รายงานวิจัยฉบับสมบูรณ์

โครงการ การศึกษาเปรียบเทียบกระบวนการทำแห้งเปลือกมังคุดเพื่ออนุรักษ์ปริมาณแซนโทน

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หัวหน้าโครงการวิจัยผู้รับทุน

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นักวิจัยที่ปรึกษา

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ABSTRACT

Rind of mangosteen is one of the best natural sources of xanthones, which have been reported to have high antioxidant activity. In general, mangosteen rind must be dried prior to extraction of the active compounds**.** However, information on the effects of different drying methods and conditions on the xanthones retention in mangosteen rind was still very limited. This work was therefore aimed to study the effects of selected drying methods and conditions on the changes of the contents as well as the antioxidant activity of xanthones in mangosteen rind. Mangosteen rind was subjected to hot air drying, vacuum drying or low-pressure superheated steam drying (LPSSD) at 60, 75 and 90°C and in the case of sub-atmospheric drying methods at an absolute pressure of 7 kPa. The xanthones contents were analyzed by HPLC while their antioxidant activity was assessed by DPPH radical scavenging capacity and ABTS assays. The results showed that the drying methods significantly affected degradation of xanthones (i.e., α-mangostin and 8-desoxygartanin) and their antioxidant activity. Either hot air drying or LPSSD at 75°C is proposed as appropriate drying technique and condition to preserve xanthones in mangosteen.

Keywords: 8-desoxygartanin; α-mangostin; Enzymatic degradation; Hot air drying; LPSSD; LC-MS; Thermal degradation; Polyphenol oxidase; Vacuum drying

บทคัดย่อ

สารแซนโทนเป็นกลุ่มสารที่มีฤทธิ์ต้านอนุมูลอิสระสูง โดยมีเปลือกมังคุดเป็นวัตถุดิบหลักในการผลิตสารแซนโทน ซึ่งในกระบวนการผลิตสารสำคัญต่างๆ จากเปลือกมังคุดจำเป็นต้องนำเปลือกมังคุดไปผ่านกระบวนการทำแห้ง อย่างไรก็ตามยังไม่มีงานวิจัยใดที่ให้ข้อมูลหรือศึกษาผลของกระบวนการ และสภาวะการอบแห้งที่มีต่อการเปลี่ยนแปลงปริมาณแซนโทนในเปลือกมังคุด ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของกระบวนการและสภาวะการอบแห้งที่มีต่อการเปลี่ยนแปลงปริมาณ และฤทธิ์ต้านอนุมูลอิสระของแซนโทนในเปลือกมังคุด โดยได้นำกระบวนการอบแห้งด้วยลมร้อน การอบแห้งด้วยสุญญากาศ และ การอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำมาใช้ในการอบแห้งเปลือกมังคุดบด ที่สภาวะ 60, 75 และ 90 องศาเซลเซียส และความดัน 7 กิโลปาสคาลสำหรับการอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ โดยใช้การวิเคราะห์ปริมาณแซนโทนด้วยวิธี HPLC และวิเคราะห์ฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging capacity และ ABTS (2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid)) โดยผลการทดลองพบว่ากระบวนการอบแห้งรูปแบบต่างๆ มีผลต่อการลดปริมาณแซนโทนซึ่งในงานวิจัยนี้ได้วิเคราะห์สารแซนโทนสองชนิด ได้แก่ α-mangostin and 8-desoxygartanin และกระบวนการอบแห้งยังมีผลต่อฤทธิ์ต้านอนุมูลอิสระ โดยกระบวนการอบแห้งแบบลมร้อนและไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำที่ใช้อุณหภูมิอบแห้ง 75 องศาเซลเซียส พบว่าเป็นกระบวนการและสภาวะที่ใช้ในอบแห้งที่เหมาะสมในการอนุรักษ์แซนโทนในเปลือกมังคุด

คำสำคัญ: การเสื่อมสลายจากเอนไซม์ / การเสื่อมสลายความร้อน / การอบแห้งแบบลมร้อน / การอบแห้งแบบสุญญากาศ / การอบแห้งแบบไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ / โพลีฟีนอลออกซิเดส / 8-desoxygartanin / α-mangostin / LC-MS

EXECUTIVE SUMMARY

ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ ประจำปี 2551

1. ความสำคัญและที่มาของปัญหา

นอกจากเป็นผลไม้ที่มีรสชาติดีเป็นที่นิยมแล้ว มังคุด (*Garcinia mangostana* Linn.) ยังถูกจัดเป็นยาสมุนไพร โดยเปลือกมังคุดเป็นส่วนสำคัญที่ถูกนำมาใช้เป็นยาสมุนไพร ผลิตภัณฑ์จากเปลือกมังคุด เช่น ผลิตภัณฑ์อาหารเสริม เครื่องดื่ม และเครื่องสำอาง กำลังได้รับความนิยมอย่างต่อเนื่องทั้งในประเทศและต่างประเทศ โดยมีงานวิจัย บทความ ข่าวสาร เกี่ยวกับผลิตภัณฑ์เปลือกมังคุดถูกตีพิมพ์เป็นจำนวนเพิ่มมากขึ้น ซึ่งกล่าวถึงสรรพคุณทางยาและประโยชน์ต่อสุขภาพในด้านต่างๆ

สารเคมีที่มีสรรพคุณทางยาที่สำคัญที่พบในเปลือกมังคุด คือ สารแทนนิน (tannin) ซึ่งเป็นสารที่มีรสฝาดมีฤทธิ์แก้อาการท้องเดิน และสารสำคัญอีกกลุ่มหนึ่งที่พบเป็นจำนวนมากเช่นกัน คือ สารแซนโธน (xanthones) ที่ประกอบด้วยสารหลัก คือ α-mangostin และยังมีสาร xanthones ตัวอื่นๆ จำนวนเล็กน้อย ได้แก่ β-mangostin, γ-mangostin, mangosharin, garcinones, gartanin, epicatechin และแซนโธนตัวอื่นๆ (Ji, Avula & Khan, 2007 และ Walker, 2007)

มีรายงานการวิจัยเป็นจำนวนมากรายงานเกี่ยวกับฤทธิ์ทางเภสัชวิทยาของ xanthones จากเปลือกผลมังคุดต่างๆ เช่น ฤทธิ์ในการต้านเชื้อแบคทีเรีย เช่น ต้านเชื้อ *Staphylococcus aureus* ได้ดี ซึ่งเป็นเชื้อที่เป็นสาเหตุสำคัญของเกิดฝีหนอง ต้านเชื้อรา ต้านเชื้อมาเลเรีย ยับยั้งการหลั่ง histamine และ serotonin ในการลดการอักเสบ มีฤทธิ์ต้านการอักเสบของข้อ มีสมบัติใช้ในการรักษาแผล ลดการเกิดแผลในทางเดินอาหาร มีฤทธิ์ในการทำให้นอนหลับสนิท และมีความสามารถในการจับกับอนุมูลอิสระและต้านออกซิเดชัน ต้านเซลล์มะเร็ง (Hay, Hélesbeux, Duval, Labaïed, Grellier & Richomme, 2004; Park et al., 2006a; Chen, Yang, & Wang, 2008; Fang, Ye, Chen, & Zhao, 2008)

จากสรรพคุณดังกล่าวจึงเริ่มมีการผลิตผลิตภัณฑ์จากเปลือกมังคุดมากขึ้น เช่น เครื่องดื่มเนื้อมังคุดผสมเปลือก ผลิตภัณฑ์อาหารเสริม และเครื่องสำอาง อย่างไรก็ดีมังคุดเป็นผลไม้ที่สามารถเก็บผลผลิตได้เพียงปีละครั้ง ดังนั้นจึงจำเป็นต้องมีกระบวนการแปรรูปเปลือกมังคุดเพื่อให้มีใช้นอกฤดู รวมถึงการผลิตเปลือกมังคุดเพื่อส่งออกเพื่อนำไปผลิตเป็นผลิตภัณฑ์ยาและอาหารเสริมต่างๆ การทำแห้งเป็นวิธีหนึ่งที่เหมาะสมสำหรับยืดอายุการเก็บเปลือกมังคุด เนื่องจากการทำแห้งสามารถลดปริมาตรและน้ำหนักในการขนส่ง และยังสะดวกต่อการนำไปผลิตผลิตภัณฑ์อื่นๆ สามารถนำไปผสมในผลิตภัณฑ์หรือสกัดสาร xanthones ออกมาได้ง่าย ซึ่งโดยทั่วไปเกษตรกรมักทำแห้งเปลือกมังคุดโดยการตากแดด หรือทำแห้งโดยใช้เครื่องอบแห้งแบบลมร้อน และไม่มีงานวิจัยใดที่ศึกษาการทำแห้งและจลนพลศาสตร์การทำแห้งเปลือกมังคุดอย่างจริงจัง รวมทั้งศึกษาทดลองทำแห้งเปลือกมังคุดโดยใช้การทำแห้งแบบอื่นๆ ที่มีศักยภาพและประสิทธิภาพในการทำแห้งและรักษาคุณค่าสาร

ออกฤทธิ์ทางชีวภาพต่างๆ ในเปลือกมังคุดได้มากกว่าการทำแห้งแบบลมร้อน ดังนั้นงานวิจัยนี้จึงได้ศึกษาการทำแห้งเปลือกมังคุดด้วยวิธีต่างๆ เพื่อคงคุณค่าของ xanthones และฤทธิ์ในการต้านอนุมูลอิสระของเปลือกมังคุด รวมถึงศึกษาอิทธิพลของปริมาณออกซิเจนที่เป็นตัวทำให้เกิดปฏิกิริยาออกซิเดชันในระหว่างการทำแห้งที่ทำให้เกิดการเสื่อมสลายของ xanthones

2. วัตถุประสงค์

2.1 เพื่อศึกษาผลของสภาวะการทำแห้งที่มีต่อจลนพลศาสตร์การทำแห้ง และปริมาณสาร xanthones ในเปลือกมังคุดที่ผ่านการทำแห้งโดยใช้การทำแห้งแบบต่างๆ

2.2 เพื่อศึกษาวิธีการต่างๆ ซึ่งนำไปสู่ความสามารถในการอนุรักษ์สาร xanthone และ α-mangostin รวมทั้งฤทธิ์ต้านอนุมูลอิสระในเปลือกมังคุดแห้งเมื่อควบคุมการถ่ายเทความชื้น

2.3 เพื่อศึกษาความคงตัวของ xanthones ในระหว่างการเก็บรักษาเปลือกมังคุดอบแห้ง

3. สรุปผลการทดลอง

งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของกระบวนการและสภาวะการอบแห้งที่มีต่อการเปลี่ยนแปลงปริมาณ และฤทธิ์ต้านอนุมูลอิสระของแซนโทนในเปลือกมังคุด โดยได้นำกระบวนการอบแห้งด้วยลมร้อน การอบแห้งด้วยสุญญากาศ และ การอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำมาใช้ในการอบแห้งเปลือกมังคุดบด ที่สภาวะ 60, 75 และ 90 องศาเซลเซียส และความดัน 7 กิโลปาสคาลสำหรับการอบแห้งด้วยไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ โดยใช้การวิเคราะห์ปริมาณแซนโทนด้วยวิธี HPLC และวิเคราะห์ฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging capacity และ ABTS (2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid)) โดยผลการทดลองพบว่ากระบวนการอบแห้งรูปแบบต่างๆ มีผลต่อการลดปริมาณแซนโทนซึ่งในงานวิจัยนี้ได้วิเคราะห์สารแซนโทนสองชนิด ได้แก่ α-mangostin and 8-desoxygartanin และกระบวนการอบแห้งยังมีผลต่อฤทธิ์ต้านอนุมูลอิสระ โดยกระบวนการอบแห้งแบบลมร้อนและไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำที่ใช้อุณหภูมิอบแห้ง 75 องศาเซลเซียส พบว่าเป็นกระบวนการและสภาวะที่ใช้ในอบแห้งที่เหมาะสมในการอนุรักษ์แซนโทนในเปลือกมังคุด

ต่อมาเป็นการศึกษาความคงตัวของแซนโทนในระหว่างการเก็บรักษาเปลือกมังคุดอบแห้ง โดยใช้สภาวะการบรรจุ 2 แบบ ได้แก่ การบรรจุที่สภาวะบรรยากาศและการบรรจุที่สภาวะสุญญากาศ และทดสอบอุณหภูมิในการเก็บรักษา 2 อุณหภูมิ ได้แก่ 45 และ 55 องศาเซลเซียส จากผลการทดลองพบว่าแซนโทนในเปลือกมังคุดมีความคงตัวในระหว่างการเก็บรักษาเป็นเวลา 30 วัน ที่ทุกสภาวะการเก็บรักษา ฤทธิ์ในการต้านอนุมูลอิสระที่วัดด้วยวิธี ABTS มีความคงตัวในระหว่าง

การเก็บรักษา แต่ในทางกลับกันฤทธิ์ต้านอนุมูลอิสระที่วัดด้วยวิธี DPPH มีค่าลดลงในระหว่างการเก็บรักษา ทั้งนี้เนื่องจากโมเลกุลของอนุมูลอิสระ DPPH เข้าทำปฏิกิริยากับสารต้านอนุมูลอิสระได้ยากกว่าอนุมูลอิสระ ABTS

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1. INTRODUCTION

Mangosteen (*Garcinia mangostana* Linn.) or “Mang Khut” in Thai is a tropical fruit and is known as the “Queen of fruits” in Asia. When mangosteen is ripe its rind becomes dark purple to red purple while the flesh is white, soft, juicy and sweet. Besides the edible flesh mangosteen rind has been used to prepare traditional medicines for treatment of diarrhea, skin infection, among other diseases, for years (Ji, Avula, & Khan, 2007; Zadernowski, Czaplicki, & Naczk, 2009).

