cl) (López-Cervantes *et al*., 2006), o-phthaldialdehyde (OPA) (Bartolomeo and Maisano, 2006), ninhydrin (Zakaria *et al*., 2007; Zuraini *et al*., 2006; Vidotti *et al*., 2003), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (Callejón *et al*., 2008; Gam *et al*., 2005; Herna´ndez-Orte *et al*., 2003; Wandelen *et al*., 1997; Díaz *et al*., 1996), 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F) (Bryan *et al*., 1999), butylisothiocyanate (BITC), 4-dimethyl-aminoazobenzene-4-sulfonyl chloride (DBS) and 1-dimethylamino-naphthalene-5-sulfonyl chloride (Dansyl-Cl) (Aristoy and Toldra, 2004). AQC which introduced by Cohen and Michaudis is the best derivatizing agent for precolumn derivatization techniques which reacts with primary and secondary amines from amino acids, peptides and proteins, broad optimum pH, yielding very stable derivatives with fluorescent properties, which are easily separated by reverse-phase HPLC, no interfere of excess reagent and reaction time is short (Aristoy and Toldra, 2004; Herna´ndez-Orte *et al*., 2003; Cohen and Michaud, 1993). Precolumn derivatization with 6-aminoquinolyl-N-

**Corresponding author:* e-mail: febriyenti74@yahoo.com

hydroxysuccinimidyl carbamate method reported by Cohen (2005) is used in this study. There are 16 amino acids in Haruan extract which could be analysed using acid hydrolysis. Cystine has to be oxidized with performic acid prior to acid hydrolysis while tryptophane on the other hand has to be hydrolysed using alkali prior to analysis.

Fatty acids should be converted into fatty acid methyl esters (FAMEs) for GC analysis. Fatty acids in Haruan extract were esterified using transesterification method to form FAMEs. Boron trifluoride-methanol reagent (12-14%) is the most often used for transesterification of all types of lipids (AOAC, 2000; Eder, 1995; Liu, 1994). Derivatization of fatty acids for GC analysis is carried out to increase the volatility of the substances, to improve separation and to reduce tailing (Brondz, 2002; Liu, 1994). GC is the most widely used technique for determining the fatty acid profile (Petrovic *et al*., 2010).

The purpose of this study is to observe the effect of formulation and other excipients in the formula of amino acids and fatty acids content in Haruan extract before and after formulated into aerosol.

MATERIALS AND METHODS

Materials, reagents and solvents

Haruan extract was supplied by Major Interest Sdn. Bhd. (Malaysia). Reagents and solvents used for amino acids analysis were hydrochloric acid (R&J Chemicals, Malaysia), α-aminobutyric acid (AABA) (Sigma-Aldrich, USA), Waters AccQ Fluor reagent (Borate buffer, AQC powder, Acetonitrile), C-18 AccQ-Tag column (3.9 × 150 mm), Waters AccQ Concentrated Eluent A, Amino acid standard Pierce (Thermofisher, USA), Acetonitrile (HPLC grade) (J&T Bakers, Germany). Reagents and solvents used for fatty acids analysis were GC Column 19091J-436 HP-5, 60 meters, ID 0.250 mm, film 0.25 µm, serial No. US8620925J (Made in USA), FAME mixture (catalog No. 47885-U) (Supelco, USA), potassium hydroxide and anhydrous sodium sulphate (R&M Chemicals, UK), commercially 50% boron trifluoride in methanol (Merck, Germany), dichloromethane (Fluka, USA), methyl nonadecanoate (Fluka, USA), hydrogen and helium gas (MOX, Malaysia). Samples for amino acid analysis were 1 g Haruan extract, 20 g concentrate of formula E2 (contained 1 g Haruan extract) and 20 g aerosol of formula E2 (contained 1 g Haruan extract). Samples for fatty acid analysis were 200 mg freeze-dried Haruan extract from 5 g Haruan extract, 100 g concentrate and 100 g aerosol. Concentrate and aerosol were formed as film prior used in analysis.

