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Rambutan (*Nephelium lappaceum*) Seed Flour Prepared by Fat Extraction of Rambutan Seeds with SC-CO_2

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Abstract

Introduction: Rambutan (*Nephelium lappaceum*) seeds are waste byproduct from fresh consumption to fruit canning industry. This seed waste contains high amounts of fat (14-41%) and carbohydrate (28-46%). After fat removal, the defatted material could be potentially used as a flour alternative for food applications. The aim of this study was to produce flour from rambutan seeds by fat extraction with SC-CO_2. The defatted flour obtained was characterized by some selected properties and evaluated for potential toxicity following acute oral exposure in rats. **Methods:** Fat was extracted from ground rambutan seeds by SC-CO_2 at 350 bar and 45 °C. Defatted flour samples were determined for proximate compositions, amylose content, paste viscosity by using RVA and evaluated for acute oral toxicity. **Results:** Ninety percent of the total rambutan fat was extracted by SC-CO_2, comparable to that of hexane extraction. Defatted rambutan seed flour had high contents of protein (10.07%), carbohydrate (87.04%) and amylose (32.16%). Fat extraction resulted in higher RVA viscosity pattern and exhibited no pasting peak with continual rise in viscosity. Compared with other sources of flours, the defatted rambutan seed flour exhibited much lower RVA viscosity pattern, suitable to apply in confectionery formulation. This flour material was non-toxic at 15000 mg/kg in acute oral toxicity testing. **Conclusion:** SC-CO_2 was capable of removing 90% of total fat available in rambutan seeds. Defatted rambutan seed flour contained high protein and carbohydrate, similar to those of all purpose wheat flour. Fat removal of rambutan seeds using SC-CO_2 extraction produced a flour exhibiting higher viscosity determined by RVA and continual rise in pattern throughout the RVA cycle. However, the viscosity pattern of defatted rambutan flour was lower when compared with rice and wheat flours. The oral acute toxicity showed that the defatted rambutan flour was safe for consumption. Based on our results, the defatted rambutan seed flour could potentially be used as a food ingredient in development of confectionery products.

Keywords: Rambutan seed flour, Supercritical carbon dioxide (SC-CO_2) extraction, Proximate composition, Pasting properties, Acute oral toxicity

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Introduction

There is growing interest in rambutan (*Nephelium lappaceum*) seeds, a waste byproduct from fresh consumption to fruit canning industry. Rambutan seed waste is usually discarded in abundance because its economic value is less. This waste material is a potential source of vegetable fat and oil. The whole seeds contain nearly equal amounts of unsaturated and saturated fats, with total fat between 14 and 41% (Sirisompong *et al*., 2011). Rambutan seed fat has the physical and chemical characteristics that can be exploited as an ingredient in manufactured food and cosmetic products (Sirisompong *et al*., 2011; Solís-Fuentes et al., 2010; Yanty *et al*., 2013).

Apart from fat, rambutan seeds have a carbohydrate ranged from 28 to 46% (Solís-Fuentes *et al*., 2010; Yanty *et al*., 2013). These seeds after fat removal could be potentially used as a flour alternative for food applications. Preliminary study showed that the rambutan seed flour could serve as a thickening agent for fat substitute in a low calorie salad dressing product (Phanthanapatet *et al*., 2012).

The removal of fat from rambutan seeds has been previously reported by conventional solvent extraction using hexane (Sirisompong *et al*., 2011; Solís-Fuentes *et al*., 2010; Yanty *et al*., 2013) and supercritical carbon dioxide (SC-CO_2) extraction (Eiamwat, 2014; Yoswathana, 2013). The use of SC-CO_2 as opposed to other organic solvents offers the minimal solvent residue after the extraction. This major advantage is due to the non-toxicity and low critical point (P_c = 73 bar, T_c = 31 °C) so that SC-CO_2 is easily evaporated. The optimum condition for fat removal from rambutan seeds with SC-CO_2 extraction has been reported at 348 bar and 56.7 °C (Yoswathana, 2013).

Currently, there is little information on rambutan seed flour as an alternative to conven tional flours, such as wheat flour. The present study was carried out with an objective to produce a flour from rambutan seeds by fat extraction with SC-CO_2. The defatted flour obtained was charac terized by some selected properties and evalu ated for potential toxicity following acute oral exposure in rats to ensure its use for consump tion.

