Fatty acid composition and acute oral toxicity of rambutan (*Nephelium lappaceum*) seed fat and oil extracted with SC-CO_2

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ABSTRACT

Seeds of rambutan (*Nephelium lappaceum*), after soaking in hot water and dry grinding, were extracted with supercritical carbon dioxide (SC-CO_2) at 35 MPa and 45°C to obtain the fat and oil. The extractable fat and oil yield ranged from 16.0-23.0% and 5.0-7.6% respectively. The fatty acid composition of SC-CO_2 extracted rambutan seed fat and oil was broadly similar, with about 45% of saturated fatty acids (SFA), 51-53% of monounsaturated fatty acids (MUFA) and 2-3% of polyunsaturated fatty acids (PUFA) respectively. Oleic (C18:1) was a major fatty acid as MUFA and present 50-52% of the total fatty acid content. By comparing the SFA amounts of SC-CO_2 extracted rambutan seed fat and oil, it was observed differences of 2-5%, mainly due to variation on arachidic acid (C20:1), behenic acid (C22:0), stearic acid (C18:0) and palmitic acid (C16:0) contents. A 14-day acute oral toxicity test revealed that SC-CO_2 extracted rambutan seed fat and oil at the limit dose of 5000 mg/kg bodyweight to rats did not cause signs of intoxication, death or gross pathological lesions. Based on these results, rambutan seeds are promising as a potential fat and oil resource for edible use. Further investigation on physical and chemical characteristics of the rambutan seed fat and oil extracted with SC-CO_2 is in process.

Keywords: Acute oral toxicity, Fatty acids, Rambutan seeds, fat and oil, Supercritical carbon dioxide (SC-CO_2)

1. INTRODUCTION

Rambutan (*Nephelium lappaceum*) is widely consumed fresh and industrially processed to canned fruit products. Rambutan seeds are usually discarded as waste. The seeds, constituting 4-9% by weight of the fruit and 14-41% fat, are an attractive source of potential natural fat and oil for human consumption and industrial applications [1]. Recovery of rambutan seed fat and oil has performed by solvent extraction using hexane [1-2] and supercritical carbon dioxide (SC-CO_2) extraction [3]. SC-CO_2 is a viable alternative to the usual hexane for extraction of rambutan seed fat and oil due to the low critical point (P_c = 7.3 MPa, T_c = 31°C) and characteristics of CO_2 that allow for efficient extraction and replacement for toxic solvents, resulting in an eco-environmental friendly process [3]. The optimum condition of rambutan seed oil extracted by SC-CO_2 was obtained at 34.8 MPa and 56.7ºC using response surface methodology [3].

Fatty acid composition is quantitatively the important indication of fatty acids in fat and oil. In general, oil has much greater proportions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) than fat which is at high contents in saturated fatty acids (SFA). The main fatty acids in the rambutan seed fat extracted with hexane were palmitic (C16:0), stearic (C18:0) and arachidic acid (C20:1) as SFA, oleic (C18:1) as MUFA, and linolenic acid (C18:3) as PUFA [1-2].

The objective of the present study, therefore, was to examine the fatty acid composition of rambutan seed fat and oil obtained by SC-CO_2 extraction at 35 MPa and 45°C, which is at lower temperature level than in the work of Yoswathana [3] and evaluate the toxicological value of the rambutan seed fat and oil by acute oral toxicity limit test.

2. MATERIAL AND METHODS

Preparation of rambutan seeds sample

Rambutan (*N. lappaceum*) seeds were obtained from a fruit processing company in Nakorn Pathom province, Thailand. The seeds were washed, soaked in hot water at 90°C for 1 h and oven-dried at 55°C for 10-12 h. The dried seeds were ground using a miller and then sieved using the sieve screens between 10 and 100 mesh. The ground seed sample of particle size ranging from 0.15 to 2.0 mm was stored in a sealed plastic bag at room temperature until used for extraction experiments.

Proximal chemical analysis

The proximate analysis of rambutan seeds sample was determined according to AOAC (2000). The analysis included moisture, crude protein, crude fat and ash. The moisture content was determined by drying in oven at 105°C to a constant weight. Total crude protein content was determined using the Kjeldahl method. The total fat content was determined by Soxhlet extraction with petroleum ether (40 to 60°C) for 3 h, and then oven-dried to dryness at 105°C for 1 h. Ash was determined by weighing the incinerated residue obtained at 550ºC for 30 min. Total available carbohydrate was calculated as 100% minus the sum of moisture, protein, fat and ash.