Mangosteen rind is known as one of the best natural sources of xanthones, which are secondary plant metabolites. Xanthones belong to a class of polyphenolic compounds commonly found in higher plant families (Peres, Nagem, & Oliveira, 2000; Zadernowski et al., 2009). Xanthones and xanthone derivatives have been reported to have high antioxidant activity (Jung, Su, Keller, Mehta, & Kinghorn, 2006; Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007; Okonogi, Duangrat, Anuchpreeda, Tachakittirungrod, & Chowwanapoonpohn, 2007), anti-inflammatory activity (Chen, Yang, & Wang, 2008; Park et al., 2006a), antibacterial activity (Fang, Ye, Chen, & Zhao, 2008), anti-atherosclerotic activity (Park et al., 2006a) and anti-malarial activities (Hay, Hélesbeux, Duval, Labaïed, Grellier, & Richomme, 2004). Therefore, xanthones from mangosteen rind have been used to produce various dietary supplement products, fortified beverages as well as antiseptic goods, e.g., soap and plaster, in many countries.

Ji et al. (2007) and Walker (2007) reported the use of high performance liquid chromatography with photodiode array detector to detect and quantify xanthones in mangosteen rind. These investigators reported the existence of six xanthones, which are α-mangostin, β-mangostin, 9-hydroxycalabaxanthone, 3-isomangostin, gartanin, and 8-desoxygartanin, in mangosteen rind.

In general, mangosteen rind must be dried prior to extraction of active compounds or even storage to extend its shelf life. Although methods and conditions of drying are known to affect differently the quality and quantity of various bioactive compounds in fruits and vegetables, there was very little information on the evolution of xanthones during drying of mangosteen rind. Only the work of Carnat, Fraisse, Carnat, Felgines, Chaud, & Lamaison (2005) is available on the study of the influences of drying methods on the amount of xanthones in root of wild gentian (*Gentiana lutea* Linn.). The results showed that the amount of xanthones was independent of the drying methods (i.e., ambient air drying in shade and hot air drying at 40°C for 5 days). However, in that study, the ranges of the tested conditions were limited; the antioxidant activity of xanthones was also not investigated.

Many research studies have been devoted to polyphenols in fruits and vegetables and their benefits, especially their antioxidant activity. In general, polyphenolic content of fresh plant materials is higher than that of dried plant materials due to phenols degradation during drying. Decline in polyphenolic content after drying has been reported for plums (Caro, Piga, Pinna, Fenu, & Agabbio, 2004), persimmons (Park et al., 2006b), mulberry leaves (Katsube, Tsurunaga, Sugiyama, Furuno, & Yamasaki, 2009), apricots (Madrau et al., 2008), olive mill waste (Obied, Bedgood, Prenzler, & Robards, 2008) and ginger leaves (Chan et al., 2009). However, some recent studies have shown that dried plant materials contain higher polyphenolics as compared to fresh plant materials. For example, an increase in polyphenolic content after drying has been reported for tomatoes (Chang, Lin, Chang, & Liu, 2006) and shiitake mushroom (Choi, Lee, Chun, Lee, & Lee, 2006). Drying has also been reported to affect the antioxidant activity of fruits and vegetables differently (Choi et al., 2006; Park et al., 2006b; Chantaro, Devahastin, & Chiewchan, 2008; Kuljarachanan, Devahastin, & Chiewchan, 2009).

The objective of this study was to investigate the effects of selected drying methods, i.e., hot air drying, vacuum drying and low-pressure superheated steam drying (LPSSD), on the amounts and antioxidant activity of xanthones in mangosteen rind. This information is needed in order to maximize the quantity and quality of xanthones in dried mangosteen rind.

2. MATERIALS AND METHODS

2.1 Chemicals

Xanthone standards, α-mangostin and 8-desoxygartanin, were purchased from ChromaDex Inc. (Irvine, CA). Ethanol, methanol and deionized water (HPLC grade) were purchased from Lab-Scan Analytical Sciences (Bangkok, Thailand). For antioxidant activity analyses, 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St. Louis, MO). Potassium persulfate ($K_2S_2O_8$) was purchased from Carlo Erba (Milan, Italy). All these chemicals were of analytical grade.

2.2 Sample preparation

Mangosteen (*Garcinia mangostana* Linn.) at a mature stage with the rind color in dark purple to red purple was purchased from a local market. Mangosteen rind was separated from the fruit flesh and stored at -18°C until further use. To perform a drying experiment frozen mangosteen rind was thawed and then gathered by trimming outer and inner skin off. The pericarp was then chopped by a chopper (Moulinex, model DPA141, Ecully, France) to obtain a particle size of about 1 mm prior to drying.

2.3 Drying of mangosteen rind

Prepared mangosteen rind was dried at temperatures of 60, 75 and 90°C in a hot air dryer (Termarks, model TS8000, Bergen, Norway). Experiments were also conducted in a vacuum dryer and low-pressure superheated steam dryer used by Devahastin, Suvarnakuta, Soponronnarit, & Mujumdar (2004); the absolute pressure in the drying chamber was fixed at 7 kPa and the drying temperatures were also 60, 75 and 90°C. Mangosteen rind was dried until reaching the final moisture content of around 0.10 kg/kg (d.b.). The moisture content of mangosteen rind was evaluated by drying the sample at 105°C for 24 h in a hot air oven (Memmert, model 800, Schwabach, Germany). The dried sample was packed in sealed aluminum bags and kept at -18°C until further analysis.

2.4 Extraction of xanthones

Extraction of xanthones was performed according to the methods of Aberham, Schwaiger, Stuppner, & Ganzera (2007) and Ji et al. (2007) with some modifications. Mangosteen rind powder (0.2 g) was mixed with 3 mL of 95% (v/v) ethanol and extracted in an ultrasonic bath (Ultrawave, model U1350, Cardiff, UK), which generated the frequency of 30 kHz, for 10 min at room temperature. The mixture was then centrifuged (Hitachi, model himacCR21, Ibaraki, Japan) at 3000 rpm for 5 min. A supernatant was collected and transferred to a 10 mL volumetric flask. The extraction was repeated thrice; all extracted solutions were combined in one 10 mL volumetric flask. The ethanolic extract was then filled up to the final volume of 10 mL with 95% (v/v) ethanol and kept at -18°C until further use.

2.5 HPLC analysis

The HPLC analysis method, which was used to detect and quantify xanthones in the ethanolic extract, was that of Walker (2007). The HPLC system consists of a pump and a controller (Waters, model 600, Milford, MA), a tunable absorbance detector (Waters, model 486, Milford, MA). The mobile phases consisted of A: 0.1% formic acid in HPLC water and B: methanol. The mobile phases were applied in the following gradient elution: 35% A/65% B (v/v) to 10% A/90% B in 30 min at a flow rate 1 mL/min. Symmetry® C_{18} 5 μm (3.9 mm × 150 mm) (Waters, Milford, MA) was used for the analysis xanthones. The detection wavelength was set at 254 nm and the injection volume was 10 μL. Prior to injection the ethanolic extract was filtered through a 0.45 μm nylon membrane filter; the mobile phases were degassed by an ultrasonic bath, which generated the frequency of 30 kHz, for 15 min at room temperature.

A typical choromatogram of α-mangostin and 8-desoxygartanin is shown in Fig. 1. In addition, α-mangostin was the most abundant xanthone in the mangosteen rind, about 24-fold higher than 8-desoxygartanin and 40-fold higher than other xanthones. Therefore, only α-mangostin and 8-desoxygartanin were monitored in this study.

The evolution of xanthones is reported as the percentage of xanthones retention:

$$\%\ \text{Xanthones retention} = \frac{\text{Xanthones content of dried (or drying) rind (mg/g d.b.)}}{\text{Xanthones content of fresh rind (mg/g d.b.)}} \times 100 \qquad (1)$$

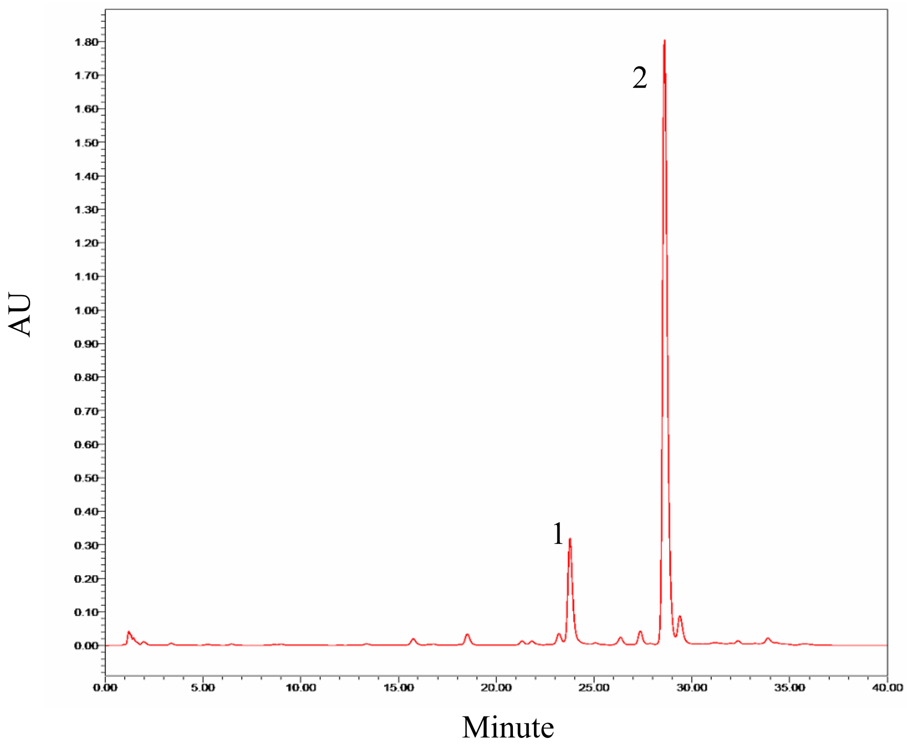


Fig. 1. Typical chromatograms of xanthones in mangosteen rind at a wavelength of 254 nm: (1) 8-desoxygartanin; (2) α-mangostin.

2.6 Antioxidant activity evaluation

2.6.1 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay

DPPH radical scavenging activity of the ethanolic extract was evaluated according to the method of Okonogi et al. (2007) with some modifications. 3.9 mL of 100 µM DPPH radical in ethanol was added to a test tube with 0.2 mL of the ethanolic extract. The mixture was mixed using a vortex mixer (Scientific Industries Inc., model G-560E, Bohemia, NY) for 10 sec and left to stand in dark at room temperature for 60 min. The absorbance was measured at 540 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific, model Genesys20, Waltham, MA). Pure ethanol was used to calibrate the spectrophotometer.

2.6.2 2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid) (ABTS) assay

The ABTS assay was performed according to the modified method of Huang, Ou, & Prior (2005). The $ABTS^{\cdot+}$ radical cation was prepared by mixing ABTS (7 mM final concentration) with potassium persulfate ($K_2S_2O_8$) (2.45 mM final concentration). The mixture was kept in dark at room temperature for 12-16 h to give a dark blue solution, which was stable for 2 days. This solution was diluted with pure ethanol until its absorbance reached 0.7 at 734 nm. One mL of the $ABTS^{\cdot+}$ radical cation solution was added to 0.02 mL of the ethanolic extract and mixed using a vortex mixer for 10 sec. The absorbance was then measured at 734 nm using a UV-visible spectrophotometer exactly 1 min after vortex mixing. Pure ethanol was again used to calibrate the spectrophotometer.

The antioxidant activity of the ethanolic extract, which was analyzed by 2 different methods (DPPH and ABTS assays), is expressed in terms of the relative inhibition (Hiranvarachat, Suvarnakuta, & Devahastin, 2008):

$$\text{Relative inhibition} = \frac{\text{Inhibition capacity of dried (or drying) mangosteen rind}}{\text{Inhibition capacity of fresh mangosteen rind}} \qquad (2)$$

Inhibition capacity of fresh and dried mangosteen rind was calculated from equation (3):

$$\text{Inhibition capacity} = \frac{\text{\% Inhibition}}{\text{Dry weight of sample kg/kg (d.b.)}} \qquad (3)$$

The percentage inhibition of the DPPH and ABTS radicals was calculated from the following equation (Okonogi et al., 2007):

$$\% \text{ Inhibition} = \frac{OD_{blank} - OD_{sample}}{OD_{blank}} \times 100 \qquad (4)$$

where OD_{blank} and OD_{sample} are the optical density of 95% ethanol and of the ethanolic extract, respectively.

2.7 Storage of Dried Mangosteen Rind

Mangosteen rind powder (2 g) was weighed and packed in aluminum bags (7.5 cm × 11.5 cm) which were either atmospherically packed (using Good Package Machinery, model GSV450-10D, Gyeongbuk, Korea) or vacuum packed (using Multivac, model A300/42, Wolfertschwenden, Germany). The samples were stored in two incubators, which maintained temperature at either 45 or 55°C for 30 days. Sampling was performed every 5 days to analyze the amount and antioxidant activity of xanthones in dried mangosteen rind during storage.

2.8 Statistical Analysis

All data were analyzed using the analysis of variance (ANOVA) and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests. Values were considered at a confidence level of 95%. Statistical program SPSS (version 15) was used to perform all statistical calculations.

3. RESULTS AND DISCUSSION

3.1 Drying kinetics of mangosteen rind

The initial moisture content of fresh mangosteen rind was approximately 1.80 kg/kg (d.b.) or 0.64 kg/kg (w.b.). The drying curves and temperature profiles of mangosteen rind undergoing hot air drying, vacuum drying and LPSSD at the temperatures of 60, 75 and 90°C are shown in Fig. 2. As expected, the drying rates increased with an increase in the drying temperature because drying at higher temperature provided larger driving force for heat transfer, which is obviously related to the rate of mass transfer. Moreover, the moisture diffusivity is also higher at higher temperature (Leeratanarak, Devahastin, & Chiewchan, 2006).

As can be seen in Fig. 2 hot air drying was the slowest drying process. This could be explained by the fact that the absolute pressure in the drying chamber in the cases of vacuum drying and LPSSD was only 7 kPa; boiling point of water at this pressure is about 40°C. Hence, vaporization of moisture occurred within the rind; moisture could move to the surface of the rind by vapor diffusion while only liquid diffusion was taking place in the case of hot air drying. Since vapor diffusion is faster than liquid diffusion, the drying rates of vacuum drying and LPSSD were higher than those of hot air drying. Although vacuum drying was a faster drying process than LPSSD, the differences between the drying time of LPSSD and vacuum drying were smaller at higher drying temperatures.

