รายงานวิจัยฉบับสมบูรณ์

การดัดแปรโครงสร้างของกากเนื้อในเมล็ดปาล์มเพื่อใช้เป็นวัตถุดิบอาหารสำหรับปลานิล (*Oreochromis niloticus*)

Structural modification of palm kernel meal as feedstuff for Nile tilapia (*Oreochromis niloticus*)

คณะนักวิจัย

ผู้ช่วยศาสตราจารย์ ดร. การุณ ทองประจุแก้ว (หัวหน้าโครงการ)

ผู้ช่วยศาสตราจารย์ ดร. ชุติมา ตันติกิตติ (อาจารย์พี่เลี้ยง)

โครงการวิจัยนี้ได้รับทุนสนับสนุนจากเงินรายได้ มหาวิทยาลัยสงขลานครินทร์

ประจำปีงบประมาณ 2555 รหัสโครงการ SCI550289S

โครงการวิจัย

การดัดแปรโครงสร้างของกากเนื้อในเมล็ดปาล์มเพื่อใช้เป็นวัตถุดิบอาหาร
สำหรับปลานิล (*Oreochromis niloticus*)

Structural modification of palm kernel meal as feedstuff
for Nile tilapia (*Oreochromis niloticus*)

คณะนักวิจัย

ผู้ช่วยศาสตราจารย์ ดร. การุณ ทองประจุแก้ว (หัวหน้าโครงการ)
ภาควิชาวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่

ผู้ช่วยศาสตราจารย์ ดร. ชุติมา ตันติกิตติ (อาจารย์พี่เลี้ยง)
ภาควิชาวาริชศาสตร์ คณะทรัพยากรธรรมชาติ
มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณโครงการทุนดรุณาจารย์ (เลขที่สัญญา SCI550289S) ที่ให้การสนับสนุนเงินทุนในการวิจัยครั้งนี้ ขอขอบคุณศูนย์เครื่องมือวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ที่ให้ความช่วยเหลือในการวิเคราะห์ตัวอย่าง หน่วย Publication Clinic สำนักวิจัยและพัฒนา มหาวิทยาลัยสงขลานครินทร์ ที่ให้ความช่วยเหลือในการเตรียมต้นฉบับสำหรับตีพิมพ์ และขอขอบคุณภาควิชาวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์ ที่ให้ความอนุเคราะห์สถานที่ สารเคมี และเครื่องมือที่ใช้ในการวิจัย

ขอขอบพระคุณ รองศาสตราจารย์ ดร. อุทัยวรรณ โกวิทวที จากภาควิชาสัตววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ ที่กรุณาให้คำปรึกษาในการวางแผนการทดลอง และการเตรียมต้นฉบับสำหรับตีพิมพ์ และขอขอบคุณ อาจารย์ ดร. สมรักษ์ รอดเจริญ จากสาขาวิทยาศาสตร์ชีวภาพ คณะวิทยาศาสตร์และเทคโนโลยีการประมง มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย ที่ช่วยอนุเคราะห์สถานที่ และทดลองเลี้ยงปลานิลเพื่อทดสอบคุณภาพของอาหาร

ผู้ช่วยศาสตราจารย์ ดร. การุณ ทองประจุแก้ว
ผู้ช่วยศาสตราจารย์ ดร. ชุติมา ตันติกิตติ

สารบัญ

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อปรับปรุงคาร์โบไฮเดรตในกากเนื้อในเมล็ดปาล์มให้อยู่ในรูปที่ใช้ประโยชน์ได้ และใช้เป็นวัตถุดิบอาหารสำหรับเลี้ยงปลานิล (*Oreochromis niloticus*) โดยดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีการทางฟิสิกส์ ได้แก่ การแช่น้ำ การใช้คลื่นไมโครเวฟ การฉายรังสีแกมมา และลำแสงอิเล็กตรอน แล้วศึกษาการเปลี่ยนแปลงองค์ประกอบทางเคมี สมบัติทางเคมีกายภาพ และประสิทธิภาพการย่อยในหลอดทดลอง ผลการศึกษาพบว่าวิธีการดัดแปรมีผลต่อการลดลงของปริมาณเยื่อใย และมีการเพิ่มขึ้นของคาร์โบไฮเดรตที่ใช้ประโยชน์ได้ ($P < 0.05$) โดยการดัดแปรด้วยวิธีการแช่น้ำ และการใช้คลื่นไมโครเวฟ สามารถปรับปรุงสมบัติทางเคมีกายภาพ (พีเอช การละลายน้ำ โครงสร้างระดับจุลภาค ความเป็นผลึกสัมพัทธ์ และองค์ประกอบของลิกโนเซลลูโลส) และประสิทธิภาพการย่อยคาร์โบไฮเดรตในหลอดทดลอง

การศึกษาผลของคุณภาพอาหารที่มีกากเนื้อในเมล็ดปาล์มเป็นองค์ประกอบ 20 เปอร์เซ็นต์ต่อการเจริญเติบโตของปลานิล (น้ำหนักเริ่มต้น 20.61 ± 0.15 กรัม และความยาวเริ่มต้น 10.45 ± 0.03 เซนติเมตร) เป็นระยะเวลา 10 สัปดาห์ ภายใต้แผนการทดลองแบบสุ่มสมบูรณ์ (4 ทรีทเมนต์ × 3 ซ้ำ) โดยให้ปลานิลได้รับอาหาร 4 สูตร ที่มีปริมาณโปรตีน ลิพิด และพลังงานใกล้เคียงกัน แต่มีกากเนื้อในเมล็ดปาล์มที่ดัดแปรด้วยกรรมวิธีที่แตกต่างกันเป็นองค์ประกอบ ได้แก่ กากเนื้อในเมล็ดปาล์มที่ไม่ผ่านการดัดแปร กากเนื้อในเมล็ดปาล์มที่ดัดแปรด้วยการแช่น้ำ กากเนื้อในเมล็ดปาล์มที่ผ่านการไมโครเวฟ และกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำร่วมกับไมโครเวฟ ผลการศึกษาพบว่าสมบัติทางเคมีกายภาพของอาหารที่มีผลต่อการไฮโดรไลท์ของเอนไซม์ย่อยอาหาร ได้แก่ พีเอช ความข่น โครงสร้างระดับจุลภาค สเปกตรัมของลิกโนเซลลูโลส รูปแบบการเลี้ยวเบนรังสีเอกซ์ และสมบัติเชิงความร้อน มีความสัมพันธ์กับการเจริญเติบโต อัตราการแลกเนื้อ และดัชนีของอวัยวะภายใน โดยปลาที่ได้รับอาหารที่มีกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ และแช่น้ำร่วมกับไมโครเวฟ มีการเจริญเติบโตและประสิทธิภาพการใช้อาหารสูงสุด มีระดับการต้านอนุมูลอิสระสูงขึ้น นอกจากนี้ ปลาดังกล่าวยังมีองค์ประกอบของซากและกล้ามเนื้อที่ดีขึ้น โดยเฉพาะปลาที่ได้รับกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ ซึ่งพบว่ามีการแสดงออกของโปรตีนในกล้ามเนื้อเพิ่มขึ้น ดังนั้น การแช่น้ำจึงเป็นกรรมวิธีที่สามารถปรับปรุงคุณภาพทางโภชนาการของกากเนื้อในเมล็ดปาล์ม และมีศักยภาพในทางเศรษฐกิจที่ใช้สำหรับพัฒนาเป็นอาหารสัตว์น้ำ

Abstract

The aim of this study was to improve available carbohydrate from PKM and used it as effective ingredient for Nile tilapia (*Oreochromis niloticus*) feed. Different physical modifications including water soaking, microwave irradiation, gamma irradiation and electron beam, were investigated in relation to chemical composition, physicochemical properties and *in vitro* carbohydrate digestibility. The findings suggest that the modified methods had significant effects on chemical composition by decreasing crude fiber and increasing available carbohydrates ($P < 0.05$). Improvements in physicochemical properties (pH, water solubility, microstructure, relative crystallinity and lignocellulosic constituents) and *in vitro* carbohydrate digestibility were mainly achieved by soaking and microwave irradiation.

The effects of 20% PKM-containing feed quality on growth performance and feed utilization of Nile tilapia were studied experimentally in a ten weeks feeding trial. The fish (20.61 ± 0.15 g initial weight and 10.45 ± 0.03 cm initial length) were reared in dietary treatment groups according to a completely randomized design (4 treatments × 3 replications). The fish were fed with isonitrogenous, isolipidic and isoenergetic feeds which differed in the form of PKM that was either unprocessed (UPKM), water-soaked (SPKM), microwave irradiated (MPKM), or water-soaked and microwave-irradiated (SMPKM). Physicochemical property changes in ways that are expected to enhance enzymatic digestion of feed were determined, namely pH, turbidity, microstructure, lignocellulosic spectra, diffraction pattern, and thermal transition property. These feed characteristics were linked with growth, feed conversion ratio, and visceral organ indices. At the end of experimentation, the fish fed with SPKM and SMPKM feeds were superior in all growth indicators and feed utilization efficiency as well as slight improvement in radical scavenging activity. Compositions of carcass and muscle were also slightly improved in fish fed with SPKM and SMPKM feeds while the quality was only archived in fish fed with the SPKM. Therefore, nutritional quality of the feed could be improved by water soaking of the PKM. This modification method is economically potential for aquafeed production.