SC-CO_2 extraction

The SC-CO_2 extraction of fat and oil from rambutan seeds sample was performed using the Speed SFE instrument with a 300 ml extraction vessel (Applied Separations Inc., Allenton, PA, USA). The vessel was heated with an oven controlled by a thermostat (±1ºC). Liquid CO_2 was delivered into the vessel and pressurized to the operating value (±10 bar) with a high pressure pump (Applied Separations Inc., Allenton, PA, USA). For each extraction, about 100 g of ground rambutan seeds were loaded into the vessel, and packed with propylene wool. SC-CO_2 was left in contact with the sample for 30 min of static extraction. After that, dynamic extraction was performed with a CO_2 flow rate ranging about 2 L/min. The SC-CO_2 with dissolved oil passed through a heated micrometering valve at 110ºC, while the SC-CO_2 was expanded to atmospheric pressure. At time intervals of about 4 h, the fatty oil was collected in a pre-weighed glass vial immersed in a water bath at about 5°C, where the oil was cooled and fat was precipitated. The extraction was set to end when the fatty oil measured was less than 0.005 g/ g dry seeds. The oil and fat were subsequently separated from each other by filtration.

Fatty acid composition

SC-CO_2 extracted fat and oil were converted into fatty acid methyl esters (FAME) according to AOAC (2000). In brief, 0.2 g of the extracted sample was dissolved in 10 mL of 1M methanolic sodium hydroxide, refluxed at 100°C for 15 min, and then 12 mL of 12% BF_3 and 5 mL of *n*-heptane was added. After cooling, 30 mL of saturated sodium chloride was added to the mixture. The upper *n*-heptane phase was transferred into a vial and injected into a GCMS-QP2010 Ultra gas chromatrograph mass spectrometer (Shimadzu, Columbia, MD, USA) equipped with a capillary column Cp-Sil 88 (100 m long, 0.25 mm i.d., 0.2 µm film thickness). The initial oven temperature was from 100°C heated to 240°C (3°C/min). Injector and detector temperatures were set at 225°C. The mass spectrometer operated at ionization energy of 70 eV with a scan range of 30-320 amu. Identification of components was carried out based on retention time and mass spectra by matching with the NIST library.

Acute oral toxicity limit test

A total of 50 male and female Wistar rats were randomly divided into five groups of ten (five male and female each). The rats were around 6-7 weeks old and their body weight range for males 261-312 g and females 182-221 g. The animals were allowed to acclimatize under controlled conditions in a ventilated room at 24±1°C with relative humidity between 50% and 70% and a constant day/night cycle for a week. Feed and water *ad libitum* were provided throughout the study period. The animals were starved for 16 h overnight prior to the experiments. Rats (5/sex/group) were treated orally with SC-CO_2 extracted rambutan seed fat and oil at dose levels of 2,000 and 5,000 mg/kg body weight, while the control group received only distilled water. In all experiments, the animals were observed daily for 14 days for any signs of toxic symptoms or mortality. Body weight of the animals was noted before administration and weekly. At completion on the 14 day, all animals were euthanized and gross pathological examinations were performed.

Statistical analysis

Values were reported as means with standard error of means (SEM). Analysis of variance (ANOVA) was used to analyze the data, where $p < 0.05$ the means were considered statistically significant difference.

3. RESULTS

The yields of fat and oil extracted with SC-CO_2 ranged from 16.0-23.0% and 5.0-7.6% respectively (data not shown), compared to the mean fat yield of 31.2% recorded by Sirisompong et al [1] and about 30.4% reported by Yoswathana [3].

The proximate composition of rambutan (*N. lappaceum*) seeds is presented in Table 1. The results are in good agreement with findings of Solís-Fuentes et al [2] who reported rambutan seeds had high contents of carbohydrate (57.60%), fat (33.40%) and protein (7.80%) but a low ash content (1.22%) on a dry basis.

Table 1. Proximate composition of rambutan (*N. lappaceum*) seeds (g/100g dry weight)

	Proximate composition				
	Moisture	Protein	Fat	Ash	Carbohydrate
Rambutan seeds	6.30±0.05	7.90±0.09	28.18±0.82	1.49±0.05	62.43±0.70

Values are means of triplicate determinations.