Materials and Methods

Preparation of raw materials

Rambutan (*Nephelium lappaceum*) seeds were obtained from a fruit processing company in Nakorn Pathom province, Thailand. They were washed, soaked in hot water at 90-95 °C for 1 h and oven-dried at 50-55 °C for 10-12 h. The dried seeds were ground using a miller and then sieved between 10 and 100 mesh. The ground rambutan material of particle size ranging from 0.15 to 2.0 mm was kept in a sealed plastic bag and stored in a desiccator until used for extraction experiments.

Fat extraction with SC-CO_2

SC-CO_2 extraction of fat was performed in the Speed SFE instrument with a 300 ml vessel (Applied Separations Inc., Allenton, PA, USA) at 350 bar and 45 °C, of which is at lower than the optimum temperature based on the work of Yoswathana (2013). In each extraction experiment, approximately 100 g of ground rambutan seeds was loaded into the vessel and filled with propylene wool. Initially, static extraction was performed for 30 min, followed by a dynamic extraction with a CO_2 flow rate ranging about 2 L/min. During the dynamic time interval of 4 h fat extract fractions were collected in a glass vial immersed in a water bath at about 5 °C, and weighed. The CO_2 was vented to atmo sphere. The extraction was monitored over time and set to end when the fraction of fat extract was less than 0.01 g/g dry seeds. The results were expressed as cumulative fat yield with extraction time.

Proximate composition

After fat removal by SC-CO_2 extraction, the defatted material was ground to a much finer powder, resulting flour that would pass through a 100 μm stainless steel sieve. The defatted and non-defatted flour samples were analyzed for their chemical compositions according to the standard methods of AOAC (2000). Amylose content in the defatted flour was determined in compliance with the guidelines described in the Thai Agricultural Standard (TAS) number 4000-2003 on Thai Hom Mali rice.

Paste viscosity

A Rapid Visco Analyser (RVA-TecMaster, Newport Scientific, Australia) was used to measure viscosity of the defatted rambutan seed flour based on the AACC method 61-02 and compared with those of the non-defatted rambutan seed flour, wheat flour and rice flour.

Acute oral toxicity

Defatted rambutan seed flour was tested for potential acute oral toxicity in accordance with Organization for Economic Co-operation and Development (OECD) guideline number 423 (OECD, 2001). Wistar rats (5/sex/group; the National Laboratory Animal Center, Salaya Mahidol Univerisity, Nakorn Pathom, Thailand) were administered a single oral dose of the defatted flour at levels of 0, 2000 and 15000 mg/kg body weight. All animals were observed daily for 15 days for any adverse reactions or mortality and body weights were recorded on days 1, 8 and 15. At completion on day 15, all the animals were euthanized and macroscopic examination was performed.

Statistical analysis

The SPSS software (version 13.0) was used to compare the difference between treatment means. The statistical difference was defined as ($p < 0.05$). All measurements were performed in triplicate.

Results

SC-CO_2 extraction of rambutan seed fat

As seen with the cumulative fat extraction in Figure 1, the total fat yield leveled out around 34.4 g/100 g after 44 h of extraction. The mass yield of defatted seeds varied from 59.5 to 68.7 g/100 g.

The extraction time and total fat yield data for SC-CO_2 extraction and hexane extraction are given in Table 1. The yield of rambutan fat with the SC-CO_2 extraction was comparable to that in hexane extraction, about 90% of the total rambutan fat was extracted. The extraction time with SC-CO_2 in present study was about 5 times longer compared to that in hexane extraction reported by other investigators (Sirisompong *et al*., 2011).