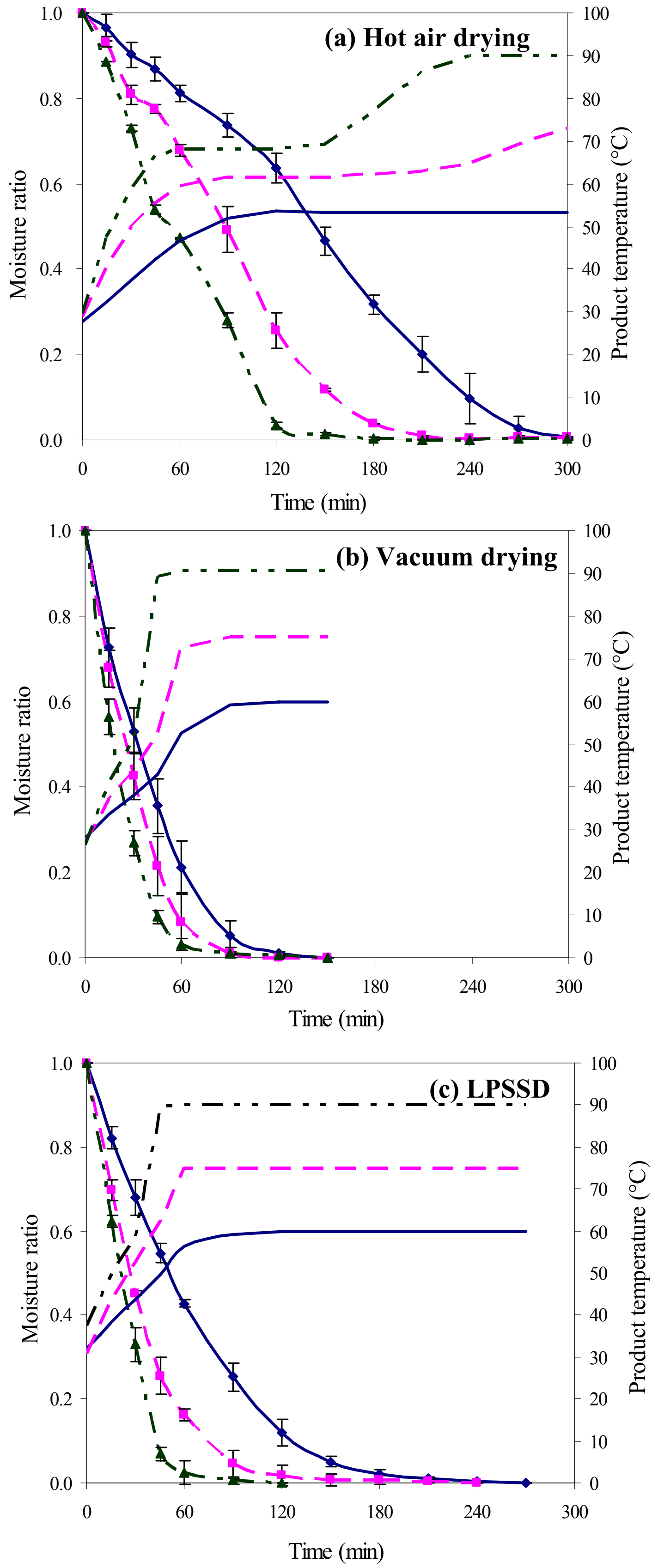


Fig. 2. Drying curves and temperature profiles of mangosteen rind undergoing (a) hot air drying; (b) vacuum drying; (c) LPSSD at 60 (▬), 75 (‒ ‒) and 90°C (..▬)

Because of the difference of the drying mechanism and drying rate, the product temperature profiles of hot air drying, vacuum drying and LPSSD were quite different. It was also observed from the temperatures profiles of mangosteen rind during various drying methods, the rind temperatures almost remained constant at wet-bulb temperature after the first 60 min in the case of hot-air drying while the temperature profiles in the case of vacuum dryings and LPSSD almost considerably increased from their initials to the medium temperatures and then remained constant within the first 60 min. However, the different temperature profiles between drying methods effect on the xanthones retention will be discuss later.

3.2 Changes of xanthones in mangosteen rind during drying

The contents of α-mangostin and 8-desoxygartanin in fresh mangosteen rind were approximately 47.82±3.76 mg/g (d.b.) and 1.43±0.30 mg/g (d.b.), respectively. The percentage of α-mangostin and 8-desoxygartanin retention in dried mangosteen rind, which had a moisture content of around 0.10 kg/kg (d.b.) is shown in Table 1. It is seen that the retention of α-mangostin and 8-desoxygartanin in mangosteen rind markedly decreased after all drying methods. The xanthones retention in mangosteen rind undergoing hot air drying and LPSSD at 75°C was, however, significant higher than that of the rind undergoing drying at other conditions.

Losses of xanthones after drying might be caused either by thermal or enzymatic degradation. It is indeed well recognized that thermal drying affects natural antioxidants in plant materials. Regarding enzymatic degradation, although thermal treatment could help inactivate degradative enzymes such as polyphenol oxidase (PPO), which is normally present in plant materials, some polyphenolics could still be degraded due to initial activity of the enzymes prior to their inactivation (Lim & Murtijaya, 2007; Obied et al., 2008). However, PPO is absolutely inhibited by heat treatment at over 85°C (Route-Meyer, Philippon & Nicolas, 1993)

Table 1. Effects of drying methods and conditions on xanthones retention

Drying method	**Drying temperature (°C)**	**% Xanthone retention***	
		α-mangostin	**8-desoxygartanin**
Hot air drying	60	67.7±1.7^{c}	66.8±1.0^{b}
	75	78.1±2.1^{a}	72.7±1.4^{a}
	90	71.9±0.1^{bc}	67.4±0.4^{b}
Vacuum drying	60	66.7±2.3^{c}	55.3±2.1^{c}
	75	73.2±1.1^{b}	66.1±1.5^{b}
	90	70.6±1.6^{bc}	67.9±0.1^{b}
LPSSD	60	62.6±1.4^{d}	57.6±2.1^{c}
	75	78.3±2.0^{a}	72.3±2.2^{a}
	90	69.9±1.6^{bc}	71.8±1.1^{a}

*The different superscripts in the same column indicate that the values are significantly different (p<0.05).

The effect of moisture content on retention of xanthones in mangosteen rind during hot air drying, vacuum drying and LPSSD is shown in Fig. 3-5, respectively.

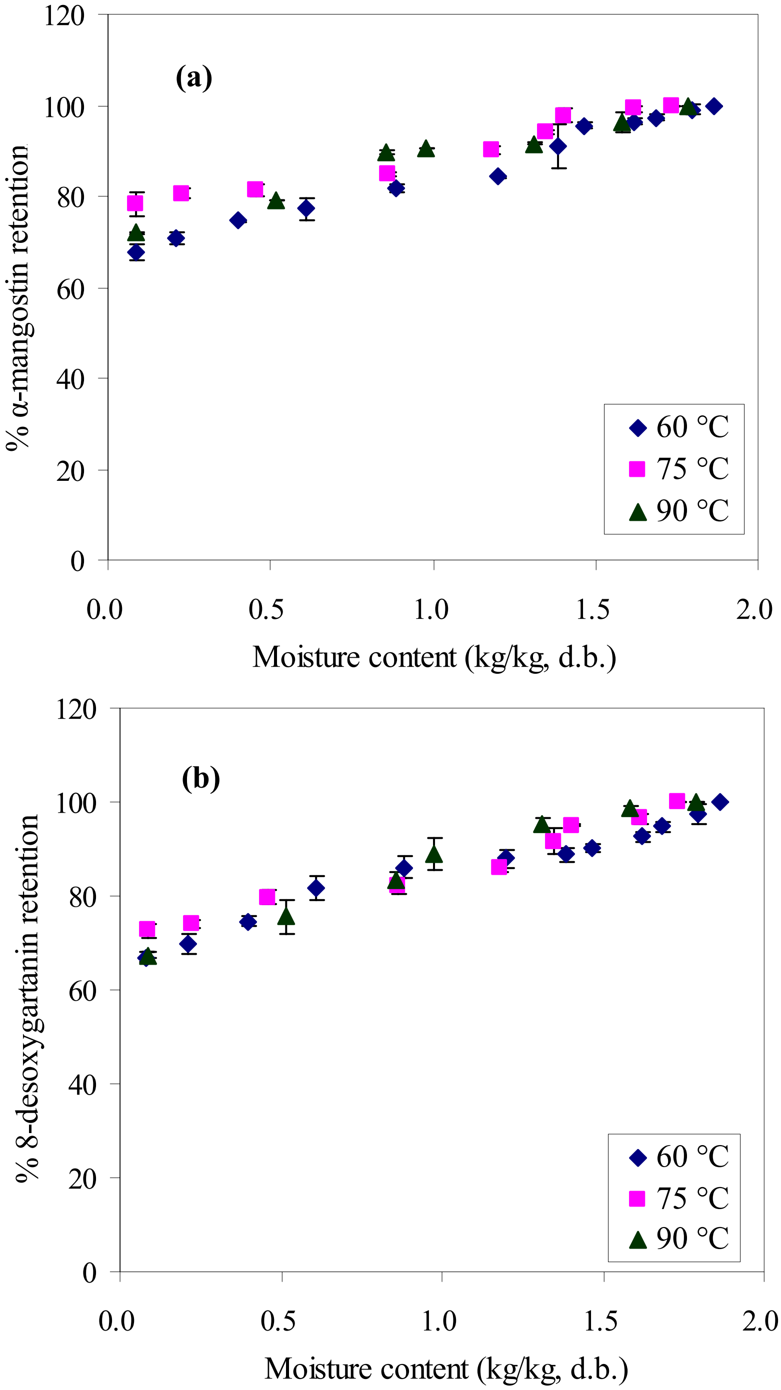


Fig. 3. Relationship between moisture content and xanthone retention in mangosteen rind during hot air drying. (a) %α-mangostin retention; (b) %8-desoxygartanin retention

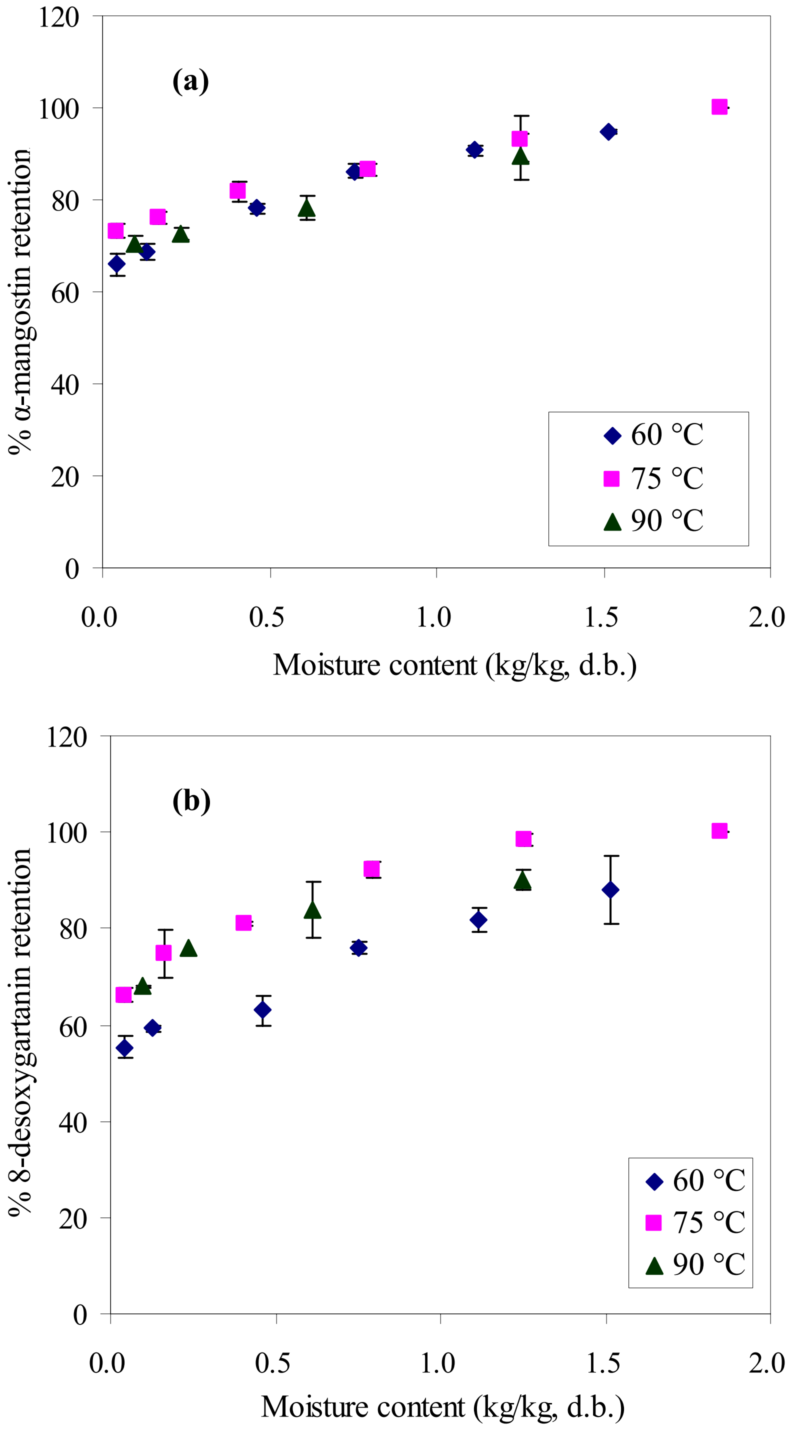


Fig. 4. Relationship between moisture content and xanthone retention in mangosteen rind during vacuum drying. (a) %α-mangostin retention; (b) %8-desoxygartanin retention