บทสรุปผู้บริหาร

1. บทนำ

การเพิ่มขึ้นของอุตสาหกรรมปาล์มน้ำมัน (oil palm) เพื่อใช้เป็นแหล่งพลังงาน และวัตถุดิบในกระบวนการผลิตของอุตสาหกรรมต่อเนื่องหลายชนิด ก่อให้เกิดผลพลอยได้ทางการเกษตร (agricultural by-products) เช่น ช่อดอกเพศผู้ ทะลายปาล์มเปล่า และเส้นใย เป็นต้น และผลพลอยได้จากอุตสาหกรรมหีบน้ำมันปาล์ม (industrial by-products) เช่น กากปาล์มทั้งผล (palm oil meal) กากเมล็ดปาล์ม (palm seed meal) และกากเนื้อในเมล็ดปาล์ม (palm kernel meal) เป็นต้น ผลพลอยได้ดังกล่าวมีการใช้ประโยชน์ได้น้อย ส่วนใหญ่มักใช้ทำปุ๋ยหมัก เพาะเห็ด หรือส่วนผสมของอาหารสัตว์ เป็นต้น อย่างไรก็ตาม การใช้ผลพลอยได้ดังกล่าวเพื่อเป็นวัตถุดิบในอาหารสัตว์พบว่าสามารถใช้ได้ในปริมาณจำกัด เนื่องจากวัตถุดิบมีคุณค่าทางโภชนาการต่ำ และมีองค์ประกอบส่วนใหญ่ที่สัตว์ไม่สามารถย่อยได้ เช่น เซลลูโลส เฮมิเซลลูโลส และลิกนิน เป็นต้น ทำให้สัตว์ที่เลี้ยงมีการเจริญเติบโตช้า อ่อนแอ อัตราการเจริญพันธุ์ต่ำและส่งผลให้ไม่คุ้มทุน นอกจากนี้ ผลพลอยได้บางส่วนที่เกิดจากการกระบวนการผลิตยังจัดเป็นของเสียที่ขาดการนำไปใช้ประโยชน์ และก่อให้เกิดปัญหาสิ่งแวดล้อมตามมา

การศึกษาในวัตถุดิบอาหารหลายชนิดพบว่าการดัดแปรโครงสร้างวัตถุดิบให้เหมาะสมจะทำให้สัตว์สามารถย่อย ดูดซึม และใช้ประโยชน์จากอาหารได้ดีขึ้น (Alajaji and El-Adawy, 2006; Sadeghi and Shawrang, 2006; El-Niely, 2007; Ebrahimi *et al*., 2009; Chung *et al*., 2010; Thongprajukaew *et al*., 2011) เนื่องจากวัตถุดิบจะเปลี่ยนแปลงสมบัติทางเคมีกายภาพให้เหมาะสมต่อการไฮโดรไลท์ของเอนไซม์ย่อยอาหาร เช่น เกิดการเจลาติไนซ์ของแป้ง (Mohapatra *et al*., 2002; Kumar *et al*., 2006; Kaur *et al*., 2010) การละลายน้ำ (Chung *et al*., 2010; Kaur *et al*., 2010) เปลี่ยนแปลงโครงสร้างของพื้นผิว (Palav and Seetharaman, 2007; Kristensen *et al*., 2008; Lopez-Rubio *et al*., 2008) และความเป็นผลึก (Lopez-Rubio *et al*., 2008; Chung *et al*., 2010; Kaur *et al*., 2010) เป็นต้น ดังนั้น การดัดแปรโครงสร้างของกากเนื้อในเมล็ดปาล์มเพื่อเพิ่มประสิทธิภาพการย่อยและใช้ประโยชน์ในสัตว์จึงเป็นแนวทางหนึ่งที่จะช่วยลดต้นทุนการผลิต ลดปัญหากากของเสียที่ส่งผลกระทบต่อสิ่งแวดล้อม และเป็นการใช้ทรัพยากรได้อย่างคุ้มค่าและยั่งยืน

การศึกษาครั้งนี้มีจุดประสงค์เพื่อนำกากเนื้อในเมล็ดปาล์มมาดัดแปรโครงสร้างโดยวิธีการทางฟิสิกส์ ได้แก่ การแช่น้ำ การใช้คลื่นไมโครเวฟ การฉายรังสีแกมมา และลำแสงอิเล็กตรอน ให้มีความเหมาะสมต่อการย่อยของเอนไซม์สัตว์น้ำ โดยประเมินจากการเปลี่ยนแปลงองค์ประกอบทางเคมี สมบัติทางเคมีกายภาพ และประสิทธิภาพการย่อยในหลอดทดลอง (*in vitro* digestibility) หลังจาก

นั้นจึงมีการทดลองเลี้ยงปลานิล (*Oreochromis niloticus*) เพื่อทดสอบประสิทธิภาพของอาหาร โดยศึกษาอัตราการรอด การเจริญเติบโต ประสิทธิภาพการแลกเนื้อ กิจกรรมของเอนไซม์ย่อยอาหาร (ทริปซิน ไคโมทริปซิน อะไมเลส เซลลูเลส และไลเปส) การต้านอนุมูลอิสระของอวัยวะที่สำคัญ ได้แก่ ตับ กระเพาะอาหาร และลำไส้ องค์ประกอบของกล้ามเนื้อและซาก และคุณภาพของกล้ามเนื้อ ผลจากการศึกษาครั้งนี้คาดว่าจะสามารถคัดเลือกวิธีการที่เหมาะสมสำหรับการดัดแปรโครงสร้างของกากเนื้อในเมล็ดปาล์ม ทราบสมบัติทางเคมีกายภาพเบื้องต้นของวัตถุดิบอาหารที่เหมาะสมต่อการย่อยและใช้ประโยชน์ ตลอดจนสามารถส่งเสริมให้มีการนำกากเนื้อในเมล็ดปาล์มไปใช้ประโยชน์เป็นวัตถุดิบอาหารให้กับสัตว์น้ำเศรษฐกิจได้อย่างครบวงจรและยั่งยืนต่อไป

2. วัตถุประสงค์

2.1 เพื่อสร้างองค์ความรู้ใหม่ในการตรวจสอบและประเมินคุณภาพการใช้ประโยชน์วัตถุดิบอาหารสัตว์ในระดับหลอดทดลอง โดยใช้เทคโนโลยีทางด้านเอนไซม์ย่อยอาหาร ร่วมกับการศึกษาการเปลี่ยนแปลงสมบัติทางเคมีกายภาพ

2.2 เพื่อปรับปรุงคุณภาพของกากเนื้อในเมล็ดปาล์มซึ่งเป็นผลพลอยได้ทางการเกษตรที่มีคุณค่าทางโภชนาการต่ำ ให้มีโครงสร้างที่เหมาะสมและสัตว์น้ำสามารถนำไปใช้ประโยชน์ได้เต็มประสิทธิภาพ

2.3 เพื่อสร้างสูตรอาหารสัตว์น้ำที่มีราคาถูก ใช้วัตถุดิบที่หาได้ง่ายในท้องถิ่น มีต้นทุนต่ำ แต่มีประสิทธิภาพสูง และสามารถผลิตได้ในเชิงอุตสาหกรรม

3. สรุป

3.1 การศึกษาที่ 1: ผลของการดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีทางฟิสิกส์ต่อการเปลี่ยนแปลงปริมาณคาร์โบไฮเดรตที่ย่อยได้ สมบัติทางเคมีกายภาพ และประสิทธิภาพการย่อยคาร์โบไฮเดรตในหลอดทดลอง (ภาคผนวก ก และ ง)

การดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีการทางฟิสิกส์มีผลอย่างมีนัยสำคัญต่อการลดลงของปริมาณเยื่อใย และทำให้ปริมาณคาร์โบไฮเดรตมีค่าเพิ่มขึ้น โดยการแช่น้ำและการไมโครเวฟจะปรับปรุงสมบัติทางเคมีกายภาพของวัตถุดิบให้เหมาะสมต่อการไฮโดรไลท์ของเอนไซม์ย่อยอาหาร และทำให้ประสิทธิภาพการย่อยคาร์โบไฮเดรตในหลอดทดลองของปลานิลมีค่าสูง ดังนั้น การดัดแปร