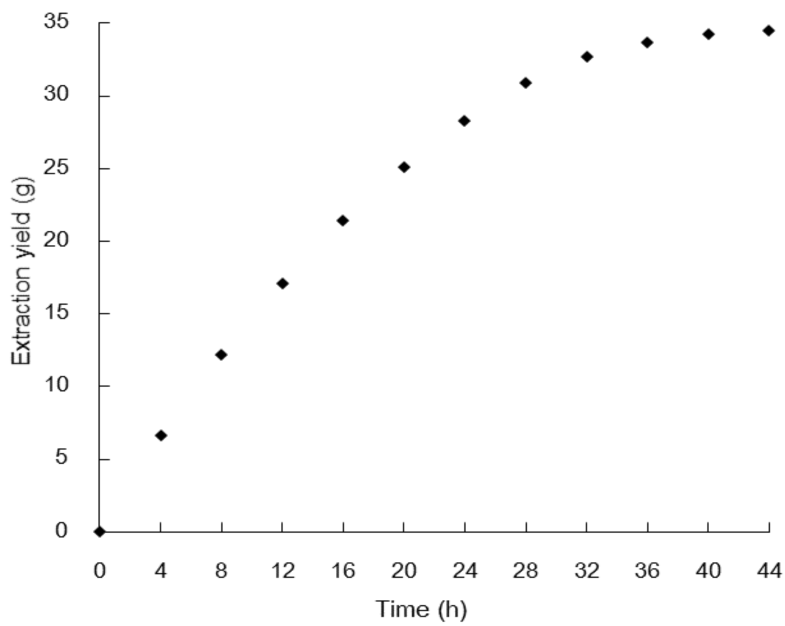

Figure 1 Cummulative extraction of fat from 100 g of rambutan seeds by SC-CO_2 extraction at 350 bar, 45 °C and CO_2 flow rate of about 2 L/min. Each point represents the average of three runs.

Table 1 Comparison of SC-CO_2 extraction with hexane extraction for fat removal from rambutan seeds

Extraction method	Extraction time (h)	Total fat yield (g/ 100g)
SC-CO_2[a]	44	34.39
Hexane[b]	9.2	37.35

[a] Determined values; [b] Sirisompong et al. (2011)

Chemical compositions

The compositions of non-defatted and defatted rambutan seed flour are shown in Table 2. It is seen that higher protein and carbohydrate contents in defatted rambutan flour directly resulted from fat removal. The defatted rambutan flour had a high content of total carbohydrate (87.04%), low contents of fat (1.99%) and ash (1.17%). The protein content of defatted rambutan flour (10.07%) was in agreement with that of commercial available all-purpose wheat flour, being normally between 9 and 12%. The amylose content value for defatted rambutan flour (32.16%) differed and was higher than that from all-purpose wheat flour with a value of 17.47% (Ng *et al*., 2014).

Table 2 Proximate composition (g/100g dry weight) of non-defatted and defatted rambutan seed flour

Components	Non-defatted flour	Defatted flour
Moisture	6.30±0.05	6.74±0.14
Protein	7.90±0.09	10.07±0.22
Fat	28.18±0.82	1.99±0.16
Ash	1.49±0.05	1.17±0.06
Total carbohydrate[a]	62.43±0.70	87.04±0.28
Amylose	ND	32.16±0.43

Values are means ± standard deviations of triplicate determinations.

[a] Calculated by difference

ND = not determined

Paste viscosity

Comparative pasting characteristics of rambutan seed flour, all-purpose wheat flour and rice flour (Saohai variety) are shown in Figure 2 Rambutan seed flour had the lowest viscosity values when compared with wheat flour, which had comparable protein content, and rice flour (Saohai variety), having similar value of amylose content (31%). Rambutan seed flour had the lowest peak viscosity (482 cP) which differed significantly from wheat flour (2,025 cP) and 3,879 cP for rice flour (Saohai variety). The final viscosity values were lowest for rambutan seed flour (543 cP), whereas wheat flour had a value of 1,729 cP and the rice flour with 5,440 cP.