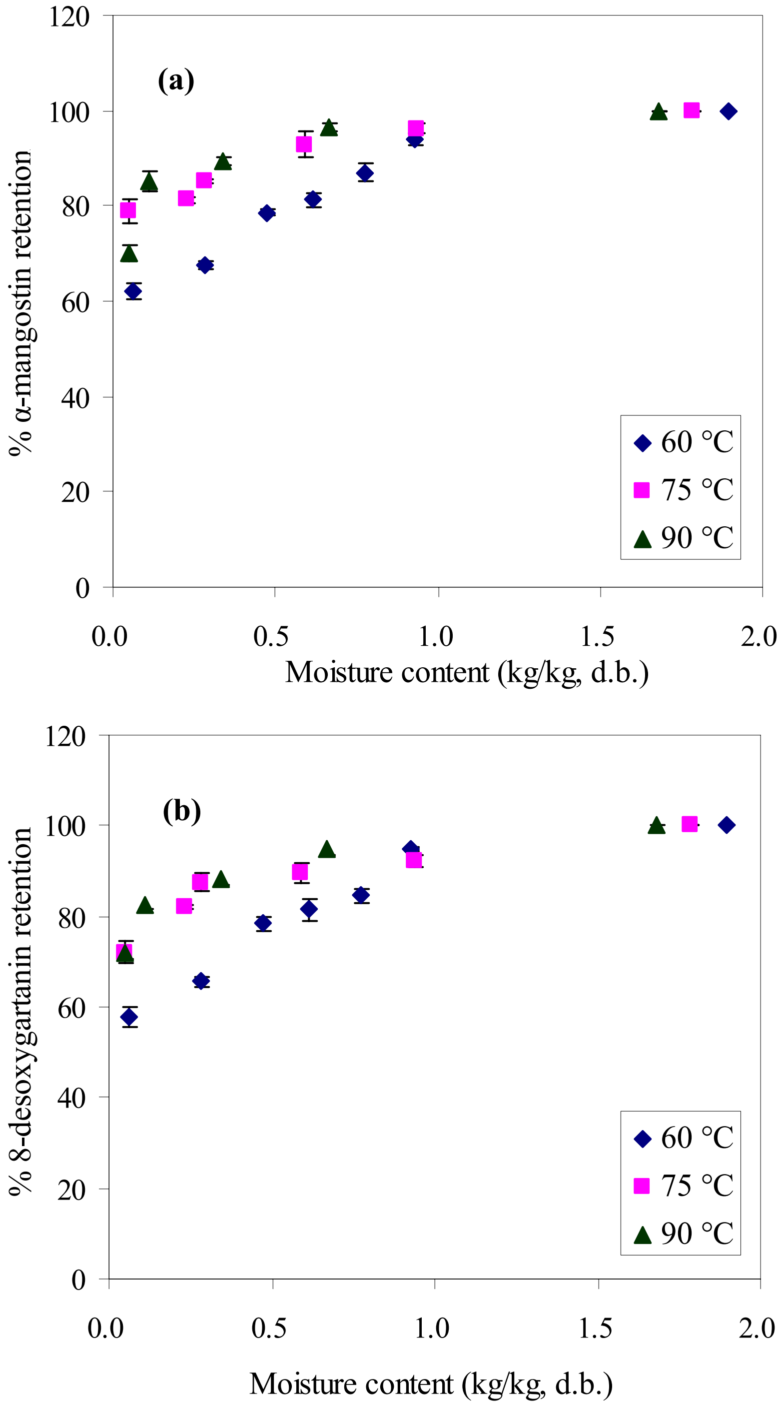


Fig. 5. Relationship between moisture content and xanthone retention in mangosteen rind during LPSSD (a) %α-mangostin retention; (b) %8-desoxygartanin retention

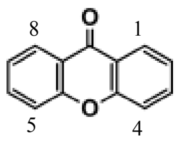
In the cases of hot air drying, the α-mangostin and 8-desoxygartanin contents decreased during drying since the product temperature was still lower than approximately 80°C (see Fig. 2). However, it took longer for the products undergoing vacuum drying and LPSSD to reach temperature of higher than 60°C compared with the case of hot air drying. Therefore, more enzymatic degradation might occur during early stages of vacuum drying and LPSSD than in the case of hot air drying, resulting in less retention of xanthones in the early stages in the cases of vacuum drying and LPSSD. In the later stages, it can be seen clearly that the thermal degradation took place when product temperatures increase instantly in the case of both of vacuum drying and LPSSD. However, comparison of final xanthones retention between vacuum drying and LPSSD revealed that the xanthone retentions differed significantly (see Table 1). This is because the product temperature of all vacuum drying cases was higher than LPSSD cases, thus thermal degradation has high effect in the case of vacuum drying. In addition, the LPSSD chamber was fully contained with superheated steam; therefore, the oxygen content remaining in the LPSSD chamber was less (none indeed) than in the case of vacuum drying chamber resulting in enzymatic degradation of xanthones.

In the case of hot air drying the α-mangostin and 8-desoxygartanin contents linearly decreased during drying. This linearity decrease was probably due to enzymatic degradation. Since the temperature of the rind reached approximately 55°C within 120 min (see Fig. 2), enzymes responsible for xanthones degradation were most activated (Vámos-Vigyázó, 1981). Therefore, beyond this initial period, especially in the case of drying at higher temperatures (i.e., 75 and 90°C), enzymatic degradation was most probably stopped. However, xanthones retention still decreased continuously after 45 min. This could be due to thermal degradation (Lim et al., 2007; Chantaro et al., 2008). In the case of drying at 60°C, on the other hand, the rind temperature was only around 50°C even during the later stage of drying. This lower rind temperature might not be enough to fully inactivate PPO; enzymatic degradation was therefore still in effect and this led to higher losses of xanthones during lower-temperature drying. Therefore, hot air drying at 60°C led to lower the xanthones retention than that at 75 and 90°C. In contrast, as mentioned earlier, thermal degradation could be mostly responsible for the losses of xanthones during drying at 75 and 90°C (Lim et al., 2007; Obied et al., 2008).

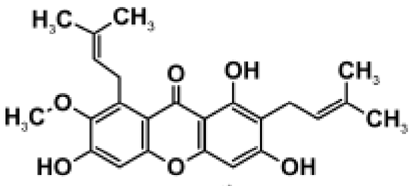
3.3 Changes of antioxidant activity of mangosteen rind during drying

IC_{50} values of the xanthone standards, i.e., α-mangostin and 8-desoxygartanin, as determined by the DPPH assay, were 25.6±2.6 and 4.2±0.3 μg/mL, respectively, while the values as determined by ABTS assay were 0.47±0.05 and 0.15±0.01 μg/mL, respectively. This shows that 8-desoxygartanin exhibits a higher antioxidant activity than α-mangostin. This is because 8-desoxygartanin has hydroxyl group at position C-5, which contributes to higher scavenging activity on DPPH and ABTS radicals than the group at position C-6 in the case of α-mangostin (see Fig. 6). It is noted that hydroxyl groups at positions C-6 and C-3 in both xanthone derivatives have less activity because of the steric resistance effect.

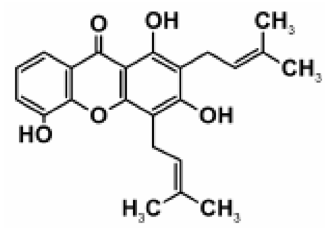
The antioxidant activity of xanthones as assessed by the ABTS assay was higher than that assessed by the DPPH assay. This observed phenomenon could be due to steric accessibility of the DPPH radicals. In addition, the active sites of DPPH radicals are in the middle of the structure as a result antioxidants hardly access to the DPPH radical sites. (Prior et al., 2005).



xanthone



α-mangostin



8-desoxygartanin

Fig. 6. Skeleton structures of xanthone and xanthone derivatives.

The total antioxidant activity of fresh mangosteen rind, which was assessed by DPPH and ABTS assays, was approximately 78.26±5.96% and 70.41±5.69%, respectively. The relative inhibitions of xanthones in mangosteen rind dried at various conditions are shown in Tables 2 and 3. The total antioxidant activity of the rind undergoing hot air drying, vacuum drying and LPSSD decreased compared with that of the fresh mangosteen rind. The total antioxidant activity of mangosteen rind during hot air drying, vacuum drying and LPSSD decreased following the patterns of the degradation kinetics of xanthones.

From Tables 2 and 3, it was found that both antioxidant assays gave similar antioxidant activity trends. This indicated that xanthones in mangosteen rind played an important role in the antioxidant activity of the rind. However, xanthones may not be the only potent antioxidants in mangosteen rind because there might also be some other phenolic compounds in the rind, which were not identified in this study.

Table 2

Antioxidant activity of xanthones in mangosteen rind during drying in terms of relative inhibition by DPPH radical scavenging capacity assay

Drying time (min)	**Relative inhibition***								
	Hot air drying			**Vacuum drying**			**LPSSD**		
	60°C	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**
15	0.99±0.01	0.97±0.05	1.00±0.01	0.93±0.02	0.97±0.07	0.80±0.01	0.93±0.01	0.92±0.01	0.89±0.02
30	0.98±0.04	0.98±0.01	0.99±0.02	0.82±0.04	0.83±0.02	0.61±0.02	0.81±0.04	0.80±0.01	0.67±0.01
45	0.98±0.03	0.99±0.06	0.95±0.03	0.69±0.01	0.66±0.01	0.47±0.02	0.76±0.04	0.64±0.01	0.56±0.01
60	0.91±0.01	0.99±0.02	0.91±0.06	0.57±0.04	0.56±0.03	0.42±0.02^{b}	0.65±0.02	0.61±0.01	0.51±0.01^{ab}
90	0.88±0.05	0.92±0.01	0.72±0.08	0.46±0.01	0.49±0.03^{ab}		0.53±0.01	0.54±0.02^{a}	
120	0.86±0.01	0.74±0.01	0.50±0.05^{ab}	0.42±0.01^{b}			0.47±0.04^{ab}		
150	0.75±0.00	0.61±0.01							
180	0.66±0.01	0.55±0.01^{a}							
210	0.55±0.03								
240	0.49±0.02								
270	0.44±0.02^{b}								

*Mean ± SD of two replicates. The different superscripts mean that the values are significantly different (p<0.05) at same final moisture contents.

Table 3

Antioxidant activity of xanthones in mangosteen rind during drying in terms of relative inhibition by ABTS assay

Drying time (min)	**Relative inhibition***								
	Hot air drying			**Vacuum drying**			**LPSSD**		
	60°C	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**
15	0.99±0.01	0.97±0.01	1.02±0.02	0.93±0.01	0.88±0.02	0.84±0.03	0.95±0.02	0.93±0.01	0.97±0.00
30	0.96±0.06	0.94±0.00	0.99±0.01	0.81±0.07	0.78±0.04	0.69±0.02	0.81±0.01	0.88±0.02	0.72±0.01
45	0.98±0.04	0.96±0.02	0.88±0.01	0.66±0.02	0.60±0.01	0.51±0.00	0.78±0.03	0.74±0.02	0.58±0.01
60	0.94±0.06	0.94±0.01	0.87±0.00	0.60±0.09	0.51±0.03	0.45±0.02^{bc}	0.65±0.01	0.65±0.02	0.54±0.01^{ab}
90	0.87±0.01	0.84±0.01	0.74±0.01	0.49±0.04	0.44±0.01^{bc}		0.56±0.01	0.60±0.02^{a}	
120	0.89±0.05	0.68±0.02	0.47±0.00^{bc}	0.47±0.02^{bc}			0.50±0.02^{b}		
150	0.79±0.03	0.58±0.02							
180	0.68±0.01	0.54±0.01^{ab}							
210	0.54±0.02								
240	0.47±0.04								
270	0.42±0.00^{c}								

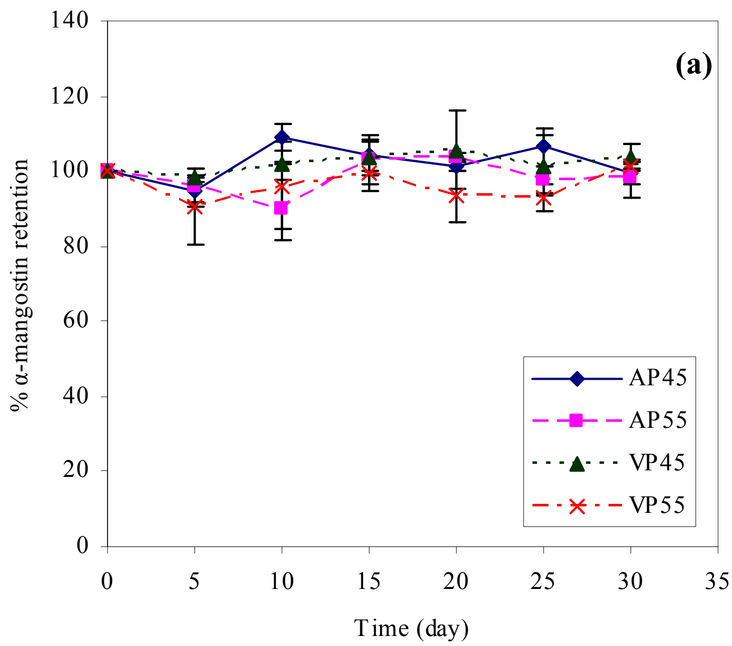
*Mean ± SD of two replicates. The different superscripts mean that the values are significantly different (p<0.05) at same final moisture contents.

3.3 Storage Stability of Xanthones in Dried Mangosteen Rind

Based on the results of xanthones retention in mangosteen rind during drying, the best treatment to preserve xanthones and their antioxidant activity is drying at 75°C. Hence, to study the storage stability of xanthones in dried mangosteen rind, mangosteen rind was dried either by hot air drying or vacuum drying at 75°C. The xanthones content and the antioxidant activity of the rind during storage at various conditions were then investigated. The moisture content of dried mangosteen rind before storage was approximately 0.09 kg/kg (d.b.) and its water activity was approximately 0.41 ± 0.02.

The changes of the percentage of xanthones retention in hot air dried and vacuum dried mangosteen rind are shown in Figs 7 and 8. It was found that the α-mangostin and 8-desoxygartanin contents in hot air dried and vacuum dried mangosteen rind did not significantly change during storage at all conditions. This might be because degradative enzymes were inhibited during drying, so enzymatic degradation did not occur during storage. Although the storage conditions were accelerated conditions, which were 45 and 55°C, these conditions still did not affect the degradation of xanthones. Also, thermal degradation might not occur during storage at 45 and 55°C due to low temperatures. For the results of the different packing conditions, xanthones retention in the rind packed in both vacuum package and atmospheric package seemed to be stable. Atmospheric package should thus be sufficient for storage of dried mangosteen rind.