The fatty acid composition of rambutan seed fat and oil extracted with SC-CO_2 at 35 MPa and 45°C is shown in Table 2. Statistically significant ($p < 0.05$) differences in several fatty acids were detected between the SC-CO_2 extracted fat and oil. In general, both the fat and oil displayed high compositions of 16:0, 18:0, 20:0 and 22:0 as SFA, 18:1 *cis*-9 as MUFA and low composition of 18:3 as PUFA. A comparison of the compositions of 16:0, 18:0, 20:0, 22:0 and 18:1 *cis*-9 fatty acids between the fat and oil were not large, about 2 to 5%.

Table 2. Fatty acid composition of rambutan seed fat and oil extracted with SC-CO_2 at 35 MPa and 45°C

	Percentage by weight of total fatty acids	
	Fat	Oil
Saturated fatty acid		
Myristic acid (C14:0)	0.01^{a}±0.01	0.04^{a}±0.01
Palmitic acid (C16:0)	7.39^{b}±0.20	10.33^{a}±0.15
Stearic acid (C18:0)	16.58^{a}±0.20	12.21^{b}±0.14
Arachidic acid (C20:0)	12.34^{b}±0.03	16.22^{a}±0.06
Behenic acid (C22:0)	8.91^{a}±0.03	6.53^{b}±0.05
Monounsaturated fatty acid		
Palmitoleic acid (C16:1)	0.49^{b}±0.03	1.35^{a}±0.05
Elaidic acid (C18:1 *trans*-9)	0.02^{a}±0.01	0.03^{a}±0.01
Oleic acid (C18:1 *cis*-9)	52.18^{a}±0.44	50.17^{b}±0.38
Polyunsaturated fatty acid		
Linolenic acid (C18:3)	2.02^{b}±0.01	3.04^{a}±0.05

Data are expressed as mean values of triplicate determinations. ^{a,b}Means with different subscripts are significantly different (I < 0.05)

The SC-CO_2 extracted fat obtained in our study had higher ($p < 0.05$) proportions of 16:0, 18:0, 22:0 and 18:1 *cis*-9 fatty acids, and lower ($p < 0.05$) proportions of 20:0 and 18:3 fatty acids, when the fatty acid composition of hexane extracted fat previously reported by Sirisompong et al. [1] and Solís-Fuentes et al. [2] was compared. The differences found with these results may be due to the solubility of fatty acids in SC-CO_2, which mainly affects the proportion of individual fatty acids in the SC-CO_2 extracted fat compared with the hexane extracted fat.

In the acute toxicity study, the 14-day observation period revealed that there was no mortality in all male and female animals. The oral LD_{50} of SC-CO_2 extracted rambutan seed fat and oil in Wistar rats was greater than 5,000 mg/kg body weight. There were no any gross pathological lesions observed on necropsy at the end of the study in male and female rats.

Data referring to the body weights of male and female rats receiving SC-CO_2 extracted fat and oil from rambutan seeds at dose levels up to 5,000 mg/kg bodyweight are presented in Table 3. There were no significant differences ($p > 0.05$) in the body weights of male and female rats when the control and treatment groups were compared. These results suggest that the extracted fat and oil were non-toxic and feeding at dose level of 5,000 mg/kg body weight to rats had no significant effect on body weights.

Table 3. Comparison of mean body weights for male and female rats during a 14-day trail toxicity study with SC-CO_2 extracted fat and oil from rambutan seeds

Day	Sex	Control	Fat (mg/kg bw)		Oil (mg/kg bw)	
			2,000	5,000	2,000	5,000
1	M	282.40±14.59	291.80±11.92	296.60±7.77	291.20±15.80	298.80±15.09
	F	192.60±4.98	194.20±12.13	203.00±10.17	194.20±6.98	201.80±9.58
8	M	308.40±21.20	316.00±18.81	328.80±13.33	316.20±16.84	324.60±13.60
	F	222.20±1.16	217.00±13.45	223.20±5.60	220.40±13.39	229.60±12.78
15	M	328.00±26.91	327.00±14.75	340.80±10.87	325.80±19.18	339.80±15.85
	F	235.60±11.33	231.80±13.70	241.40±13.54	226.20±14.48	243.80±15.12

Values are mean ± SEM for 5 rats in each group.

4. CONCLUSIONS

Rambutan seed fat and oil obtained by SC-CO_2 at 35 MPa and 45°C contained mainly palmitic (7.39 vs 10.33%), stearic (16.58 vs 12.21%), arachidic (12.34 vs 16.22%) and behenic (8.91 vs 6.53%) as SFA, oleic (52.18 vs 50.17%) as MUFA, and linolenic (2.02 vs 3.04%) as PUFA. The SC-CO_2 extracted rambutan fat and oil are safe to use and may be considered for edible purposes.

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