ด้วยการแช่น้ำหรือการไมโครเวฟจึงเป็นทางเลือกหนึ่งที่จะช่วยปรับปรุงคุณภาพของกากเนื้อในเมล็ดปาล์มให้เหมาะสมต่อการย่อยของสัตว์น้ำมากขึ้น

3.2 การทดลองที่ 2 (ภาคผนวก ข)

3.2.1 การทดลองที่ 2.1: ผลของการดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีทางฟิสิกส์ต่อ สมบัติทางเคมีกายภาพของอาหาร และการเจริญเติบโตของปลานิล

พีเอชและความข่นสามารถใช้แยกความแตกต่างระหว่างอาหารที่มีส่วนผสมของกากเนื้อในเมล็ดปาล์มที่ผ่านการดัดแปร และกากเนื้อในเมล็ดปาล์มในชุดควบคุมได้ ขณะที่สมบัติทางเคมีกายภาพของอาหาร ได้แก่ โครงสร้างระดับจุลภาค สเปกตรัมของลิกโนเซลลูโลส รูปแบบการเลี้ยวเบนรังสีเอกซ์ และสมบัติทางความร้อน มีความสัมพันธ์กับการเจริญเติบโตและใช้ประโยชน์จากอาหารของปลานิล ผลการทดลองแสดงให้เห็นว่าสมบัติทางเคมีกายภาพมีความสำคัญต่อคุณภาพของอาหาร และคุณลักษณะดังกล่าวสามารถใช้ในการควบคุมคุณภาพในกระบวนการผลิตอาหารสัตว์น้ำได้

3.2.2 การทดลองที่ 2.2: ผลของการดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีทางฟิสิกส์ต่อ การเจริญเติบโต การใช้อาหาร การต้านอนุมูลอิสระ กิจกรรมของเอนไซม์ย่อยอาหาร องค์ประกอบของซาก และคุณภาพกล้ามเนื้อของปลานิล

การดัดแปรกากเนื้อในเมล็ดปาล์มด้วยการแช่น้ำ หรือแช่น้ำร่วมกับไมโครเวฟ ช่วยปรับปรุงคุณภาพของอาหารปลานิลได้ โดยปลาที่ได้รับอาหารที่มีกากเนื้อในเมล็ดปาล์มที่ดัดแปรด้วยวิธีดังกล่าวจะมีการเจริญเติบโตและมีประสิทธิภาพการใช้อาหารสูงกว่าในทรีทเมนต์อื่น ปลาในกลุ่มดังกล่าวมีการย่อยโปรตีนและคาร์โบไฮเดรตได้ดี ส่วนการย่อยลิพิดพบว่าปลาที่ได้รับกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำจะมีการตอบสนองที่ดี เมื่อศึกษาการต้านอนุมูลอิสระพบว่าปลาทั้ง 2 กลุ่ม มีความสามารถในการต้านอนุมูลอิสระในระดับคงที่ทั้งในตับและลำไส้เล็ก แต่มีระดับที่สูงขึ้นในกระเพาะอาหาร นอกจากนี้ ยังมีองค์ประกอบของซากและกล้ามเนื้อที่ดีขึ้น โดยเฉพาะปลาที่ได้รับกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ ซึ่งพบว่ามีการแสดงออกของโปรตีนบางชนิดเพิ่มมากขึ้นในกล้ามเนื้อ ดังนั้น การแช่น้ำกากเนื้อในเมล็ดปาล์มก่อนการผสมวัตถุดิบจึงเหมาะสมต่อการผลิตอาหารปลานิล

ภาคผนวก

ภาคผนวก ก

สำเนาบทความที่ได้รับการตีพิมพ์แล้ว

Research Article

Received: 17 December 2012 Revised: 10 June 2013 Accepted article published: 23 July 2013 Published online in Wiley Online Library: 20 August 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6314

Physical modification of palm kernel meal improved available carbohydrate, physicochemical properties and *in vitro* digestibility in economic freshwater fish

Karun Thongprajukaew,[a,d]* Pinya Yawang,[a] Lateepah Dudae,[a] Husna Bilanglod,[a] Terdtoon Dumrongrittamatt,[a] Chutima Tantikitti[b] and Uthaiwan Kovitvadhi[c,d]

Abstract

BACKGROUND: Unavailable carbohydrates are an important limiting factor for utilization of palm kernel meal (PKM) as aquafeed ingredients. The aim of this study was to improve available carbohydrate from PKM. Different physical modifications including water soaking, microwave irradiation, gamma irradiation and electron beam, were investigated in relation to chemical composition, physicochemical properties and *in vitro* carbohydrate digestibility using digestive enzymes from economic freshwater fish.

RESULTS: Modified methods had significant ($P < 0.05$) effects on chemical composition by decreasing crude fiber and increasing available carbohydrates. Improvements in physicochemical properties of PKM, such as water solubility, microstructure, relative crystallinity and lignocellulosic spectra, were mainly achieved by soaking and microwave irradiation. Carbohydrate digestibility varied among the physical modifications tested ($P < 0.05$) and three fish species had different abilities to digest PKM. Soaking was the appropriate modification for increasing carbohydrate digestion specifically in Nile tilapia (*Oreochromis niloticus*), whereas either soaking or microwave irradiation was effective for striped snakehead (*Channa striata*). For walking catfish (*Clarias batrachus*), carbohydrate digestibility was similar among raw, soaked and microwave-irradiated PKM.

CONCLUSION: These findings suggest that soaking and microwave irradiation could be practical methods for altering appropriate physicochemical properties of PKM as well as increasing carbohydrate digestibility in select economic freshwater fish.

Keywords: palm kernel meal; soaking; microwave irradiation; physicochemical properties; carbohydrate digestibility; economic fish

INTRODUCTION

The global production rate of oil palm industry maintains an increasing trend. Palm kernel meal (PKM) is a common by-product from palm oil extraction (Fig. 1), and is commonly used as a feed ingredient for ruminants as well as for non-ruminants, such as swine[1] and poultry.[2] For aquatic animals, little information is currently available on the use of PKM in diets. Insufficient nutrient content and low digestibility of PKM due to large amounts of non-starch polysaccharides in cell wall constituents, including mannans, celluloses and xylans, are the main problem.[3] These components impair the digestibility and utilization of nutrients, by encapsulation of nutrients or by increasing the viscosity of intestinal content.[4] However, substitution levels of PKM in aquafeed have been optimized in Nile tilapia (*Oreochromis niloticus*)[5] and hybrid Asian–African catfish (*Clarias macrocephalus* × *C. gariepinus*).[6] Moreover, palm-originated oil exhibited significant benefits for replacing fish oil whereas the carbohydrate problem in many feedstuffs occurs due to limited amounts of lipid.[7]

Increased PKM utilization by some biological modifications has been reported, such as commercial enzyme supplementation and fungal fermentation.[5] These methods are time consuming and probably contaminate with unfavorable microorganisms.

* *Correspondence to: Karun Thongprajukaew, Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand, E-mail: karun.t@psu.ac.th*

a *Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla, 90112, Thailand*

b *Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90112, Thailand*

c *Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand*

d *Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand*

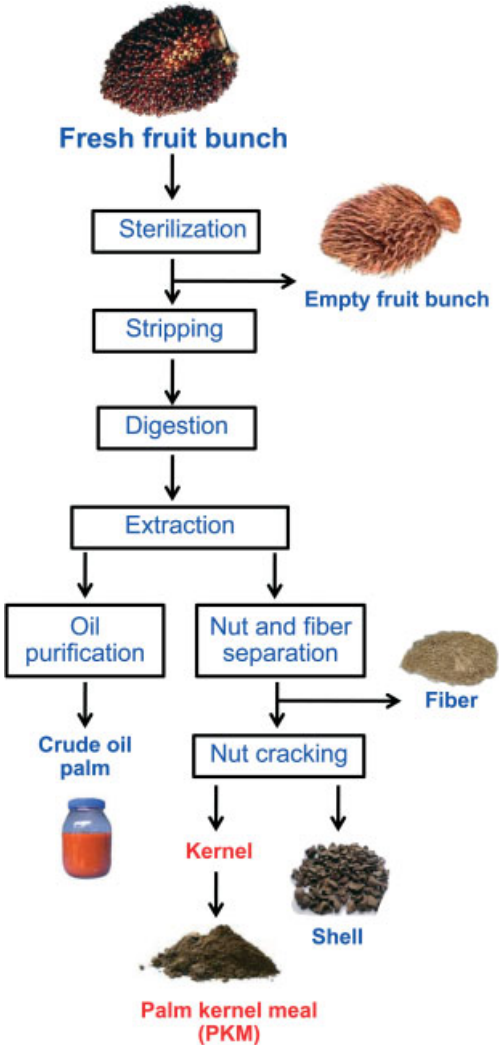


Figure 1. PKM generated from palm oil processing.