As can be seen in Figs 9 and 10, the antioxidant activity, when assessed by the ABTS assay, of both hot air dried and vacuum dried mangosteen rind did not significantly change during storage, whereas the activity decreased when assessed by the DPPH assay. Some fluctuations in the antioxidant activity results were also observed, as was also noted by Patthamakanokporn et al. (2009). Therefore, the antioxidant activity of mangosteen rind during storage as assessed by the ABTS assay might be higher than that assessed by the DPPH assay due to steric inaccessibility of the DPPH radical (Prior et al., 2005).



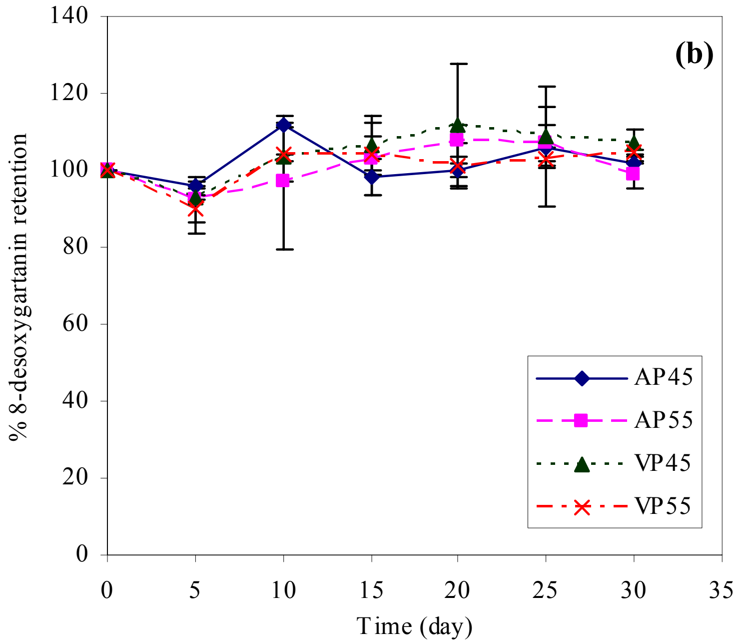


Fig. 7. Percentage of xanthone retention in hot air dried mangosteen rind during storage in atmospheric-packed package at 45°C (AP45), atmospheric-packed package at 55°C (AP55), vacuum-packed package at 45°C (VP45) and vacuum-packed package at 55°C (VP55).

(a) % α-mangostin retention; (b) % 8-desoxygartanin retention

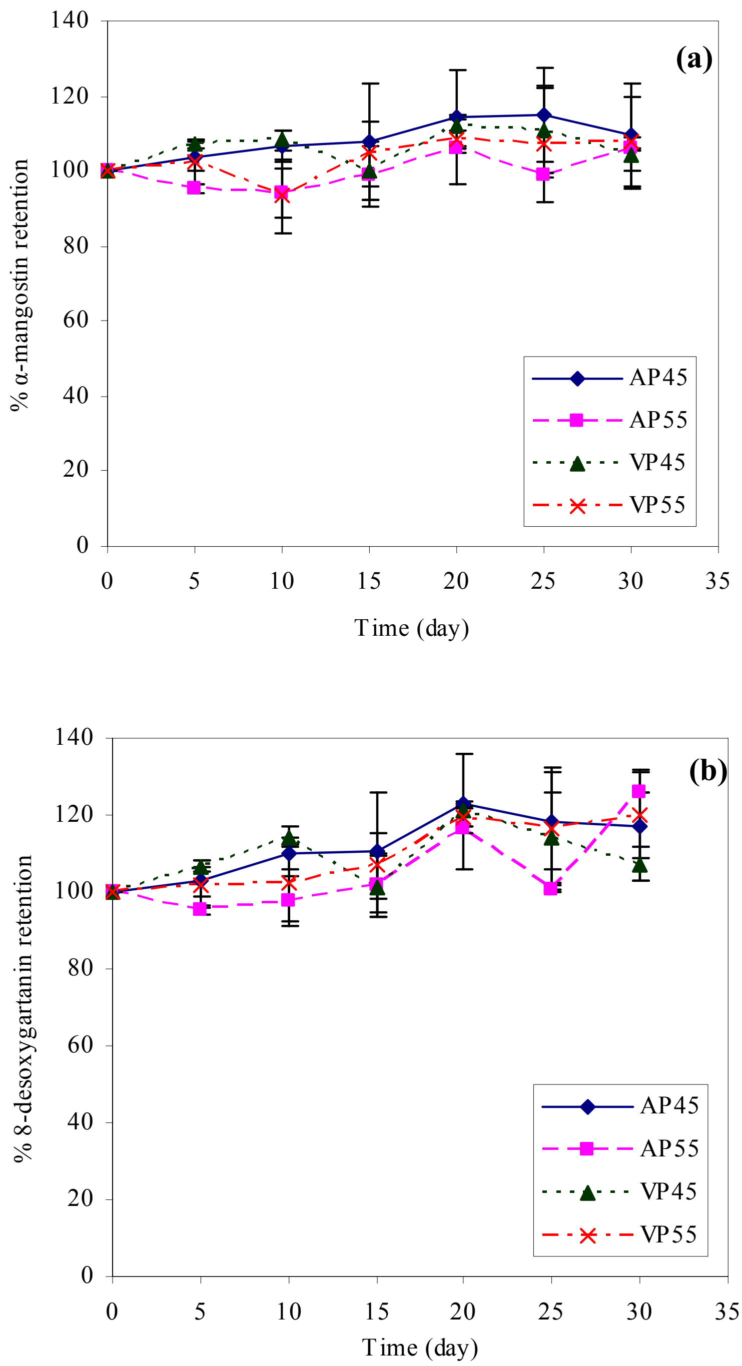


Fig. 8. Percentage of xanthone retention in vacuum dried mangosteen rind during storage in atmospheric-packed package at 45°C (AP45), atmospheric-packed package at 55°C (AP55), vacuum-packed package at 45°C (VP45) and vacuum-packed package at 55°C (VP55).

(a) % α-mangostin retention; (b) % 8-desoxygartanin retention

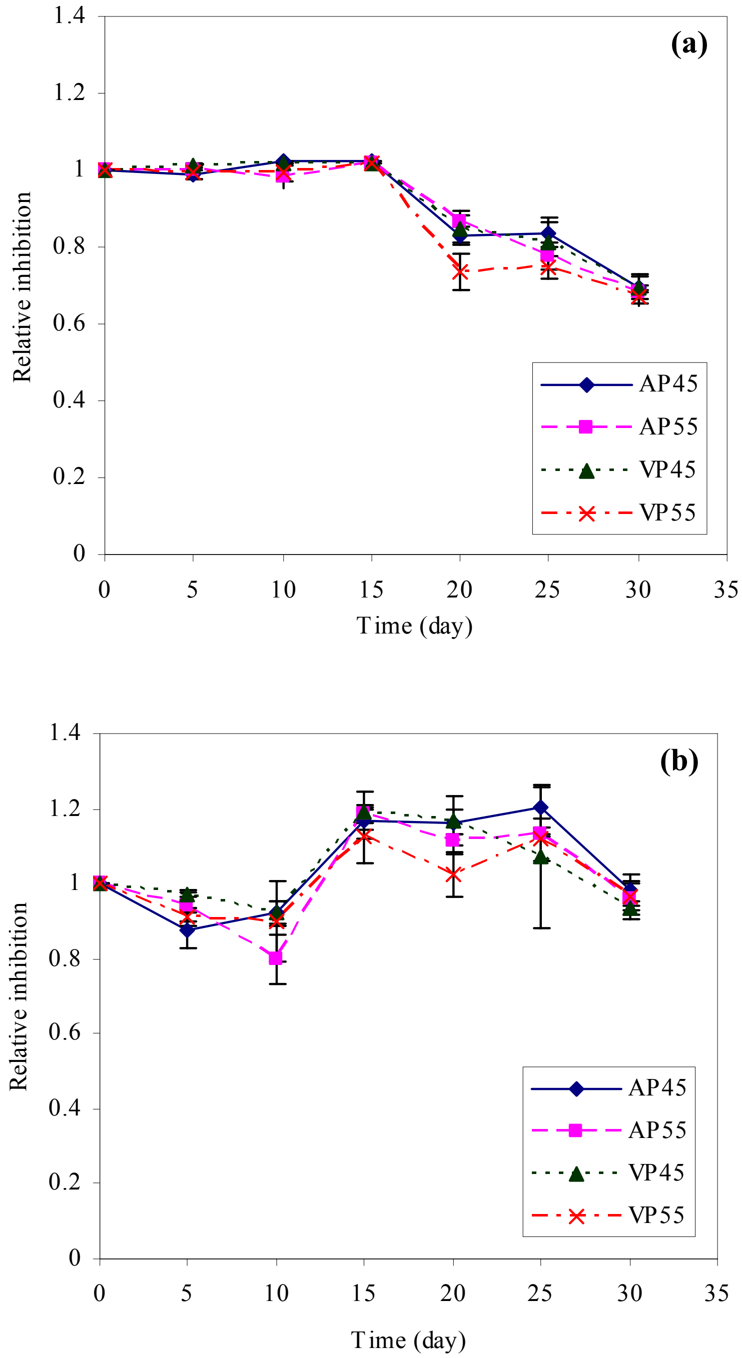


Fig. 9. Total antioxidant activity of hot air dried mangosteen rind during storage in atmospheric-packed package at 45°C (AP45), atmospheric-packed package at 55°C (AP55), vacuum-packed package at 45°C (VP45) and vacuum-packed package at 55°C (VP55). (a) DPPH radical scavenging capacity assay; (b) ABTS assay

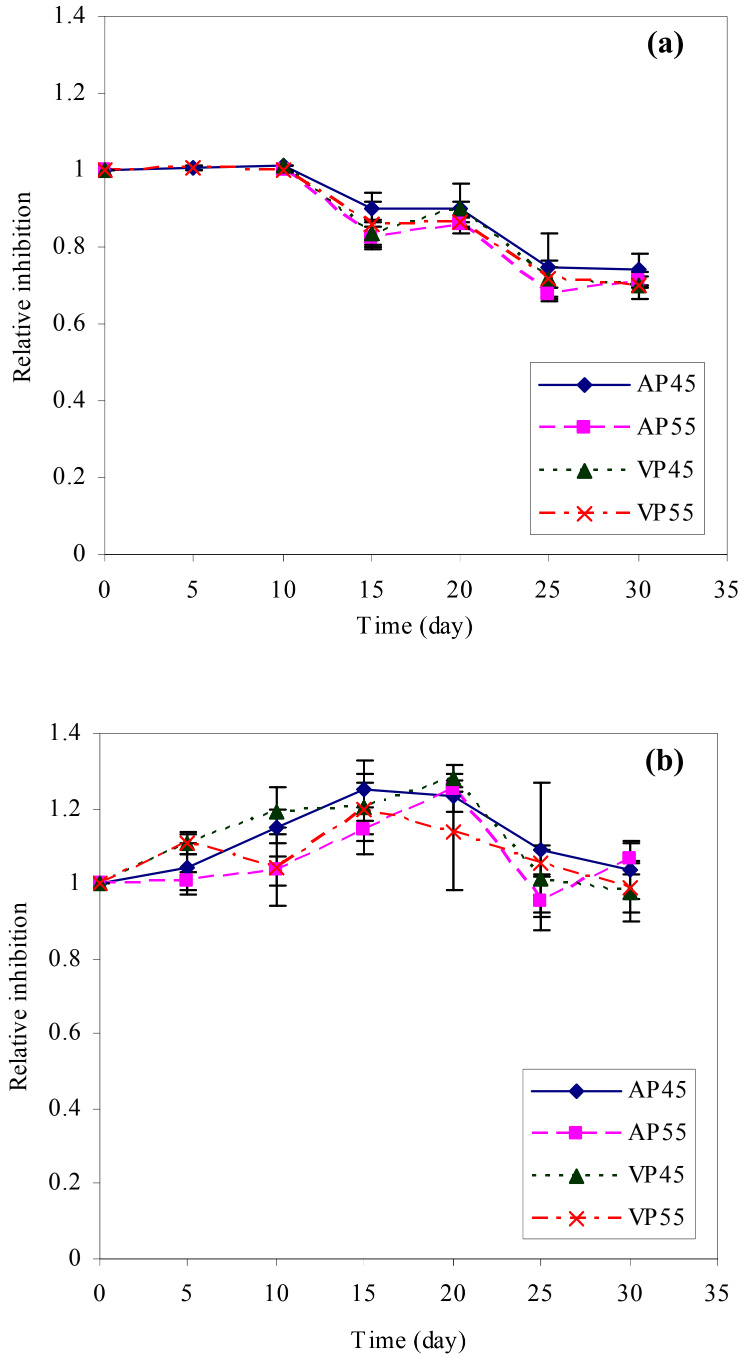


Fig. 10. Total antioxidant activity of vacuum dried mangosteen rind during storage in atmospheric-packed package at 45°C (AP45), atmospheric-packed package at 55°C (AP55), vacuum-packed package at 45°C (VP45) and vacuum-packed package at 55°C (VP55). (a) DPPH radical scavenging capacity assay; (b) ABTS assay

4. CONCLUSION

The effects of drying methods and conditions on the xanthones contents in mangosteen rind undergoing hot air drying, vacuum drying and LPSSD were investigated in this study. The results showed that the contents of xanthones in mangosteen rind significantly decreased during all drying methods due either to enzymatic degradation or thermal degradation. Because of the similar trends of the xanthones content and antioxidant activities, all drying methods was also found to reduce the antioxidant activity of mangosteen rind due to the losses of xanthones during drying. Hot air drying and Low-pressure superheated steam dryings at operating temperature of 75°C is the two best conditions to maximize the quantity and quality of xanthones in mangosteen rind because it provides the proper drying time and temperature to help inactivate PPO and also minimize thermal degradation.

The stability of xanthones in dried mangosteen rind was evaluated during storage at various conditions for 30 days. It was found that the α-mangostin and 8-desoxygartanin content and the antioxidant activity of both hot air dried and vacuum dried mangosteen rind, as assessed by the ABTS assay, did not change during storage. On the other hand the antioxidant activity of the rind, when assessed by the DPPH assay, decreased during storage. This might be due to steric inaccessibility of the DPPH radicals. Since storage temperature and packing conditions did not significantly affect the xanthones contents, atmospheric packing and room-temperature storage are sufficient for storing dried mangosteen rind.