Amino acids analysis

Hydrolysis Samples

1 gram (contain protein approximately 40 mg) Haruan extract was hydrolysed with 5 ml HCl 6 M in an electric oven for 24 hours at 110^{o}C. The sample was then cooled to room temperature and 400 µl of internal standard

Table 1: Instrument and experimental condition of HPLC for amino acid analysis

Instrument	Water System (e2695 separation module) equipped with quaternary pump delivery system, auto sampler, column heater
Detector	Waters 2475 Multi λ fluorescence, λem = 248 nm, λex = 395 nm
Processing Software	Empower System
Injection volume	5µL
Column	AccQ-Tag column, 3.9 µm, 150 x 4.6 mm (Waters, USA)
Column Temperature	38^{o}C
Mobile phase	Eluent A (acetate phosphate buffer), Eluent B (acetonitrile), Eluent C (Milli-Q water)
Flow rate	1 mL/min

Table 2: Gradient table for amino acids analysis using HPLC

Time (Min)	Flow rate (ml/min)	%A	%B	%C	Curve
Initial	1.0	100.0	0.0	0.0	-
0.50	1.0	99.0	1.0	0.0	6
18.0	1.0	95.0	5.0	0.0	6
19.0	1.0	91.0	9.0	0.0	6
29.5	1.0	83.0	17.0	0.0	6
33.0	1.0	0.0	60.0	40.0	11
36.0	1.0	100.0	0.0	0.0	11

solution (AABA, 2.5 μmole/ml) was added. The content of the tube were quantitatively transferred to a 100 ml clean volumetric flask and diluted to 100 ml with distilled water. After through mixing, 1 ml of dilute sample was filtered, and 10 μl filtrate was placed in a derivatizing tube (Cohen, 2005; Gam et al., 2005).

Derivatization of amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)
Ten microliters filtrate of the above acid hydrolysis was added with 70 μl of AccQ Fluor Borate Buffer (Waters Corporation, USA). The sample tube was vortexed briefly prior to adding of 20 μl of reconstituted AccQ Fluor Reagent to the sample tube. After vortexing for several seconds, the sample tube was left to stand for 1 minute at room temperature and then heated in oven at 50°C for 10 min. The mixture was transferred to LVI vial before injection to HPLC (Callejón et al., 2008; Cohen, 2005; Wandelen et al., 1997).

Fatty acids analysis
Base hydrolysis (saponification) and esterification process to produce fatty acid methyl esters (FAMEs)
Haruan extract was freeze-dried in a Vacuum Freeze Dryer (Hetovac) prior used as sample for fatty acids analysis. Approximately 200 mg freeze-dried Haruan extract (equal with 5g liquid Haruan extract) was weighed accurately and put into a hydrolysis tube and 2 ml 0.5 M methanolic-potassium hydroxide was added. The mixture was refluxed for 10 to 15 minutes (until the oil globules have gone). Two millilitres of a 14% boron trifluoride in methanol solution was added and boiling was continued for another 5 minutes. Two millilitres of dichloromethane was added and boiling was continued for further 2 minutes. The boiled contents were allowed to cool and 3 ml of saturated NaCl was added. The tube was then stoppered and was shaked vigorously for 15 seconds while solution was still tepid. The content was let to settle and the organic layer was taken and transferred to other test tube. A small amount of anhydrous sodium sulphate was added and the mixture was then shaken well to clear the sample. The clear solution (organic layer) was transferred to vial and added with 200 μl of internal standard solution. Mixture of the sample and internal standard in vial was dried under nitrogen. The dried sample was reconstituted with 1 ml dichloromethane prior analysis. A 1 μl of the reconstituted sample was injected to GC apparatus equipped with Flame Ionization Detector (FID) (AOAC, 2000; Eder, 1995; Liu, 1994).

For concentrate and aerosol, prior to the analysis, fatty acids were extracted from the sample using 30 ml chloroform for 72 hours at room temperature. Films and non-lipid material were removed by filtration. Chloroform was removed in a stream of nitrogen prior to the analysis (Petrovic et al., 2010; Basconcillo and McCarry, 2008; Bond et al., 2005; Seppänen-Laakso and Laakso, 2002).

Column specification and optimization of instrumental conditions
Table 3: Experimental condition of GC-FID to analyze FAMEs

Column	GC Column J&W Scientific Agilent Technologies 19091J-436 HP-5, 60 meters, ID 0.250 mm, film 0.25 μm, Serial No. US8620925J (Made in USA)
Inlet temperature	300°C
Injection volume	1 μl
Split ratio	1:50
Carrier gas	Helium
Head pressure	Constant pressure mode 230 kPa at 50°C, 33 cm/s at 50°C
Oven temperature	50°C for 2 min, 15 °C/min to 225°C, 10°C /min to 300°C, hold for 0.5 min
Detector temperature	320°C
Detector gases	Hydrogen: 60 kPa Air: 50 kPa Helium make up gas: 100 and 230 kPa

RESULTS

Amount of amino acid before and after formulated into aerosol have tabulated in table 4. Only tyrosine and methionine concentrations were different after formulated into concentrate and aerosol.