Recently, physical modifications for increasing enzymatic hydrolysis in food or feed ingredients have been used worldwide, such as water soaking,[8] microwave irradiation,[8–10] gamma irradiation[11,12] and electron beam.[13–15] These procedures can improve some physicochemical properties of raw materials, such as water solubility,[16] crystallinity[17] and lignocellulosic contents,[18] as well as protein and starch degradation,[10] which is important for increasing nutrient utilization. However, little is known about appropriate modifications of PKM using physical methods that contribute to better feedstuff quality.

The goal of this study was to investigate the effects of different modification methods including water soaking, microwave irradiation, gamma irradiation and electron beam, on chemical composition, physicochemical properties and *in vitro* carbohydrate digestibility of PKMs. Three freshwater fish with high economic values, namely Nile tilapia (*Oreochromis niloticus*), walking catfish (*Clarias batrachus*) and striped snakehead (*Channa striata*), were used as sources of digestive enzymes for *in vitro* digestibility. Carbohydrate digestibility is determined because PKM contains a large amount of structural carbohydrate. The findings from the present study might be used for improving PKM feed quality in aquatic production.

EXPERIMENTAL

PKM modifications

PKMs were obtained from an industrial factory in Hat Yai, Songkhla, Thailand. Unprocessed PKM was used as a control. Modifications of PKM using different physical methods were performed following previous studies in feed or food ingredients: (i) water soaking – PKM was soaked in distilled water (1:2, w/v) for 12 h at room temperature;[8] (ii) microwave irradiation – 100 g PKM was placed in a plastic box (20 cm diameter × 10 cm height), mixed with distilled water (1:2 w/v) and then cooked at 800 W in a microwave oven (MW 71B, Samsung, Malaysia) under agitation for 4 min;[8] (iii) gamma irradiation – PKM was irradiated at a dose of 30 kGy[14] using ^{60}Co from a carrier-type gamma irradiator (JS 8900 IR-155, MDS Nordion, Ottawa, ON, Canada) as irradiation source; and (iv) electron beam irradiation – PKM was irradiated at dose of 30 kGy at a fixed beam energy of 10 MeV[15] by electron accelerator (TT-200, IBA Co. Ltd, Louvain, Belgium). Modifications by gamma and electron beam were conducted at the Institute of Nuclear Technology (Public Organization), Thailand.

PKM preparation

Raw and modified PKMs were dried using a freeze dryer (Delta 2-24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h, ground and then kept in a desiccator for later analysis of physicochemical properties and *in vitro* digestibility. For chemical composition analysis, raw and modified PKMs were dried in an air oven at 105 °C for 24 h to remove moisture and kept in a desiccator until analysis.

Chemical composition

Raw and modified PKMs were analyzed for proximate composition including protein, lipid, ash and fiber according to standard AOAC methods.[19] Nitrogen-free extract (NFE) was calculated from the results. All chemical compositions are reported on a dry matter basis.

Physicochemical characteristics

Determination of pH

Suspension of PKM was prepared by adding 1 g of sample in 25 mL of water at 25 °C and agitating for 10 min.[20] Measurement of pH in suspended samples was conducted using a pH meter (CyberScan 510, Eutech Instruments, Singapore).

Determination of water solubility

Water solubility was determined according to the method of Chung *et al.*[12] One gram of PKM was mixed with 10 mL water, gently stirred for 1 h at room temperature and centrifuged at 1500 × *g* for 10 min. Subsequently, the supernatant was collected, dried at 60 °C for 48 h and weighed. Solubility of the sample was calculated from the ratio between weight of dissolved solids in the supernatant and weight of dry solids in the original sample.

Microscopic observation

Microstructure (including shape, surface and roughness) of raw and modified PKMs was studied using scanning electron micrographs (Quanta 400, FEI, Brno, Czech Republic) at 200×, 2000× and 8000× magnification. The PKM samples were mounted using double-sticky tape on an aluminium stub and coated with gold. An energy potential of 15 kV was used during micrography.

X-ray diffraction pattern

X-ray diffraction patterns of PKM were determined using an X-ray diffractometer (X'Pert MPD, Philips, Amsterdam, Netherlands) operated at 40 kV voltage and 40 mA current. Diffractograms were recorded for 2θ between 4° and 35° with a scanning rate of 2° min^{-1}.

Fourier transform infrared (FTIR)

Alterations in lignocellulosic constituents were analyzed using an FTIR spectrometer (Equinox 55, Bruker, Bremen, Germany). Sample discs were prepared by mixing 1 mg dried PKM with 100 mg KBr in mortar and then pressing the mixture at 10 MPa for 5 min. FTIR spectra were taken for each sample from 4000 to 400 cm^{-1}. Specific spectra for each component were measured following previous reports.[21–24]

Determination of *in vitro* carbohydrate digestibility

Fish preparation

Adult Nile tilapia (*O. niloticus*), walking catfish (*C. batrachus*) and striped snakehead (*C. striata*) were obtained from a farm in Songkhla Province, Thailand. The fish ($n = 4$) were acclimatized for 14 days in tanks (80 cm diameter × 40 cm height) and maintained at a water temperature of 27.4 ± 0.3 °C, pH 7.03 ± 0.07 and diurnal cycle 12 h light:12 h dark. The fish were fed *ad libitum*, twice daily (08:00 and 18:00 h) with a commercial diet according to their feeding habits (20% crude protein for Nile tilapia, and 37% crude protein for walking catfish and striped snakehead). All fish were starved for 24 h prior to sampling and then sacrificed by chilling in ice according to 'Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes', National Research Council, Thailand. Intestines of the fish were carefully collected and then kept at −20 °C until digestive enzyme extraction.

Digestive enzyme extraction

Small intestines were extracted with 50 mmol L^{-1} Tris–HCl buffer pH 8 containing 200 mmol L^{-1} NaCl (1:4, w/v) using a micro-homogenizer (THP-220; Omni International, Kennesaw, GA, USA). The homogenate was centrifuged at 15 000 × *g* for 30 min at 4 °C. The supernatant was collected and then kept at −20 °C.

Determination of α-amylase activity (EC 3.2.1.1)

Amylase activity was assayed at pH 8 and 25 °C. The reactions were performed according to Thongprajukaew[25] using 2% soluble starch (final concentration) as substrate. The absorbance at 540 nm was determined with a spectrophotometer and compared to standard maltose. Quantification of amylase activity was expressed as 'U' units.

In vitro carbohydrate digestibility

Digestive enzyme extracts were dialyzed overnight against extraction buffer. Carbohydrate digestibility of the modified PKM was determined using the method described by Thongprajukaew *et al.*[16] The reaction mixture contained 5 mg PKM, 10 mL 50 mmol L^{-1} phosphate buffer pH 8.2, 50 µL 0.5% chloramphenicol and 125 µL dialyzed crude enzyme extract, and was incubated at 25 °C for 24 h. Blanks without crude enzyme extracts were used as controls to measure the levels of sugar liberated from the PKM. Carbohydrate digestibility was determined by measuring the increase in reducing sugar after enzymatic reaction and then compared with maltose standard curve. Digestibility values of carbohydrate in each fish species were standardized by amylase activity and were expressed as µmol maltose g^{-1} PKM.

Statistical analysis

The experiments followed a completely randomized design (CRD). Data are reported as mean ± SEM. Significant differences between means were analyzed and ranked by one-way analysis of variance and by Duncan's multiple range test (DMRT) at 95% confidence levels, respectively.

RESULTS

Chemical composition

Differences in chemical composition between raw and modified PKMs are shown in Table 1. Crude protein was the highest in gamma-irradiated PKM. Increased lipid concentrations were found after electron beam and microwave modifications. Ash content decreased significantly in modified PKMs (except for electron beam modification). The largest decrease in ash was in soaked PKM, followed by microwave and gamma irradiation. Crude fiber from raw PKM decreased dramatically in soaked (17.62%), microwave-irradiated (33.95%), gamma–irradiated (37.72%) and electron beam modified (42.87%) PKM. This caused 1.09- and 1.17-fold increased carbohydrate components (nitrogen-free extract) in soaked and irradiated PKM, respectively.