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OUTPUT

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Effects of Drying Methods on Retention and Antioxidant Activity of Xanthones in Mangosteen Rind

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Abstract

Rind of mangosteen is one of the best natural sources of xanthones, which have been reported to have high antioxidant activity. In general, mangosteen rind must be dried prior to extraction of the active compounds. However, information on the effects of different drying methods and conditions on the xanthones retention in mangosteen rind was still very limited. This work was therefore aimed to study the effects of selected drying methods and conditions on the changes of the contents as well as the antioxidant activity of xanthones in mangosteen rind. Mangosteen rind was subjected to hot air drying, vacuum drying or low-pressure superheated steam drying (LPSSD) at 60, 75 and 90°C and in the case of sub-atmospheric drying methods at an absolute pressure of 7 kPa. The xanthones contents were analyzed by HPLC while their antioxidant activity was assessed by DPPH radical scavenging capacity and ABTS assays. The results showed that the drying methods significantly affected degradation of xanthones (i.e., α-mangostin and 8-desoxygartanin) and their antioxidant activity. Either hot air drying or LPSSD at 75°C is proposed as appropriate drying technique and condition to preserve xanthones in mangosteen.

Keywords: 8-desoxygartanin; α-mangostin; Enzymatic degradation; Hot air drying; LPSSD; LC-MS; Thermal degradation; Polyphenol oxidase; Vacuum drying

1. Introduction

Mangosteen (*Garcinia mangostana* Linn.) or “Mang Khut” in Thai is a tropical fruit and is known as the “Queen of fruits” in Asia. When mangosteen is ripe its rind becomes dark purple to red purple while the flesh is white, soft, juicy and sweet. Besides the edible flesh mangosteen rind has been used to prepare traditional medicines for treatment of diarrhea, skin infection, among other diseases, for years (Ji, Avula, & Khan, 2007; Zadernowski, Czaplicki, & Naczk, 2009).

Mangosteen rind is known as one of the best natural sources of xanthones, which are secondary plant metabolites. Xanthones belong to a class of polyphenolic compounds commonly found in higher plant families (Peres, Nagem, & Oliveira, 2000; Zadernowski et al., 2009). Xanthones and xanthone derivatives have been reported to have high antioxidant activity (Jung, Su, Keller, Mehta, & Kinghorn, 2006; Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007; Okonogi, Duangrat, Anuchpreeda, Tachakittirungrod, & Chowwanapoonpohn, 2007), anti-inflammatory activity (Chen, Yang, & Wang, 2008; Park et al., 2006a), antibacterial activity (Fang, Ye, Chen, & Zhao, 2008), anti-atherosclerotic activity (Park et al., 2006a) and anti-malarial activities (Hay, Hélesbeux, Duval, Labaïed, Grellier, & Richomme, 2004). Therefore, xanthones from mangosteen rind have been used to produce various dietary supplement products, fortified beverages as well as antiseptic goods, e.g., soap and plaster, in many countries.

Ji et al. (2007) and Walker (2007) reported the use of high performance liquid chromatography with photodiode array detector to detect and quantify xanthones in mangosteen rind. These investigators reported the existence of six xanthones, which are α-mangostin, β-mangostin, 9-hydroxycalabaxanthone, 3-isomangostin, gartanin, and 8-desoxygartanin, in mangosteen rind.

In general, mangosteen rind must be dried prior to extraction of active compounds or even storage to extend its shelf life. Although methods and conditions of drying are known to affect differently the quality and quantity of various bioactive compounds in fruits and vegetables, there was very little information on the evolution of xanthones during drying of mangosteen rind. Only the work of Carnat, Fraisse, Carnat, Felgines, Chaud, & Lamaison (2005) is available on the study of the influences of drying methods on the amount of xanthones in root of wild gentian (*Gentiana lutea* Linn.). The results showed that the amount of xanthones was independent of the drying methods (i.e., ambient air drying in shade and hot air drying at 40°C for 5 days). However, in that study, the ranges of the tested conditions were limited; the antioxidant activity of xanthones was also not investigated.

Many research studies have been devoted to polyphenols in fruits and vegetables and their benefits, especially their antioxidant activity. In general, polyphenolic content of fresh plant materials is higher than that of dried plant materials due to phenols degradation during drying. Decline in polyphenolic content after drying has been reported for plums (Caro, Piga, Pinna, Fenu, & Agabbio, 2004), persimmons (Park et al., 2006b), mulberry leaves (Katsube, Tsurunaga, Sugiyama, Furuno, & Yamasaki, 2009), apricots (Madrau et al., 2008), olive mill waste (Obied, Bedgood, Prenzler, & Robards, 2008) and ginger leaves (Chan et al., 2009). However, some recent studies have shown that dried plant materials contain higher polyphenolics as compared to fresh plant materials. For example, an increase in polyphenolic content after drying has been reported for tomatoes (Chang, Lin, Chang, & Liu, 2006) and shiitake mushroom (Choi, Lee, Chun, Lee, & Lee, 2006). Drying has also been reported to affect the antioxidant activity of fruits and vegetables differently (Choi et al., 2006; Park et al., 2006b; Chantaro, Devahastin, & Chiewchan, 2008; Kuljarachanan, Devahastin, & Chiewchan, 2009).

The objective of this study was to investigate the effects of selected drying methods, i.e., hot air drying, vacuum drying and low-pressure superheated steam drying (LPSSD), on the amounts and antioxidant activity of xanthones in mangosteen rind. This information is needed in order to maximize the quantity and quality of xanthones in dried mangosteen rind.

2. Materials and methods

2.1 Chemicals

Xanthone standards, α-mangostin and 8-desoxygartanin, were purchased from ChromaDex Inc. (Irvine, CA). Ethanol, methanol and deionized water (HPLC grade) were purchased from Lab-Scan Analytical Sciences (Bangkok, Thailand). For antioxidant activity analyses, 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St. Louis, MO). Potassium persulfate ($K_2S_2O_8$) was purchased from Carlo Erba (Milan, Italy). All these chemicals were of analytical grade.

2.2 Sample preparation

Mangosteen (*Garcinia mangostana* Linn.) at a mature stage with the rind color in dark purple to red purple was purchased from a local market. Mangosteen rind was separated from the fruit flesh and stored at -18°C until further use. To perform a drying experiment frozen mangosteen rind was thawed and then gathered by trimming outer and inner skin off. The pericarp was then chopped by a chopper (Moulinex, model DPA141, Ecully, France) to obtain a particle size of about 1 mm prior to drying.

2.3 Drying of mangosteen rind

Prepared mangosteen rind was dried at temperatures of 60, 75 and 90°C in a hot air dryer (Termarks, model TS8000, Bergen, Norway). Experiments were also conducted in a vacuum dryer and low-pressure superheated steam dryer used by Devahastin, Suvarnakuta, Soponronnarit, & Mujumdar (2004); the absolute pressure in the drying chamber was fixed at 7 kPa and the drying temperatures were also 60, 75 and 90°C. Mangosteen rind was dried until reaching the final moisture content of around 0.10 kg/kg (d.b.). The moisture content of mangosteen rind was evaluated by drying the sample at 105°C for 24 h in a hot air oven (Memmert, model 800, Schwabach, Germany). The dried sample was packed in sealed aluminum bags and kept at -18°C until further analysis.

2.4 Extraction of xanthones

Extraction of xanthones was performed according to the methods of Aberham, Schwaiger, Stuppner, & Ganzera (2007) and Ji et al. (2007) with some modifications. Mangosteen rind powder (0.2 g) was mixed with 3 mL of 95% (v/v) ethanol and extracted in an ultrasonic bath (Ultrawave, model U1350, Cardiff, UK), which generated the frequency of 30 kHz, for 10 min at room temperature. The mixture was then centrifuged (Hitachi, model himacCR21, Ibaraki, Japan) at 3000 rpm for 5 min. A supernatant was collected and transferred to a 10 mL volumetric flask. The extraction was repeated thrice; all extracted solutions were combined in one 10 mL volumetric flask. The ethanolic extract was then filled up to the final volume of 10 mL with 95% (v/v) ethanol and kept at -18°C until further use.

2.5 HPLC analysis

The HPLC analysis method, which was used to detect and quantify xanthones in the ethanolic extract, was that of Walker (2007). The HPLC system consists of a pump and a controller (Waters, model 600, Milford, MA), a tunable absorbance detector (Waters, model 486, Milford, MA). The mobile phases consisted of A: 0.1% formic acid in HPLC water and B: methanol. The mobile phases were applied in the following gradient elution: 35% A/65% B (v/v) to 10% A/90% B in 30 min at a flow rate 1 mL/min. Symmetry® C_{18} 5 μm (3.9 mm × 150 mm) (Waters, Milford, MA) was used for the analysis xanthones. The detection wavelength was set at 254 nm and the injection volume was 10 μL. Prior to injection the ethanolic extract was filtered through a 0.45 μm nylon membrane filter; the mobile phases were degassed by an ultrasonic bath, which generated the frequency of 30 kHz, for 15 min at room temperature.

From our preliminary experiments it was found from liquid chromatography-mass spectrometry (LC-MS) that the ethanolic extract of mangostin rind consisted of only α-mangostin and 8-desoxygartanin (see Fig. 1). In addition, α-mangostin was the most abundant xanthone in the mangosteen rind, about 24-fold higher than 8-desoxygartanin and 40-fold higher than other xanthones. Therefore, only α-mangostin and 8-desoxygartanin were monitored in this study.

The evolution of xanthones is reported as the percentage of xanthones retention:

$$\%\ \text{Xanthones retention} = \frac{\text{Xanthones content of dried (or drying) rind (mg/g d.b.)}}{\text{Xanthones content of fresh rind (mg/g d.b.)}} \times 100 \qquad (1)$$

2.6 Antioxidant activity evaluation

2.6.1 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay

DPPH radical scavenging activity of the ethanolic extract was evaluated according to the method of Okonogi et al. (2007) with some modifications. 3.9 mL of 100 μM DPPH radical in ethanol was added to a test tube with 0.2 mL of the ethanolic extract. The mixture was mixed using a vortex mixer (Scientific Industries Inc., model G-560E, Bohemia, NY) for 10 sec and left to stand in dark at room temperature for 60 min. The absorbance was measured at 540 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific, model Genesys20, Waltham, MA). Pure ethanol was used to calibrate the spectrophotometer.

2.6.2 2,2'-azinobis(3-ethylbenzithiazoline-6-sulfonic acid) (ABTS) assay

The ABTS assay was performed according to the modified method of Huang, Ou, & Prior (2005). The $ABTS^{\cdot+}$ radical cation was prepared by mixing ABTS (7 mM final concentration) with potassium persulfate ($K_2S_2O_8$) (2.45 mM final concentration). The mixture was kept in dark at room temperature for 12-16 h to give a dark blue solution, which was stable for 2 days. This solution was diluted with pure ethanol until its absorbance reached 0.7 at 734 nm. One mL of the $ABTS^{\cdot+}$ radical cation solution was added to 0.02 mL of the ethanolic extract and mixed using a vortex mixer for 10 sec. The absorbance was then measured at 734 nm using a UV-visible spectrophotometer exactly 1 min after vortex mixing. Pure ethanol was again used to calibrate the spectrophotometer.

The antioxidant activity of the ethanolic extract, which was analyzed by 2 different methods (DPPH and ABTS assays), is expressed in terms of the relative inhibition (Hiranvarachat, Suvarnakuta, & Devahastin, 2008):

$$\text{Relative inhibition} = \frac{\text{Inhibition capacity of dried (or drying) mangosteen rind}}{\text{Inhibition capacity of fresh mangosteen rind}} \quad (2)$$

Inhibition capacity of fresh and dried mangosteen rind was calculated from equation (3):

$$\text{Inhibition capacity} = \frac{\%\ \text{Inhibition}}{\text{Dry weight of sample kg/kg (d.b.)}} \tag{3}$$

The percentage inhibition of the DPPH and ABTS radicals was calculated from the following equation (Okonogi et al., 2007):

$$\%\ \text{Inhibition} = \frac{OD_{\text{blank}} - OD_{\text{sample}}}{OD_{\text{blank}}} \times 100 \tag{4}$$

where OD_{blank} and OD_{sample} are the optical density of 95% ethanol and of the ethanolic extract, respectively.

2.7 Statistical Analysis

All data were analyzed using the analysis of variance (ANOVA) and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests. Values were considered at a confidence level of 95%. Statistical program SPSS (version 15) was used to perform all statistical calculations.

3. Results and discussion

3.1 Drying kinetics of mangosteen rind

The initial moisture content of fresh mangosteen rind was approximately 1.80 kg/kg (d.b.) or 0.64 kg/kg (w.b.). The drying curves and temperature profiles of mangosteen rind undergoing hot air drying, vacuum drying and LPSSD at the temperatures of 60, 75 and 90°C are shown in Fig. 2. As expected, the drying rates increased with an increase in the drying temperature because drying at higher temperature provided larger driving force for heat transfer, which is obviously related to the rate of mass transfer. Moreover, the moisture diffusivity is also higher at higher temperature (Leeratanarak, Devahastin, & Chiewchan, 2006).

As can be seen in Fig. 2 hot air drying was the slowest drying process. This could be explained by the fact that the absolute pressure in the drying chamber in the cases of vacuum drying and LPSSD was only 7 kPa; boiling point of water at this pressure is about 40°C. Hence, vaporization of moisture occurred within the rind; moisture could move to the surface of the rind by vapor diffusion while only liquid diffusion was taking place in the case of hot air drying. Since vapor diffusion is faster than liquid diffusion, the drying rates of vacuum drying and LPSSD were higher than those of hot air drying. Although vacuum drying was a faster drying process than LPSSD, the differences between the drying time of LPSSD and vacuum drying were smaller at higher drying temperatures.

Because of the difference of the drying mechanism and drying rate, the product temperature profiles of hot air drying, vacuum drying and LPSSD were quite different. It was also observed from the temperatures profiles of mangosteen rind during various drying methods, the rind temperatures almost remained constant at wet-bulb temperature after the first 60 min in the case of hot-air drying while the temperature profiles in the case of vacuum dryings and LPSSD almost considerably increased from their initials to the medium temperatures and then remained constant within the first 60 min. However, the different temperature profiles between drying methods effect on the xanthones retention will be discuss later.