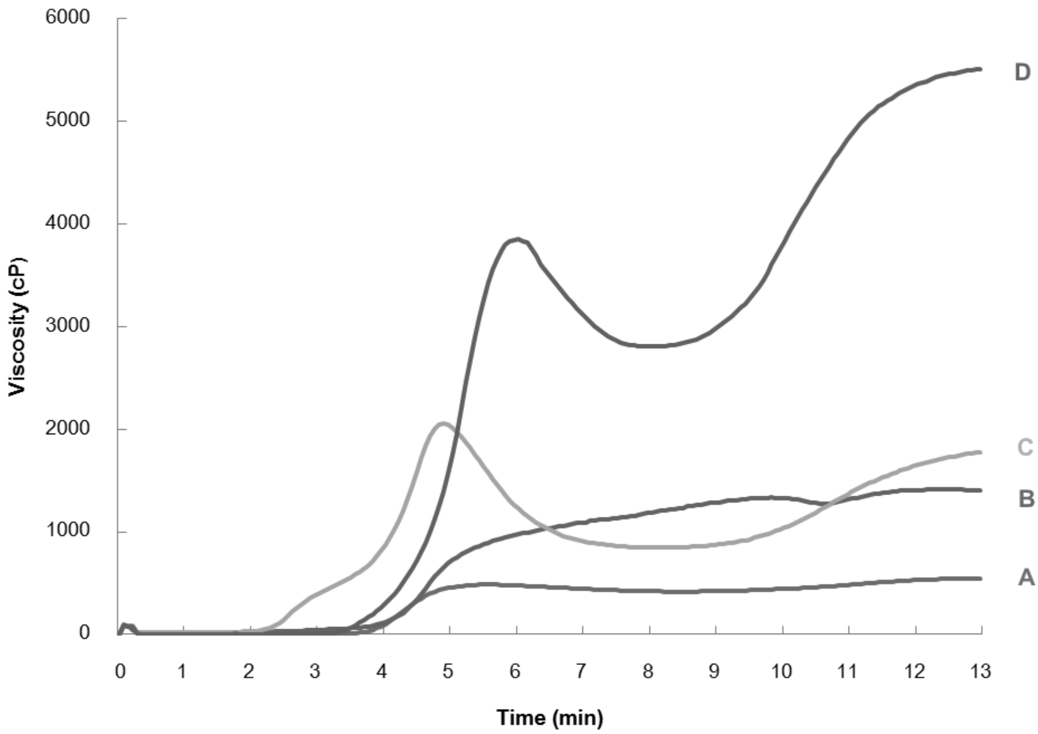

Figure 2 Pasting properties of rambutan seed flour compared with other sources (A) non-defatted rambutan seed flour (B) defatted rambutan seed flour (C) all-purpose wheat flour (D) rice flour

Acute oral toxicity

Mean body weights for male and female rats following administrations of defatted rambutan seed flour at different doses (0–15000 mg/kg) are shown in Table 3. Group mean body weights in male rats were decreased in all groups throughout the study. A significant decrease in the mean values of body weight was noted in the males by comparison with the control groups observed on day 15. In female rats with lower body weights, mean values of body weight during the course of study were comparable to the control groups. The noted significant changes were not seen in both sexes and were not dose-related. There were no any unusual signs of toxicity at the end of 15 day study, nor were there any abnormalities with all animals sacrificed on gross examination. Hence, the above changes were considered as incidental, not toxicological significance. The oral LD_{50} of rambutan seed flour in Wistar rats was greater than 15000 mg/kg bodyweight.

Table 3 Mean body weights (g) for male and female rats administered defatted rambutan seed flour during 15-day oral (gavage) toxicity study

Dose (mg/kg)		0	2000	150000
Males	Day			
	1	252.80±6.38	241.00±8.72	240.80±5.45
	8	289.80±6.06	279.20±7.53	280.20±5.17
	15	348.80±5.45	302.60±13.13*	301.20±8.44*
Females	Day			
	1	201.20±4.66	205.00±6.44	203.00±6.56
	8	219.40±3.36	215.60±5.68	214.40±6.07
	15	230.20±3.19	232.80±5.40	233.40±5.27

Values are means ± standard deviations (5 rats/sex/group).
Significant difference from control (* $p < 0.05$).

Discussion

SC-CO_2 extraction was efficient for fat removal from rambutan seeds without the use of a co-solvent to obtain defatted rambutan flour with fat content less than 5%. The removal of rambutan fat with SC-CO_2 was conducted at 45 °C in the present study, while the optimum temperature was at 56.7 °C reported by Yoswathana (2013). This could partially explain variation in the extraction time with a similar yield, as higher temperature than 45 °C would likely enhance the extractability of rambutan fat. It is also important to note that the use of SC-CO_2 obviates a risk of solvent residue in the defatted material. Whereas the conventional hexane solvent leaves adsorbed residues behind and elevated temperatures at the desolventisation process can cause chemical transformation. The solvent residues must be reduced to small concentration, generally less than 25-30 ppm (Reverchon and Marco, 2006)

Defatting of rambutan seeds by SC-CO_2 extraction contributed to additional total protein and carbohydrate contents in rambutan seed flour. Defatted rambutan flour had comparable compositions to the commercially available all-purpose wheat flour. From a nutritional point of view, the low fat content, together with the increased protein and carbohydrate fractions, might provide the defatted rambutan flour as a potential food ingredient.