Amount of fatty acid before and after formulated into aerosol have tabulated in Table 5. There are six fatty acids have amount that significantly different after formulated into concentrate and aerosol i.e. lauric acid, arachidic acid, eicosapentaenoic acid, erucic acid, lignoceric acid and nervonic acid.

DISCUSSION

Haruan extract contain amino acids and fatty acids that important for wound healing process. Haruan extract have been formulated into aerosol for drug delivery system to wound and burn treatment (Febriyenti *et al.*, 2011; Febriyenti *et al.,* 2008). Evaluation of the film properties from concentrate of aerosol had been done in other study (Febriyenti *et al*., 2010). A good formulation should not affect the quantity of the active ingredient. The quantity of the active ingredient should be the same before and after formulated. In Table 4, concentration of tyrosine and methionine were decrease after formulated. Generally, the aerosol formulation did not affect the amino acids concentrations in Haruan extract.

Contents of six fatty acids in Haruan extract were increase after formulated. Generally, fatty acids which had content increased were the long-chain fatty acids. This might be because of chain extension process. This process might be due to the extraction process of fatty acids from samples prior to analysis and other compound in formula. Saponification and decarbooxylation would give the chain extended product. This is an efficient route to C_{20} polyenes, not easily isolated from natural sources, starting from readily available C_{18} sources (Basconcillo and McCarry, 2008; Bond et al., 2005; Scrimgeour, 2005; Seppänen-Laakso and Laakso, 2002). Therefore, the content of long-chain fatty acids was increased.

Omega-3 and omega-6 fatty acids were important fatty acids in wound healing process. Omega-6 is required for production of prostaglandin which could induce inflammation in the early phase of wound healing process. But if the omega-6 is too many, it could prolong the inflammatory phase and prolong the wound healing

Table 4: Amount of amino acid in Haruan extract, concentrate and aerosol formula E2, Mean ± SD, n = 6

No.	Amino Acid	Amount (mg) amino acid/1 g Haruan extract in:		
		Haruan extract	Concentrate	Aerosol
1	Aspartic acid	2.5558[a] ± 0.6183	2.6123[a] ± 1.2239	2.0178[a] ± 0.5653
2	Serine	1.4251[a] ± 0.3031	1.5183[a] ± 0.6835	1.2946[a] ± 0.2541
3	Glutamic acid	4.5565[a] ± 1.1293	4.6910[a] ± 2.2730	3.7010[a] ± 1.0491
4	Glycine	6.0078[a] ± 1.1711	6.2085[a] ± 2.9078	5.5486[a] ± 1.0066
5	Histidine	0.5529[a] ± 0.1344	0.5349[a] ± 0.2838	0.4847[a] ± 0.1101
6	Arginine	3.0240[a] ± 0.5615	3.8922[a] ± 1.6661	2.5202[a] ± 0.7248
7	Threonine	1.1298[a] ± 0.2686	1.2675[a] ± 0.5903	1.0373[a] ± 0.2207
8	Alanine	3.3387[a] ± 0.7086	3.5479[a] ± 1.5780	2.9272[a] ± 0.6421
9	Proline	2.9134[a] ± 0.5873	2.9181[a] ± 1.5027	2.6022[a] ± 0.5700
10	Tyrosine	0.2410[b] ± 0.0994	0.0633[a] ± 0.0790	0.0127[a] ± 0.0140
11	Valine	0.9574[a] ± 0.2373	1.0872[a] ± 0.5303	0.8674[a] ± 0.2174
12	Methionine	0.6972[b] ± 0.2128	0.2421[a] ± 0.1715	0.1962[a] ± 0.0716
13	Lysine	2.0568[a] ± 0.5342	2.3595[a] ± 1.0632	1.7373[a] ± 0.4604
14	Isoleucine	0.6725[a] ± 0.1810	0.7645[a] ± 0.3784	0.6004[a] ± 0.1556
15	Leucine	1.6501[a] ± 0.4390	1.8740[a] ± 0.8724	1.4830[a] ± 0.3655
16	Phenylalanine	1.0120[a] ± 0.2505	1.0829[a] ± 0.4948	0.9325[a] ± 0.1837