Table 1. Chemical composition and some physicochemical properties of raw and pretreated PKM. Data were calculated from triplicate observations and are expressed on a dry matter basis

Parameter	Unmodified	Soaking	Microwave irradiation	Gamma irradiation	Electron beam
Chemical composition (g kg^{-1})					
Crude protein	144.8 ± 0.6b	150.1 ± 0.1ab	144.0 ± 2.4b	153.9 ± 1.5a	148.4 ± 3.0ab
Crude lipid	100.6 ± 0.1bc	99.3 ± 0.6c	101.4 ± 0.4b	99.0 ± 0.6c	106.2 ± 0.6a
Crude fiber	217.4 ± 5.6a	179.1 ± 0.3b	143.6 ± 0.3c	135.4 ± 4.0 cd	124.2 ± 5.2d
Ash	55.6 ± 0.4a	45.0 ± 1.0d	49.7 ± 0.8c	51.7 ± 0.8bc	53.4 ± 1.1ab
Nitrogen free extract	481.6 ± 5.6c	526.5 ± 1.2b	560.0 ± 2.6a	560.0 ± 4.4a	567.8 ± 6.1a
Physicochemical properties					
pH	5.23 ± 0.01b	5.36 ± 0.01a	5.37 ± 0.01a	5.21 ± 0.01b	5.23 ± 0.01b
Water solubility (g kg^{-1})	90.8 ± 2.2	101.2 ± 12.0	95.3 ± 5.2	94.7 ± 16.5	78.3 ± 5.7
Relative crystallinity (g kg^{-1})[a]	366.0 ± 0.1d	335.2 ± 0.1e	382.2 ± 0.1b	379.4 ± 0.1c	412.4 ± 0.1a

[a] Relative crystallinity was calculated from duplicate analysis.
Values with different letters in the same row indicate a significant difference ($P < 0.05$).

Physicochemical properties

pH

The modified method had a significant effect on pH of PKM ($P < 0.05$, Table 1). The highest values were observed in soaked and microwave-irradiated PKM. Gamma and electron beam irradiation appeared to have no effect on pH in this study ($P > 0.05$).

Water solubility

Solubility in water was significantly different among treated and untreated PKM ($P < 0.05$). However, no difference was observed between the modified groups ($P > 0.05$). Higher solubility was found in soaked PKM, followed by microwave-irradiated PKM, whereas lower values were found in electron beam modification.

Microstructure

Microstructure was clearly different among treated and untreated PKM (Fig. 2). Rough surface was mainly observed in soaked (Fig. 2D–F) and microwave-irradiated PKM (Fig. 2G–I) whereas smooth surface was formed after modification by gamma irradiation (Fig. 2J–L) and electron beam (Fig. 2M–O). Microstructure of raw and modified PKM was generally irregular.

Diffraction pattern

Modification of PKM affected the amorphous and crystalline regions. Similar diffraction patterns of main peaks (15.9°, 19.9° and 26.6°) were also found between treated and untreated PKM (Fig. 3). However, small differences in peaks were observed at diffraction angles (2θ) of 17.2–18.7° and 20.5–21.6°. Relative crystallinity (RC) of PKM was affected by modified methods (Table 1). The value decreased significantly by 8.4% with water soaking, whereas it increased by 3.7%, 4.45% and 12.7% with gamma irradiation, microwave irradiation and electron beam, respectively, compared with raw PKM.

FTIR spectra

FTIR spectra of raw and modified PKM were different (Fig. 4). Removal of waxes was determined by CH_2 stretching bands at

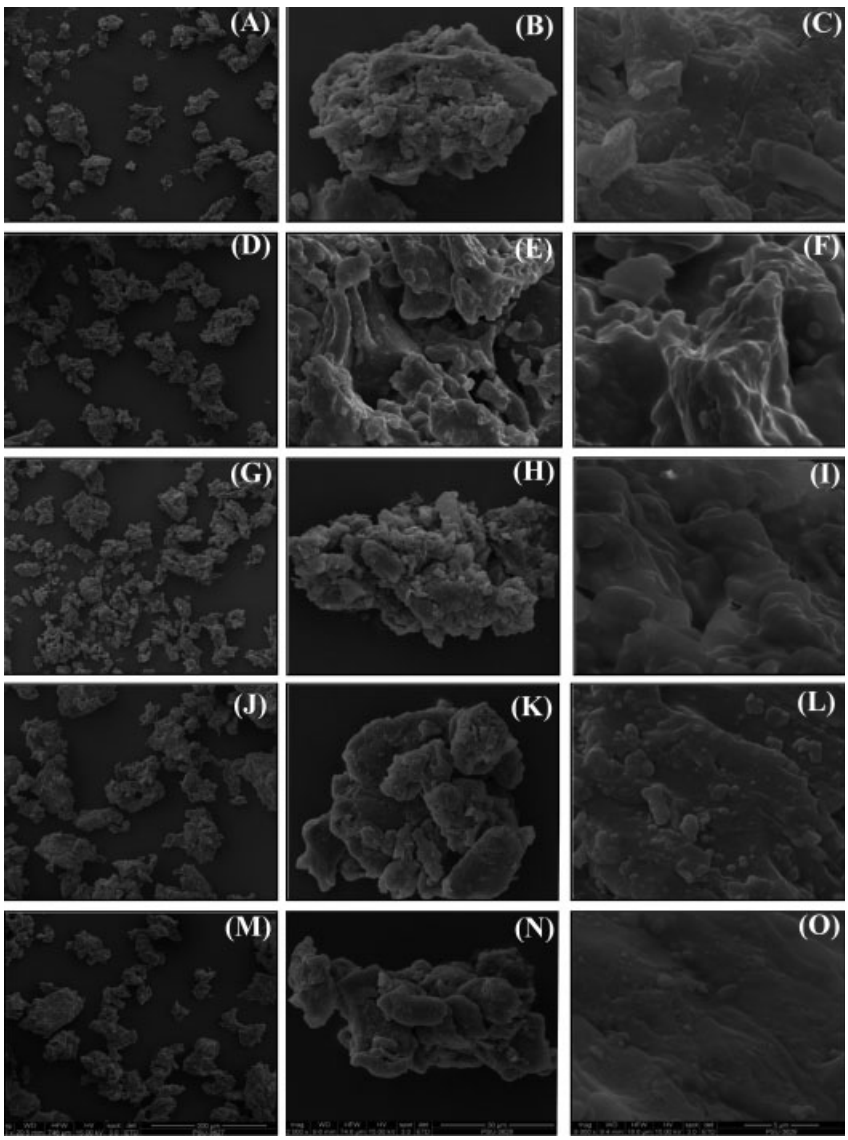


Figure 2. Microscopic structures of raw (A–C) and modified PKM: soaking (D–F), microwave irradiation (G–I), gamma irradiation (J–L) and electron beam (M–O). Magnifications of photographs are 200× (left), 2000× (middle) and 8000× (right).

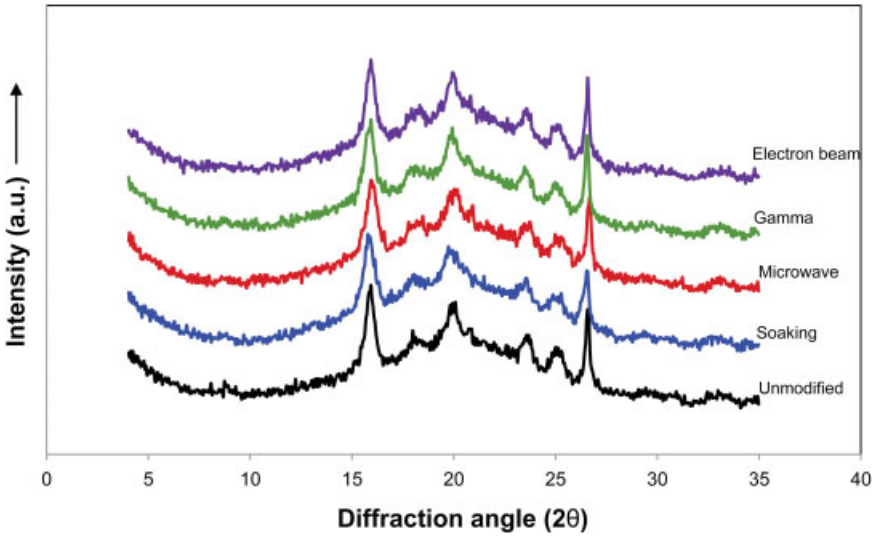


Figure 3. X-ray diffractograms of PKM: raw, modified by soaking, microwave irradiation, gamma irradiation and electron beam.

approximately 2850 and 2920 cm^{-1}.[21] Some spectral peaks of cellulose are incurred by C&bond;H vibration at 1320 cm^{-1},[23] and by other structural elements at 1381 and 1154 cm^{-1}.[22,24] The spectrum at 1745 cm^{-1} represents acetyl and uronic ester groups of hemicelluloses,[22] 1244 cm^{-1} represents the syringyl ring and C&bond;O stretch in lignin and xylan, and other hemicellolose spectral peaks occur at 1738 and 1090 cm^{-1} – these were also determined.[22] Findings from all FTIR spectra showed that soaking, gamma irradiation and microwave irradiation decreased waxes (Fig. 4A), cellulose, hemicelluloses and lignin in PKMs, whereas electron beam did not when compared with unmodified PKM (Fig. 4B). Similar results were found for the peak ratio between 1429 cm^{-1} (crystalline) and 893 cm^{-1} (amorphous). The ratios were lower in soaked (3.46), gamma-irradiated (2.35) and microwave-irradiated (3.13) PKM than in the raw PKM (3.67).