3.2 Changes of xanthones in mangosteen rind during drying

The contents of α-mangostin and 8-desoxygartanin in fresh mangosteen rind were approximately 47.82±3.76 mg/g (d.b.) and 1.43±0.30 mg/g (d.b.), respectively. The percentage of α-mangostin and 8-desoxygartanin retention in dried mangosteen rind, which had a moisture content of around 0.10 kg/kg (d.b.) is shown in Table 1. It is seen that the retention of α-mangostin and 8-desoxygartanin in mangosteen rind markedly decreased after all drying methods. The xanthones retention in mangosteen rind undergoing hot air drying and LPSSD at 75°C was, however, significant higher than that of the rind undergoing drying at other conditions.

Losses of xanthones after drying might be caused either by thermal or enzymatic degradation. It is indeed well recognized that thermal drying affects natural antioxidants in plant materials. Regarding enzymatic degradation, although thermal treatment could help inactivate degradative enzymes such as polyphenol oxidase (PPO), which is normally present in plant materials, some polyphenolics could still be degraded due to initial activity of the enzymes prior to their inactivation (Lim & Murtijaya, 2007; Obied et al., 2008). However, PPO is absolutely inhibited by heat treatment at over 85°C (Route-Meyer, Philippon & Nicolas, 1993)

The effect of moisture content on retention of xanthones in mangosteen rind during hot air drying, vacuum drying and LPSSD is shown in Fig. 3-5, respectively.

In the cases of hot air drying, the α-mangostin and 8-desoxygartanin contents decreased during drying since the product temperature was still lower than approximately 80°C (see Fig. 2). However, it took longer for the products undergoing vacuum drying and LPSSD to reach temperature of higher than 60°C compared with the case of hot air drying. Therefore, more enzymatic degradation might occur during early stages of vacuum drying and LPSSD than in the case of hot air drying, resulting in less retention of xanthones in the early stages in the cases of vacuum drying and LPSSD. In the later stages, it can be seen clearly that the thermal degradation took place when product temperatures increase instantly in the case of both of vacuum drying and LPSSD. However, comparison of final xanthones retention between vacuum drying and LPSSD revealed that the xanthone retentions differed significantly (see Table 1). This is because the product temperature of all vacuum drying cases was higher than LPSSD cases, thus thermal degradation has high effect in the case of vacuum drying. In addition, the LPSSD chamber was fully contained with superheated steam; therefore, the oxygen content remaining in the LPSSD chamber was less (none indeed) than in the case of vacuum drying chamber resulting in enzymatic degradation of xanthones.

In the case of hot air drying the α-mangostin and 8-desoxygartanin contents linearly decreased during drying. This linearity decrease was probably due to enzymatic degradation. Since the temperature of the rind reached approximately 55°C within 120 min (see Fig. 2), enzymes responsible for xanthones degradation were most activated (Vámos-Vigyázó, 1981). Therefore, beyond this initial period, especially in the case of drying at higher temperatures (i.e., 75 and 90°C), enzymatic degradation was most probably stopped. However, xanthones retention still decreased continuously after 45 min. This could be due to thermal degradation (Lim et al., 2007; Chantaro et al., 2008). In the case of drying at 60°C, on the other hand, the rind temperature was only around 50°C even during the later stage of drying. This lower rind temperature might not be enough to fully inactivate PPO; enzymatic degradation was therefore still in effect and this led to higher losses of xanthones during lower-temperature drying. Therefore, hot air drying at 60°C led to lower the xanthones retention than that at 75 and 90°C. In contrast, as mentioned earlier, thermal degradation could be mostly responsible for the losses of xanthones during drying at 75 and 90°C (Lim et al., 2007; Obied et al., 2008).

3.3 Changes of antioxidant activity of mangosteen rind during drying

IC_{50} values of the xanthone standards, i.e., α-mangostin and 8-desoxygartanin, as determined by the DPPH assay, were 25.6±2.6 and 4.2±0.3 μg/mL, respectively, while the values as determined by ABTS assay were 0.47±0.05 and 0.15±0.01 μg/mL, respectively. This shows that 8-desoxygartanin exhibits a higher antioxidant activity than α-mangostin. This is because 8-desoxygartanin has hydroxyl group at position C-5, which contributes to higher scavenging activity on DPPH and ABTS radicals than the group at position C-6 in the case of α-mangostin (see Fig. 6). It is noted that hydroxyl groups at positions C-6 and C-3 in both xanthone derivatives have less activity because of the steric resistance effect.

The antioxidant activity of xanthones as assessed by the ABTS assay was higher than that assessed by the DPPH assay. This observed phenomenon could be due to steric accessibility of the DPPH radicals. In addition, the active sites of DPPH radicals are in the middle of the structure as a result antioxidants hardly access to the DPPH radical sites. (Prior et al., 2005).

The total antioxidant activity of fresh mangosteen rind, which was assessed by DPPH and ABTS assays, was approximately 78.26±5.96% and 70.41±5.69%, respectively. The relative inhibitions of xanthones in mangosteen rind dried at various conditions are shown in Tables 2 and 3. The total antioxidant activity of the rind undergoing hot air drying, vacuum drying and LPSSD decreased compared with that of the fresh mangosteen rind. The total antioxidant activity of mangosteen rind during hot air drying, vacuum drying and LPSSD decreased following the patterns of the degradation kinetics of xanthones.

From Tables 2 and 3, it was found that both antioxidant assays gave similar antioxidant activity trends. This indicated that xanthones in mangosteen rind played an important role in the antioxidant activity of the rind. However, xanthones may not be the only potent antioxidants in mangosteen rind because there might also be some other phenolic compounds in the rind, which were not identified in this study.

4. Conclusion

The effects of drying methods and conditions on the xanthones contents in mangosteen rind undergoing hot air drying, vacuum drying and LPSSD were investigated in this study. The results showed that the contents of xanthones in mangosteen rind significantly decreased during all drying methods due either to enzymatic degradation or thermal degradation. Because of the similar trends of the xanthones content and antioxidant activities, all drying methods was also found to reduce the antioxidant activity of mangosteen rind due to the losses of xanthones during drying. Hot air drying and Low-pressure superheated steam dryings at operating temperature of 75°C is the two best conditions to maximize the quantity and quality of xanthones in mangosteen rind because it provides the proper drying time and temperature to help inactivate PPO and also minimize thermal degradation.

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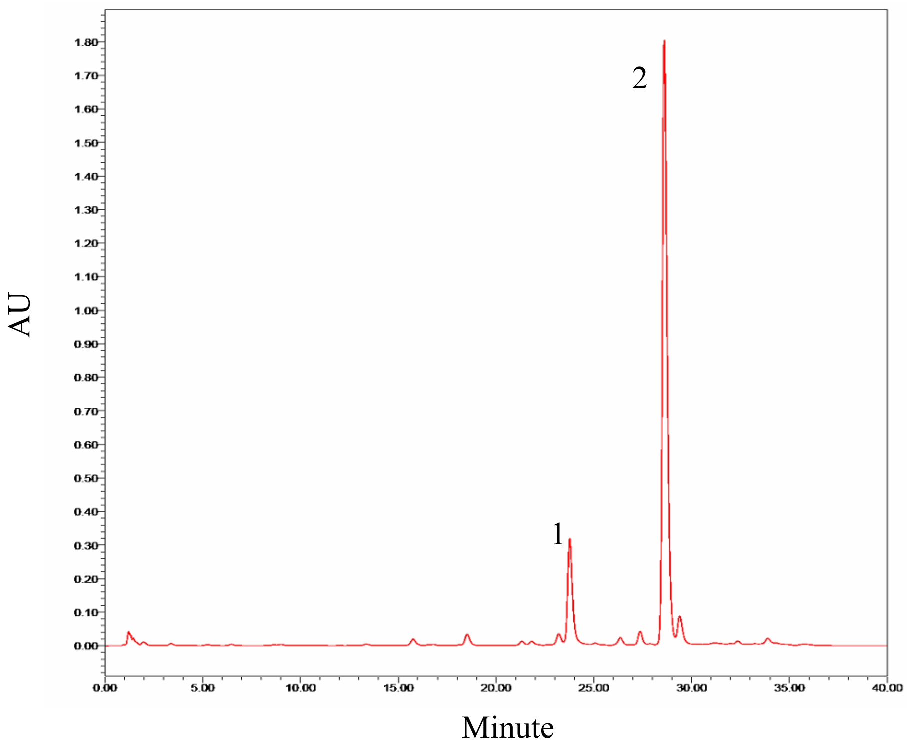


Fig. 1. Typical chromatograms of xanthones in mangosteen rind at a wavelength of 254 nm: (1) 8-desoxygartanin; (2) α-mangostin.

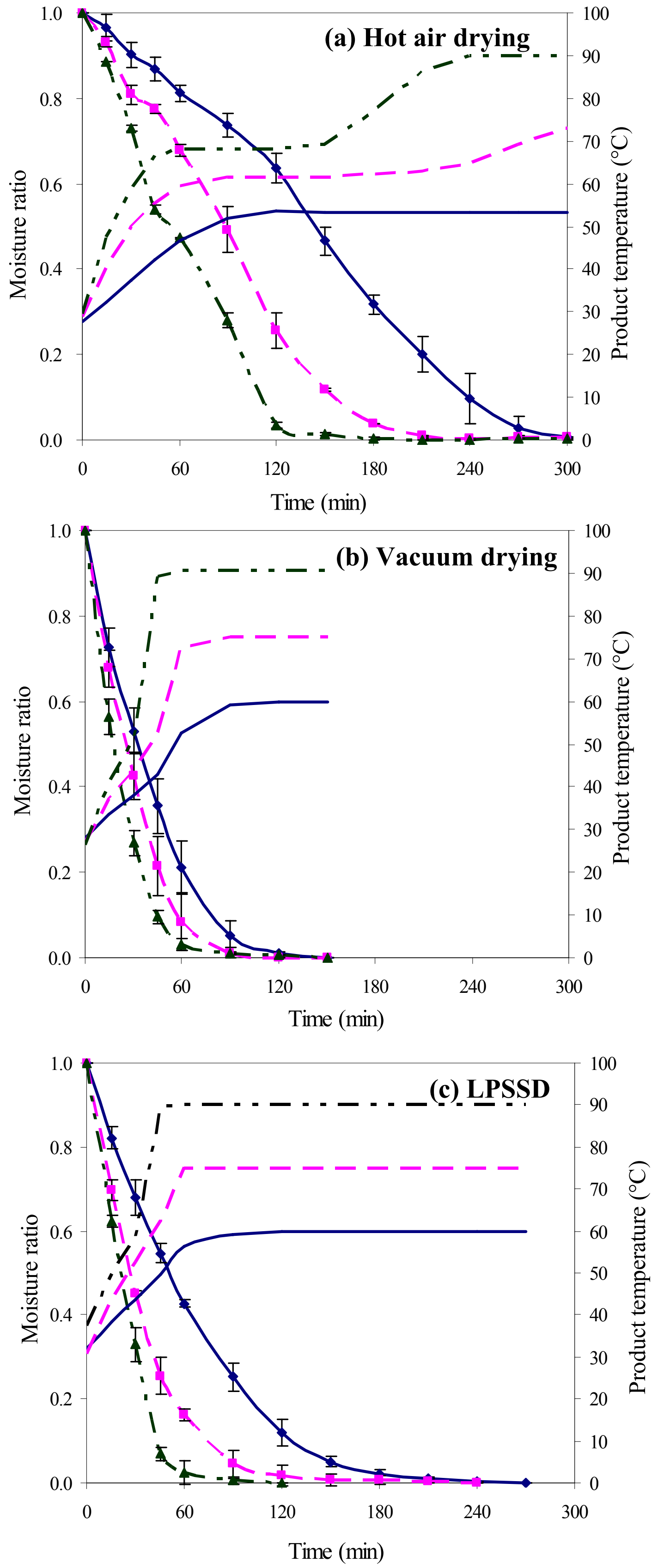


Fig. 2. Drying curves and temperature profiles of mangosteen rind undergoing (a) hot air drying; (b) vacuum drying; (c) LPSSD at 60 (▬), 75 (‒ ‒) and 90°C (‥▬)

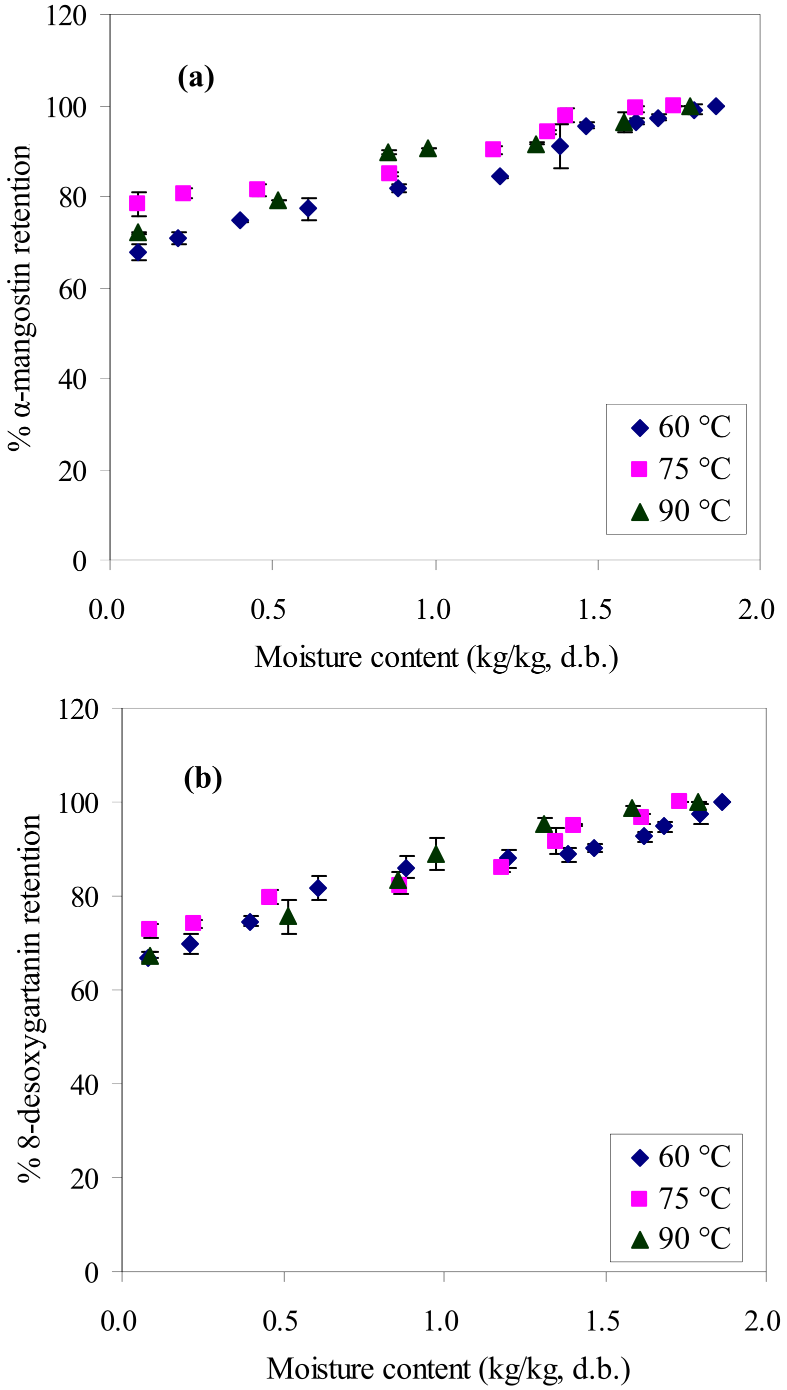


Fig. 3. Relationship between moisture content and xanthone retention in mangosteen rind during hot air drying. (a) %α-mangostin retention; (b) %8-desoxygartanin retention