Means within a row with a different letter are significantly different ($P<0.05$)

Table 5: Amount of fatty acid in Haruan extract, concentrate and aerosol formula E2, Mean ± SD, n = 6

No.	Fatty Acid		Amount (µg) fatty acid / 5 g Haruan extract in:		
			Haruan extract	Concentrate	Aerosol
1	Lauric Acid	C12:0	1.84[a] ± 0.80	4.81[b] ± 2.05	4.91[b] ± 2.39
2	Myristic Acid	C14:0	26.69[a] ± 12.26	37.68[a] ±18.39	41.57[a] ± 26.13
3	Myristoleic Acid	C14:1	16.08[a] ± 4.37	13.67[a] ± 5.45	11.77[a] ± 5.29
4	Palmitic Acid	C16:0	246.83[a] ± 100.35	385.39[a] ± 232.37	360.20[a] ± 231.24
5	Palmitoleic Acid	C16:1	75.32[a] ± 32.99	72.45[a] ± 67.64	73.80[a] ± 73.56
6	Heptadecanoic Acid	C17:0	22.45[a] ± 6.95	15.50[a] ± 8.89	17.68[a] ± 9.16
7	Heptadecenoic Acid	C17:1	5.41[a] ± 2.86	3.49[a] ± 1.56	6.61[a] ± 5.69
8	Stearic Acid	C18:0	53.55[b] ± 37.84	9.15[a] ± 3.47	11.66[a] ± 5.67
9	Elaidic Acid (trans)	C18:1n-9	17.49[a] ± 5.74	32.72[a] ± 18.06	31.27[a] ± 19.44
10	Oleic Acid (cis)	C18:1n-9	36.53[a] ± 10.81	32.58[a] ± 14.21	45.83[a] ± 29.21
11	Arachidic Acid	C20:0	4.11[a] ± 1.97	30.32[b] ± 9.63	27.83[b] ± 14.01
12	Eicocenoic Acid	C20:1	6.71[a] ± 2.52	11.77[a] ± 5.96	12.93[a] ± 9.01
13	Arachidonic Acid	C20:4n-6	48.22[a] ± 20.25	30.63[a] ± 20.28	29.08[a] ± 22.99
14	Eicosapentaenoic Acid	C20:5n-3	12.44[a] ± 4.62	37.10[b] ± 14.06	35.36[b] ± 18.06
15	Erucic Acid	C22:1n-9	2.86[a] ± 1.76	23.05[c] ± 8.41	14.07[b] ± 2.26
16	Docosahexanoic Acid	C22:6n-3	28.59[a] ± 8.87	44.38[a] ± 25.75	35.62[a] ± 23.22
17	Lignoceric Acid	C24:0	3.33[a] ± 1.25	68.95[b] ± 40.44	74.02[b] ± 29.80
18	Nervonic Acid	C24:1	5.33[a] ± 1.79	58.73[b] ± 21.92	54.43[b] ± 35.37

Means within a row with a different letter are significantly different ($P<0.05$)

Table 6: Five major rating of fatty acids in Haruan from references and in Haruan extract

No.	(Mat Jais *et al.*, 1998)	(Mat Jais *et al.*, 1994)	(Zuraini *et al.*, 2006)	(Endinkeau and Kiew, 1993)	(Salimon and Rahman, 2008)	Haruan extract
	Mucus	Fillet	Fillet	Fish oil	Fillet	Water extract
1.	C18:2n-6	C16:0	C16:0	C16:0	C18:1	C16:0
2.	C18:1n-9	C22:6n-3	C20:4n-6	C18:1	C16:0	C16:1
3.	C16:0	C18:1	C18:0	18:4n-6	C18:2n-6	C18:0
4.	C18:0	C20:4n-6	C22:6n-3	C18:0	C16:1	C20:4n-6
5.	C20:4n-6	C18:0	C18:1n-9	18:6n-3	C14:0	C18:1n-9
	% of total fatty acid					
Omega-3	2.05	17.72	15.18	2.66	1.24	6.68
Omega-6	34.00	12.70	19.02	4.33	15.13	7.86

process. Omega-3 is required to decrease production of prostaglandin (Calder, 2005; Simopoulos, 2002). The results in Table 6 showed that Haruan extract contained omega-6 more than omega-3. Ratio between omega-3 and omega-6 fatty acids was 0.85. Omega-6 fatty acid is needed as inflammatory inducer in the early phase of wound healing process.