In vitro carbohydrate digestibility

Different modifications had a significant effect on carbohydrate digestibility (CD) in three fish species (Fig. 5). Gamma and electron beam modifications decreased the CD in walking catfish ($P < 0.05$, Fig. 5B) and striped snakehead ($P < 0.05$, Fig. 5C), whereas digestibility increased in Nile tilapia ($P < 0.05$, Fig. 5A). Soaking significantly increased CD in Nile tilapia ($P < 0.05$) and striped snakehead ($P < 0.05$) by 1.35- and 1.23-fold on average, respectively, when compared with control. However, no differences were observed among soaked, microwave-irradiated and raw PKM in walking catfish. Microwave irradiation significantly increased CD in striped snakehead ($P < 0.05$), whereas a slight increase was found in walking catfish ($P > 0.05$), when compared with raw PKM. On the other hand, significantly decreased CD of microwave-irradiated PKM was prominently found in Nile tilapia (Fig. 5A).

DISCUSSION

Chemical compositions of PKM

Alteration from crude fiber into available carbohydrates was not proportionally correlated with the increase of CD. This indicates that carbohydrate quality plays a key role in CD and is also more important than the quantity. Significant changes in chemical composition between raw and modified PKM were similar to findings for various foods or feed ingredients after modification, such as in chickpea[26] and fish feed mixtures.[16] However, no differences between treated and untreated raw materials were reported in Bengal gram, green gram, horse gram, canola seed and sorghum grain.[9,11,15] Slight decreases in protein contents after microwave modification in PKM are probably due to loss during cooking. This occurred concurrently with changed protein quality observed by differential scanning calorimeter (DSC) and *in vitro* protein digestibility (unpublished data).

Changes in lipid constituents are affected by modified methods mainly at the double bonds of unsaturated fatty acids. The changes may cause formation of saturated fatty acids or provide hydroperoxides and secondary oxidation products, as reported by Thongprajukaew[25] and Stewart *et al.*,[27] respectively. However, the occurrence depends on time and temperature of processing.[27,28] For ash, a significant decrease after modification is in agreement with other studies on boiling, autoclaving and microwave irradiation[26] and water soaking for 72 h.[29]

Crude fiber analysis and FTIR spectra indicated significant reductions in main cell wall constituents (cellulose, hemicelluloses and lignin) and waxes in modified PKM. Similar results have been reported in pretreated, delignified and steam-exploded rice straws[18] and gamma-irradiated wheat straw, cotton seed shell, peanut shell, soybean shell, extracted olive cake and extracted unpeeled sunflower seeds.[30] These changes are probably due to the destruction of lignocellulosic materials during modification. Reduction of structural carbohydrate can cause an increase in available nitrogen-free extract. This result indicates higher carbohydrate utilization for modified PKM than for the raw material.

Physicochemical properties and *in vitro* digestibility of PKM

Some factors affecting carbohydrate digestibility are summarized in Table 2. These data indicate that the relevant changes in physicochemical properties of PKM occurred after water soaking and microwave irradiation. The change in pH can play an important role in determining the liberation of functional groups from macromolecule breakdown after modification. Decreased pH could be due to the breakdown of starch molecules by

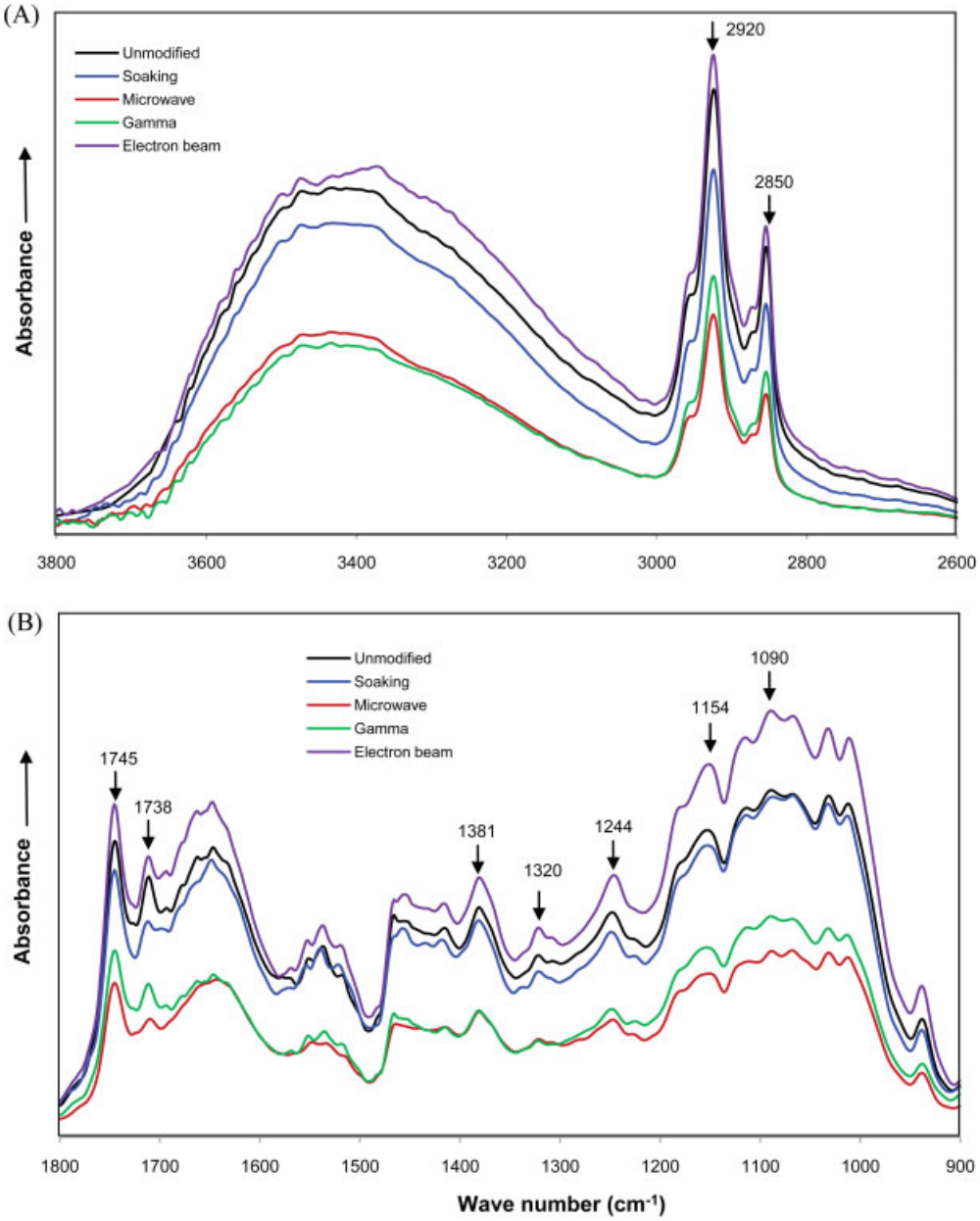


Figure 4. FTIR spectra of PKM: raw, modified by soaking, microwave irradiation, gamma irradiation and electron beam.

the action of free radicals, and then induce the formation of carboxyl groups.[17] On the other hand, an increase in pH of soaked PKM by release of hydroxyl groups from lignocellulosic degradation is postulated. Increased water solubility was also found in soaked PKM. This characteristic could contribute to the hydrolytic capacity of digestive enzymes.[12,16] Therefore, increased solubility in this modified method could improve the enzymatic digestion of PKM *in vitro*. Higher hydrolytic properties, enhancing enzymatic reactions of the PKM, are also indicated by the changes in morphology. Increased surface roughness after modification has been reported to improve the digestive capacity in various feed ingredients.[18,25] Therefore, modifications of PKM by this method might contribute to enzymatic hydrolysis in the alimentary tract of animals. Moreover, decreased RC in soaked PKM indicates an increase in the amorphous region which could contribute to enzymatic hydrolysis of carbohydrate, due to the negative correlation reported between RC and *in vitro* digestibility of rapidly and slowly digestible starches.[31]

Therefore, changes in physicochemical properties after water soaking support improving CD in Nile tilapia and striped snakehead. This finding is in agreement with prior findings in moth bean, black grams and chick pea.[8,32] This method has been widely used for reducing some antinutritional compounds, i.e. polyphenol, tannin, phytic acid and α-amylase inhibitor.[33] In an *in vivo* trial, supplementation of soaked *Sesbania* seeds in the diet of common carp (*Cyprinus carpio*) significantly improved growth performance and feed utilization.[34] Such prior success suggests that the use of soaked PKM for rearing Nile tilapia and striped snakehead, and optimizing the conditions for CD, such as soaking time and feedstuff–water ratio, should be further investigated.