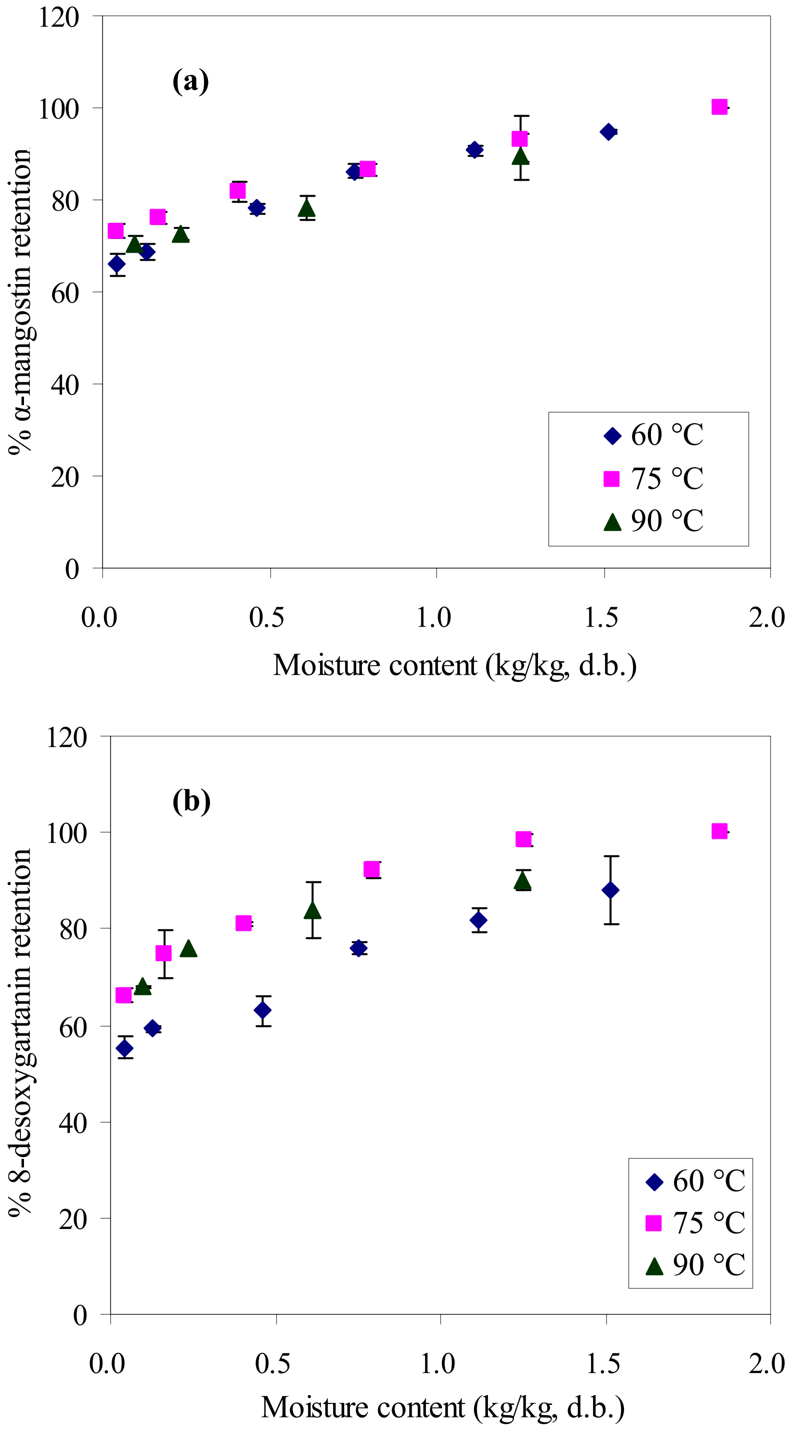


Fig. 4. Relationship between moisture content and xanthone retention in mangosteen rind during vacuum drying. (a) %α-mangostin retention; (b) %8-desoxygartanin retention

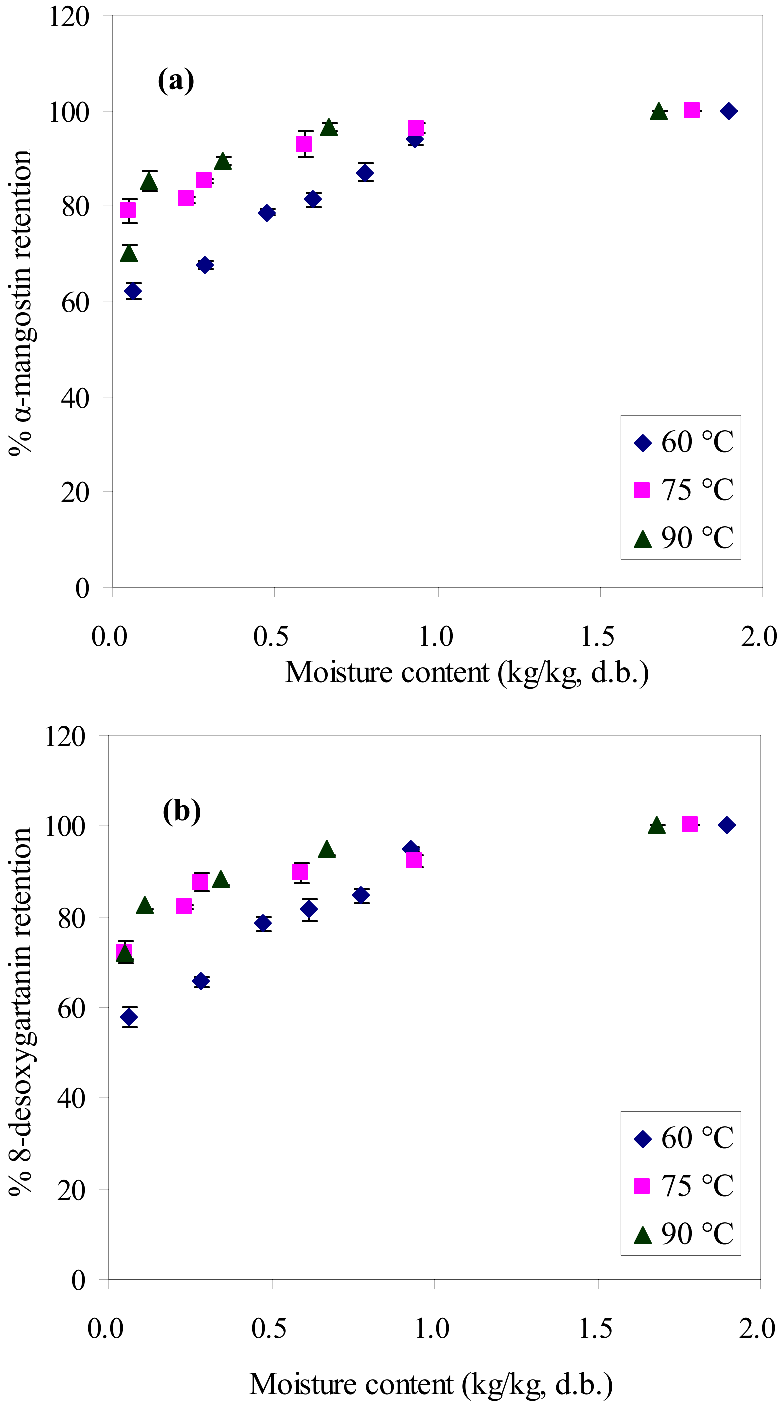
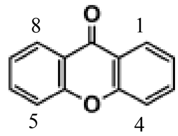
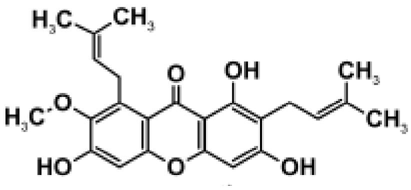
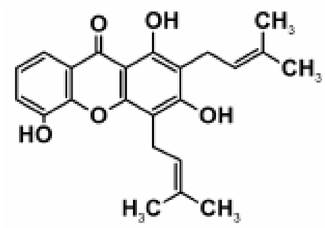


Fig. 5. Relationship between moisture content and xanthone retention in mangosteen rind during LPSSD (a) %α-mangostin retention; (b) %8-desoxygartanin retention



xanthone





α-mangostin 8-desoxygartanin

Fig. 6. Skeleton structures of xanthone and xanthone derivatives.

Table 1

Effects of drying methods and conditions on xanthones retention

Drying method	**Drying temperature (°C)**	**% Xanthone retention***	
		α-mangostin	**8-desoxygartanin**
Hot air drying	60	67.7 ± 1.7^{c}	66.8 ± 1.0^{b}
	75	78.1 ± 2.1^{a}	72.7 ± 1.4^{a}
	90	71.9 ± 0.1^{bc}	67.4 ± 0.4^{b}
Vacuum drying	60	66.7 ± 2.3^{c}	55.3 ± 2.1^{c}
	75	73.2 ± 1.1^{b}	66.1 ± 1.5^{b}
	90	70.6 ± 1.6^{bc}	67.9 ± 0.1^{b}
LPSSD	60	62.6 ± 1.4^{d}	57.6 ± 2.1^{c}
	75	78.3 ± 2.0^{a}	72.3 ± 2.2^{a}
	90	69.9 ± 1.6^{bc}	71.8 ± 1.1^{a}

*The different superscripts in the same column indicate that the values are significantly different (p<0.05).

Table 2

Antioxidant activity of xanthones in mangosteen rind during drying in terms of relative inhibition by DPPH radical scavenging capacity assay

Drying time (min)	Relative inhibition*								
	Hot air drying			Vacuum drying			LPSSD		
	60°C	75°C	90°C	60°C	75°C	90°C	60°C	75°C	90°C
15	0.99±0.01	0.97±0.05	1.00±0.01	0.93±0.02	0.97±0.07	0.80±0.01	0.93±0.01	0.92±0.01	0.89±0.02
30	0.98±0.04	0.98±0.01	0.99±0.02	0.82±0.04	0.83±0.02	0.61±0.02	0.81±0.04	0.80±0.01	0.67±0.01
45	0.98±0.03	0.99±0.06	0.95±0.03	0.69±0.01	0.66±0.01	0.47±0.02	0.76±0.04	0.64±0.01	0.56±0.01
60	0.91±0.01	0.99±0.02	0.91±0.06	0.57±0.04	0.56±0.03	0.42±0.02^{b}	0.65±0.02	0.61±0.01	0.51±0.01^{ab}
90	0.88±0.05	0.92±0.01	0.72±0.08	0.46±0.01	0.49±0.03^{ab}		0.53±0.01	0.54±0.02^{a}	
120	0.86±0.01	0.74±0.01	0.50±0.05^{ab}	0.42±0.01^{b}			0.47±0.04^{ab}		
150	0.75±0.00	0.61±0.01							
180	0.66±0.01	0.55±0.01^{a}							
210	0.55±0.03								
240	0.49±0.02								
270	0.44±0.02^{b}								

*Mean ± SD of two replicates. The different superscripts mean that the values are significantly different (p<0.05) at same final moisture contents.

Table 3

Antioxidant activity of xanthones in mangosteen rind during drying in terms of relative inhibition by ABTS assay

Drying time (min)	**Relative inhibition***								
	Hot air drying			**Vacuum drying**			**LPSSD**		
	60°C	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**	**60°C**	**75°C**	**90°C**
15	0.99±0.01	0.97±0.01	1.02±0.02	0.93±0.01	0.88±0.02	0.84±0.03	0.95±0.02	0.93±0.01	0.97±0.00
30	0.96±0.06	0.94±0.00	0.99±0.01	0.81±0.07	0.78±0.04	0.69±0.02	0.81±0.01	0.88±0.02	0.72±0.01
45	0.98±0.04	0.96±0.02	0.88±0.01	0.66±0.02	0.60±0.01	0.51±0.00	0.78±0.03	0.74±0.02	0.58±0.01
60	0.94±0.06	0.94±0.01	0.87±0.00	0.60±0.09	0.51±0.03	0.45±0.02^{bc}	0.65±0.01	0.65±0.02	0.54±0.01^{ab}
90	0.87±0.01	0.84±0.01	0.74±0.01	0.49±0.04	0.44±0.01^{bc}		0.56±0.01	0.60±0.02^{a}	
120	0.89±0.05	0.68±0.02	0.47±0.00^{bc}	0.47±0.02^{bc}			0.50±0.02^{b}		
150	0.79±0.03	0.58±0.02							
180	0.68±0.01	0.54±0.01^{ab}							
210	0.54±0.02								
240	0.47±0.04								
270	0.42±0.00^{c}								

*Mean ± SD of two replicates. The different superscripts mean that the values are significantly different (p<0.05) at same final moisture contents.