CONCLUSION

Aerosol formulation did not affect the amino acids concentrations in Haruan extract. However there were six fatty acids have their amount significantly different after formulated into concentrate and aerosol i.e. lauric acid, arachidic acid, eicosapentaenoic acid, erucic acid, lignoceric acid and nervonic acid. Contents of these fatty acids were increased. Fatty acids which had content increased after formulated were the long-chain fatty acids. This might due to the extraction process of fatty acids from concentrate and aerosol prior to analysis and other compound in formula.

ACKNOWLEDGEMENT

This study was supported by Universiti Sains Malaysia (USM) Fellowship, Andalas University-Indonesia and The Ministry of National Education of The Republic of Indonesia.

REFERENCES

AOAC (2000). AOAC Official Method 969.33, *Fatty Acids in Oils and Fats* Gaithersburg, AOAC International Press, Maryland, USA, pp.19-20.

Aristoy MC and Toldra F (2004). Amino acids. *In*: LML Nollet (Ed.), Handbook of Food Analysis, Vol.1, Marcel Dekker, Inc., New York:, pp.83-123.

Bartolomeo MP and Maisano F (2006). Validation of a Reversed-Phase HPLC method for quantitative amino acid analysis. *J. Biomol. Tech.,* **17**(2): 131-137.

Basconcillo LS and McCarry BE (2008). Comparison of three GC/MS methodologies for the analysis of fatty acids in *Sinorhizobium meliloti:* Development of a micro-scale, one-vial method. *J. Chromatogr. B,* **871**: 22-31.

Bond B, Fernandeza DR, VanderJagta DJ, Williamsb M, Huangc YS, Chuangc LT, Millsond M, Andrewsd R and Glewa RH (2005). Fatty acid, amino acid and trace mineral analysis of three complementary foods from Jos, Nigeria. *J. Food Compos. Anal.,* **18**: 675-690.

Brondz I (2002). Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography and related techniques. *Anal. Chim. Acta,* **465**: 1-37.

Bryan PD, Emry ML and El-Shourbagy TA (1999). Determination of ABT-089 in human plasma by high performance liquid chromatography using in situ precolumn derivatization with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole. *J. Pharmaceut. Biomed.,* **20**(1-2): 49-63.

Calder PC (2005). Polyunsaturated fatty acids and inflammation. *Biochem. Soc. T.,* **33**(2): 423-427.

Callejón R, Tesfaye W, Torija M, Mas A, Troncoso A and Morales M (2008). HPLC determination of amino acids with AQC derivatization in vinegars along submerged and surface acetifications and its relation to the microbiota. *Eur. Food Res. Technol.,* **227**(1): 93-102.

Callejón RM, Troncoso AM and Morales ML (2010). Determination of amino acids in grape-derived products: A review. *Talanta,* **81**(4-5): 1143-1152.

Cohen SA (2005). Amino acid analysis using precolumn derivatization with 6-Aminoquinolyl-N-Hydroxysuccinimydyl Carbamate. *In:* C. Cooper, N. Packer and K. Williams (Eds.). Methods in Molecular Biology: Amino Acid Analysis Protocols, Vol.159, Totowa: Humana Press Inc., pp.39-47.

Cohen SA and Michaud DP (1993). Synthesis of a Fluorescent Derivatizing Reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.,* **211**(2): 279-287.

Díaz J, Lliberia JL, Comellas L and Broto-Puig F (1996). Amino acid and amino sugar determination by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate followed by high-performance liquid chromatography and fluorescence detection. *J. Chromatogr. A,* **719**(1): 171-179.

Eder K (1995). Gas chromatographic analysis of fatty acid methyl esters. *J. Chromatogr. B,* **671**: 113-131.

Endinkeau K and Kiew TK (1993). Profile of Fatty Acid Contents in Malaysian Freshwater Fish. *Pertanika J. Trop. Agric. Sci.,* **16**(3): 215-221.

Febriyenti, Azmin MN and Baie S@B (2010). Mechanical properties and water vapour permeability of film from Haruan *(Channa striatus)* and fusidic acid spray for wound dressing and wound healing. *Pak. J. Pharm. Sci.,* **23**(2): 155-159.