Positive changes in physicochemical properties of microwave-irradiated PKM were similar to those observed in soaked PKM,

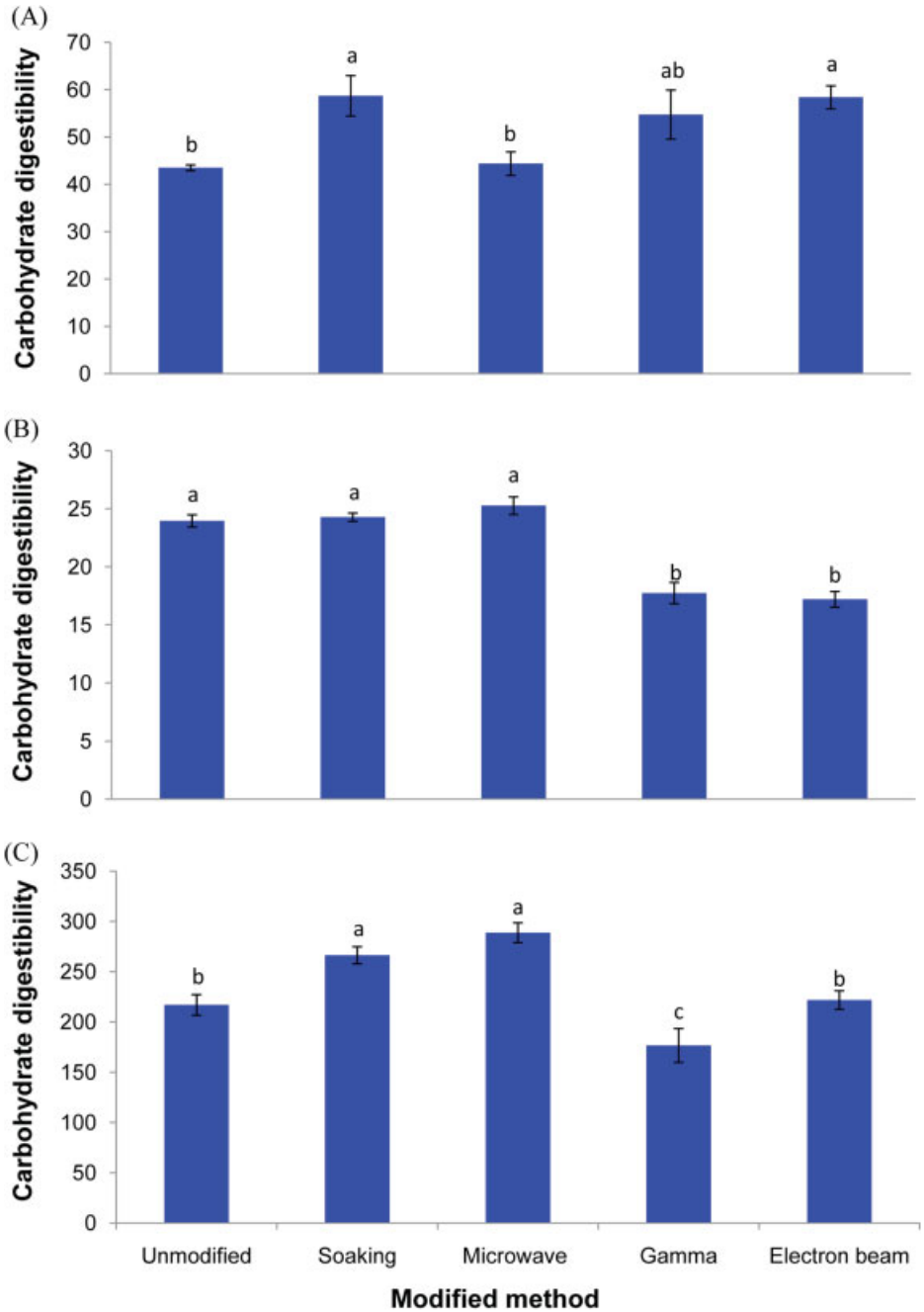


Figure 5. *In vitro* carbohydrate digestibility (μmol maltose g^{-1} PKM) of raw and modified PKMs, using digestive enzyme extracts from Nile tilapia (A, amylase 2000 U), walking catfish (B, amylase 1000 U) and striped snakehead (C, amylase 100 U). Analysis was performed in quadruplicate. Data with different superscripts are significantly different ($P < 0.05$).

whereas RC was negative (Table 2). These characteristics supported a positive effect on CD in walking catfish and striped snakehead, whereas there was a prominently negative result in Nile tilapia. These results indicate that microwave modification is effective for specific fish species, depending on their feeding habits. Increased apparent CD incurred by microwave modification has been reported in various legume seeds, such as moth bean, green gram, Bengal gram and horse gram,[8,9] as well as in the diet for Siamese fighting fish.[16] Increases in CD may be affected by starch gelatinization, amylose content, starch diameter and starch degradation.[10,35] Therefore, methods with optimal microwave modification, i.e. irradiation intensity, cooking time and feedstuff–water ratio, can probably further improve PKM quality.

Negative findings were similar between PKM modified by gamma and electron beam irradiation. The change in smooth surfaces was greater with electron beam than with gamma irradiation, as seen from a denser surface. This characteristic has been similarly found in gamma-irradiated starches from corn and potato when compared with native starches.[17,35] Increase of RC in both methods is similar to that found in rice straw modified by electron beam.[13] However, decreased RC of raw materials after modification by gamma irradiation has been reported.[17,35] The reported results may differ because the studies differ in the

Table 2. Summary of factors affecting carbohydrate digestibility of pretreated PKM in three fish species. Significantly positive (+) or negative (−) changes when compared with unmodified PKM ($P < 0.05$)

Positive finding	Soaking	Microwave irradiation	Gamma irradiation	Electron beam
Chemical composition				
Crude fiber	+	+	+	+
Nitrogen free extract	+	+	+	+
Physicochemical properties				
pH	+	+	ns	ns
Water solubility	ns	ns	ns	ns
Microstructure[a]	+	+	−	−
Relative crystallinity	+	−	−	−
Lignocellulosic constituent	+	+	+	−
In vitro carbohydrate digestibility				
Nile tilapia	+	ns	ns	+
Walking catfish	ns	ns	−	−
Striped snakehead	+	+	−	ns

[a] Positive (+) or negative (−) changes indicated by rougher surface structure than in unmodified PKM.
ns, not significant when compared with unmodified PKM ($P > 0.05$).

crystalline constituents of the raw materials and the conditions used. However, although gamma and electron beam modifications increased CD in Nile tilapia, both methods have high energy and equipment costs compared with conventional methods that had similar CD.

Digestibility among fish species was not comparable owing to their differences in various factors that affected carbohydrate utilization, such as growth stage, sex, genetics, feeding habit and rearing condition. Generally, omnivorous fish (Nile tilapia) is higher in amylase activity than in omnivorous fish with major meat-eating (walking catfish) and carnivorous fish (striped snakehead), respectively. However, basal amylase activity among each individual fish were adjusted to reduce the variation occurs within species. Therefore, utilizing *in vitro* digestibility techniques using fish crude enzyme extracts from specific fish and standardized by amylase activity is important in screening feedstuffs for developing formulated diets with high nutritional qualities for optimizing growth.[16,25]

CONCLUSION

Physical modifications of PKM had significant effects on chemical composition by decreasing crude fiber and increasing available carbohydrates. Soaking and microwave irradiation improved the determined physicochemical properties in ways that are expected to enhance enzymatic hydrolysis. Digestive enzymes extracted from Nile tilapia showed the highest efficiency for hydrolyzing carbohydrate from soaked PKM, whereas either soaked or microwave-irradiated PKM was best for striped snakehead. These *in vitro* findings indicate that soaking and microwave irradiation can improve CD in select economic freshwater fish. Therefore, both these methods for modifying PKM quality in aquaculture appear to have significant potential.

ACKNOWLEDGEMENTS

This research is partially supported by project grants from the Research and Development Office (PRPM ID 11908) and Department of Applied Science, Prince of Songkla University, Songkhla, Thailand. We acknowledge the Thailand Institute of Nuclear Technology (Public Organization) for kindly preparing gamma and electron beam irradiated PKM; and the Scientific Equipment Center, Prince of Songkla University, for help in sample analysis. The authors would like to thank Assoc. Prof. Seppo Karrila for proofreading and the Publication Clinic, Prince of Songkla University, for help in manuscript preparation.