The low viscosity behavior of rambutan seed flour may be attributed to the presence and interaction of high fat and protein fraction with starch. After fat removal, RVA viscosity pattern of rambutan seed flour changed and exhibited no pasting peak but rather continual rise during cooking and cooling. This pasting profile was found to be similar to those of cross-linked starches, enhancing the starch granule to withstand cooking by adding covalent bonds. However, by comparison with those of rice and wheat flour, the viscosity profile of defatted rambutan flour was somewhat lower. The defatted rambutan seed flour may be suitable to confectionery application, which needs low viscosity and weak gel formation (Taggart, 2004)

In the acute oral study, the defatted flour was shown to be non-toxic. There were no treatment-related effects on body weight were seen for either sex as well as no evidence of mortality in the 15-day study. Therefore, the defatted rambutan flour was safe for consumption.

Conclusion

The pre5sent study demonstrated the fat removal from currently low value rambutan seeds with SC-CO_2. Defatted rambutan flour had the protein, fat or ash content similar to that of wheat flour. Fat removal using the SC-CO_2 extraction resulted in the change of RVA pasting pattern to the continuous rise in viscosity throughout RVA cycle and the higher viscosity. When compared with those of wheat and rice flours, defatted rambutan seed flour exhibited relatively lower in viscosity, suitable for development in confectionery products. A non-toxic level of the defatted rambutan flour at 15000 mg/kg allows safe use for consumption. However, there is a need of information on physicochemical and functional properties supporting the virtue of rambutan seed flour that can be used for further applications.

Acknowledgements

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References

AACC. Approved Methods of the American Association of Cereal Chemists. 10th ed., American Association of Cereal Chemists, St. Paul, MN; 2000.

AOAC. Official method of analysis. 16th ed., Association of Official Analytical Che mists; Arlington, VA; 2000.

OECD. OECD Guidelines for Testing of Chemicals: Acute Oral Toxicology. Guideline No. 423; 2001.

Eiamwat J, Pengprecha P, Tiensong B, et al. Fatty acid composition and acute oral toxicity of rambutan (*Nephelium lappaceum*) seed fat and oil extracted with SC-CO_2. Proceedings of the 5th International Conference on Natural Products for Health and Beauty, 2014 May 6-8; Phuket, Thailand.

Ng KF, Abbas FMA, Tan TC, Azhar ME. Physi cochemical, pasting and gel textural properties of wheat-ripe Cavendish banana composite flours. *IFRJ* 2014; 21(2): 655-662.

Phanthanapratet W, Trangwacharakul S, Wanlapa S, et al. Application of rambutan seed flour for low calorie salad dressing product. *Agricultural Sci. J*. 2012; 43 Suppl 2: 517-520.

Reverchon E, Macro IDe. Supercritical fluid extraction and fractionation of natural matter. *J. of Supercitical Fluid* 2006; 38: 146-166.

Sirisompong W, Jirapakkul W, Klinkesorn U. Response surface optimization and characteristics of rambutan (*Nephelium lappaceum*) kernel fat by hexane extraction. *LWT – Food Science and Technology* 2011; 44: 1946-1951.

Solís-Fuentes JA, Camey-Ortíz G, Hernández-Medel MDR, Pérez-Mendoza F, Durán-de-Bazúa C. Composition, phase behavior and thermal stability of natural edible fat from rambutan (*Nephelium lappaceum*) seeds. *Bioresource Technology* 2010; 101: 799-803.

Taggart P. Starch as an ingredient: manufacture and implication. In: Elliason AC, editor. *Starch in Food.* England: CRC Press; 2004: 363-392.

Thai Agricultural Standard. Thai Hom Mali Rice. Guideline No.: TAS 4000-2003. Available from:http://www.acfs.go.th/standard/download/eng/Thai_Hom_Mali.pdf

Yanty NAM, Marikkar JMN, Long K, Ghazali HM. Physico-chemical characterization of the fat from red-skin rambutan (*Nephelium lappaceum*) seed. *J. Oleo. Sci*. 2013; 62(6): 335-343.

Yoswathana N. Optimization of supercritical carbon dioxide (SC-CO_2) extraction of rambutan (*Nephelium lappaceum*) seed oil using surface response methodology. *IJCEA* 2013; 4: 187-190.