Febriyenti, Azmin MN and Baie S@B (2008). Formulation of aerosol concentrates containing Haruan (*Channa striatus*) for wound dressing. *Malay. J. Pharm. Sci.,* **6**(1): 43-58.

Febriyenti, Noor AM and Baie S@B (2011). Physical evaluations of Haruan spray for wound dressing and wound healing. *Int. J. Drug Del.,* **3**: 115-124.

Gam LH, Leow CY and Baie S@B (2005). Amino acid composition of snakehead fish *(Channa striatus)* of various sizes obtained at different times of the year. *Malay. J. Pharm. Sci.,* **3**(2): 19-30.

Heinrikson RL and Meredith SC (1984). Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.,* **136**(1): 65-74.

Herna´ndez-Orte P, Ibarz MJ, Cacho J and Ferreira V (2003). Amino acid determination in grape juices and wines by HPLC using a modification of the 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate (AQC) method. *Chromatographia,* **58**(1-2): 29-35.

Liu KS (1994). Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. *JAOCS,* **71**(11): 1179-1187.

López-Cervantes J, Sánchez-Machado DI and Rosas-Rodríguez JA (2006). Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. *J. Chromatogr. A,* **1105**(1-2): 106-110.

Marrubini G, Caccialanza G and Massolini G (2008). Determination of glycine and threonine in topical dermatological preparations. *J. Pharmaceut. Biomed.,* **47**(4-5): 716-722.

Mat Jais and AM (2007). Pharmacognosy and pharmacology of Haruan *(Channa striatus)*: A medicinal fish with wound healing properties. [Review]. *Bol. La. y del Caribe de Plant. Med. y Aromatic.,* **6**(3): 52-60.

Mat Jais AM, Matori MF, Kittakoop P and Sowanborirux K (1998). Fatty acid compositions in mucus and roe of Haruan, *Channa striatus,* for wound healing. *Gen. Pharmacol.,* **30**(4): 561-563.

Mat Jais AM, McCulloch R and Croft K (1994). Fatty acid and amino acid composition in haruan as a potential role in wound healing. *Gen. Pharmacol.-Vasc. S.,* **25**(5): 947-950.

Petrovic M, Kezic N and Bolanca V (2010). Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. *Food Chem.,* **122**: 285-291.

Ponce-Soto LA, Lomonte B, Gutiérrez JM, Rodrigues-Simioni L, Novello JC and Marangoni S (2007). Structural and functional properties of BaTX, a new Lys49 phospholipase A2 homologue isolated from the venom of the snake Bothrops alternatus. *Biochimica et Biophysica Acta,* **1770**: 585-593.

Salimon J and Rahman NA (2008). Fatty acids composition of selected farmed and wild freshwater fishes. *Sains Malaysiana,* **37**(2): 149-153.

Scrimgeour C (2005). Chemistry of fatty acids. *In:* F. Shahidi (Ed.), *Bailey's Industrial Oil and Fat Products,* Vol. 6, John Wiley & Sons, Inc., pp.1-43).

Seppänen-Laakso T and Laakso RHI (2002). Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. *Anal. Chim. Acta,* **465**: 39-62.

Simopoulos AP (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. *Am. College of Nutr.,* **21**(6): 495-505.

Vidotti RM, Viegas EMM and Carneiro DJ (2003). Amino acid composition of processed fish silage using different raw materials. *Anim. Feed Sci. Tech.,* **105**(1-4): 199-204.

Wandelen CV, Cohen SA and Hancock WS (1997). Using quaternary high-performance liquid chromatography eluent systems for separating 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. *J. Chromatogr. A,* **763**: 11-22.

Zakaria ZA, Mat Jais AM, Goh YM, Sulaiman MR and Somchit MN (2007). Amino acid and fatty acid composition of an aqueous extract of *Channa striatus* (Haruan) that exhibits antinociceptive activity *Clin. Exp. Pharmacol. P.,* **34**(3): 198-204.

Zuraini A, Somchit MN, Solihah MH, Goh YM, Arifah AK, Zakaria MS, Somchit N, Rajion MA, Zakaria ZA and Mat Jais AM (2006). Fatty acid and amino acid composition of three local *Malaysian Channa* spp. fish. *Food Chem.,* **97**(4): 674-678.