REFERENCES

1 Agunbiade JA, Wiseman J and Cole DJA, Energy and nutrient use of palm kernels, palm kernel meal and palm kernel oil in diets for growing pigs. *Anim Feed Sci Technol* **80**:165–181 (1999).
2 Khadijat BM, Enoch OO and Suleiman BE, Performance, carcass characteristics and blood composition of broilers fed varying levels of palm kernel meal (*Elaise guinensis*) supplemented with different levels of fish meal. *Int J Poult Sci* **11**:73–77 (2012).
3 Dusterhoft EM and Voragen AGJ, Nonstarch polysaccharides from sunflower (*Helianthus annuus*) and palm kernel (*Elaeis guineensis*) meal: preparation of cell wall material and extraction of polysaccharide fractions. *J Sci Food Agric* **55**:411–422 (1991).
4 Choct M and Annison G, Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. *Br Poult Sci* **33**:821–834 (1992).
5 Ng WK, Lim HA, Lim SL and Ibrahim CO, Nutritive value of palm kernel meal pretreated with enzymes or fermented with *Trichoderma koningii* (Oudemans) as a dietary ingredient for red hybrid tilapia (*Oreochromis* sp.). *Aquacult Res* **33**:1199–1207 (2002).
6 Ng WK and Chen ML, Replacement of soybean meal with palm kernel meal in practical diets for hybrid Asian–African catfish, *Clarias macrocephalus* × *C. gariepinus*. *J Appl Aquacult* **12**:67–76 (2002).
7 Nasopoulou C and Zabetakis I, Benefits of fish oil replacement by plant originated oils in compounded fish feeds: a review. *LWT – Food Sci Technol* **47**:217–224 (2012).
8 Negi A, Boora P and Khetarpaul N, Effect of microwave cooking on the starch and protein digestibility of some newly released moth bean (*Phaseolus aconitifolius* Jacq.) cultivars. *J Food Comp Anal* **14**:541–546 (2001).
9 Khatoon N and Prakash J, Nutrient retention in microwave cooked germinated legumes. *Food Chem* **97**:115–121 (2006).
10 Sadeghi AA and Shawrang P, Effects of microwave irradiation on ruminal protein and starch degradation of corn grain. *Anim Feed Sci Technol* **127**:113–123 (2006).
11 Ebrahimi SR, Nikkhah A, Sadeghi AA and Raisali G, Chemical composition, secondary compounds, ruminal degradation and *in vitro* crude protein digestibility of gamma irradiated canola seed. *Anim Feed Sci Technol* **151**:184–193 (2009).

12 Chung HJ, Lee SY, Kim JH, Lee JW, Byun MW and Lim ST, Pasting characteristics and *in vitro* digestibility of γ-irradiated RS_4 waxy maize starches. *J Cereal Sci* **52**:53–58 (2010).
13 Bak JS, Ko JK, Han YH, Lee BC, Choi IG and Kim KH, Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation pretreatment. *Biores Technol* **100**:1285–1290 (2009).
14 Shawrang P, Mansouri MH, Sadeghi AA and Ziaie F, Evaluation and comparison of gamma and electron beam irradiation effects on total and free gossypol of cottonseed meal. *Radiat Phys Chem* **80**:761–762 (2011).
15 Shawrang P, Sadeghi AA, Behgar M, Zareshahi M and Shahhoseini G, Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chem* **125**:376–379 (2011).
16 Thongprajukaew K, Kovitvadhi U, Kovitvadhi S, Somsueb P and Rungruangsak-Torrissen K, Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910). *Aquaculture* **322–323**:1–9 (2011).
17 Chung HJ and Liu Q, Molecular structure and physicochemical properties of potato and bean starches as affected by gamma-irradiation. *Int J Biol Macromol* **47**:214–222 (2010).
18 Kristensen JB, Thygesen LG, Felby C, JØrgensen H and Elder T, Cell-wall structural changes in wheat straw pretreated for bioethanol production. *Biotechnol Biofuel* **1**:5–14 (2008).
19 AOAC, *Official Methods of Analysis of AOAC International* (18th edn). Association of Official Analytical Chemists, Gaithersburg, MD (2005).
20 Sokhey AS and Chinnaswamy R, Chemical and molecular properties of irradiated starch extrudates. *Cereal Chem* **70**:260–268 (1993).
21 Merk S, Blume A and Riederer M, Phase behavior and crystallinity of plant cuticular waxes studies by Fourier transform infrared spectroscopy. *Planta* **204**:44–53 (1998).
22 Fang JM, Sun RC and Tomkinson J, Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction. *Cellulose* **7**:87–107 (2000).
23 Pandey KK and Pitman AJ, FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *Int Biodeter Biodegr* **52**:151–160 (2003).
24 Rana R, Langenfeld-Heyser R, Finkeldey R and Polle A, FTIR spectroscopy, chemical and histochemical characterisation of wood and lignin of five tropical timber wood species of the family of Dipterocarpaceae. *Wood Sci Technol* **44**:225–242 (2010).
25 Thongprajukaew K, Feed development using digestive enzyme technology for successive growth in Siamese fighting fish (*Betta splendens* Regan, 1910). PhD thesis, Kasetsart University, Thailand (2011).
26 Alajaji SA and El-Adawy TA, Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *J Food Compos Anal* **19**:806–812 (2006).
27 Stewart OJ, Raghavan GSV, Orsat V and Golden KD, The effect of drying on unsaturated fatty acids and trypsin inhibitor activity in soybean. *Proc Biochem* **39**:483–489 (2003).
28 Malheiro R, Oliveira I, Vilas-Boas M, Falcão S, Bento A and Pereira JA, Effect of microwave heating with different exposure times on physical and chemical parameters of olive oil. *Food Chem Toxicol* **47**:92–97 (2009).
29 Akingbade AA, Sodeinde FG, Olaniyi CO, Oyetayo TS, Fadare OR and Rabiu AO, Proximate and mineral elements composition of water soaked *Carnavalia ensiformis* seeds. *Pakistan J Nutr* **8**:1401–1403 (2009).
30 Al-Masri MR and Guenther KD, Changes in digestibility and cell-wall constituents of some agricultural by-products due to gamma irradiation and urea treatments. *Radiat Phys Chem* **55**:323–329 (1999).
31 Kaur M, Sandhu KS and Lim ST, Microstructure, physicochemical properties and *in vitro* digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars. *Carbohyd Polym* **79**:349–355 (2010).
32 Rehman ZU, Domestic processing effects on available carbohydrate content and starch digestibility of black grams (*Vigna mungo*) and chick peas (*Cicer arietium*). *Food Chem* **100**:764–767 (2007).
33 Khandelwal S, Udipi SA and Ghugre P, Polyphenols and tannins in Indian pulses: effect of soaking, germination and pressure cooking. *Food Res Int* **43**:526–530 (2010).
34 Hossain MA, Focken U and Becker K, Effect of soaking and soaking followed by autoclaving of Sesbania seeds on growth and feed utilisation in common carp, *Cyprinus carpio L. Aquaculture* **203**:133–148 (2001).
35 Chung HJ and Liu Q, Effect of gamma irradiation on molecular structure and physicochemical properties of corn starch. *J Food Sci* **74**: 353–361 (2009).

ภาคผนวก ข

สำเนาบทความที่อยู่ระหว่างการแก้ไข

Physicochemical modifications of dietary palm kernel meal affect growth and feed utilization of Nile tilapia (*Oreochromis niloticus*)

Karun Thongprajukaew[a, b, *], Somrak Rodjaroen[c], Chutima Tantikitti[d], Uthaiwan Kovitvadhi[b, e]

[a] *Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand*

[b] *Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

[c] *Department of Bioscience, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang 92150, Thailand*

[d] *Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand*

[e] *Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

* Corresponding author at: Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand. Tel.: +66 74288562; Fax: +66 74446681.

E-mail address: karun.t@psu.ac.th (K. Thongprajukaew).

ABSTRACT

The effects of feed quality on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) were studied experimentally in a two-month feeding trial. Fish with an initial weight of 20.61 ± 0.15 g and initial length of 10.45 ± 0.03 cm were reared for 8 weeks in dietary treatment groups according to a completely randomized design (4 treatments × 3 replications). The isonitrogenous, isolipidic and isoenergetic feeds contained 200 g/kg palm kernel meal (PKM), and differed in the form of PKM that was either unprocessed (UPKM), water-soaked (SPKM), microwave-irradiated (MPKM), or water-soaked and microwave-irradiated (SMPKM). Physicochemical property changes in ways that are expected to enhance enzymatic digestion of the feed were determined, including pH, turbidity, microstructure, lignocellulosic spectra, diffraction pattern, and thermal transition parameters. These feed characteristics were linked with growth, feed conversion ratio, and visceral organ indices. The best fish growth and feed utilization were attained with SMPKM, followed by SPKM, and this matched the expectations from the feed physicochemical properties.

Keywords: Feed utilization; *Oreochromis niloticus*; Nile tilapia; Palm kernel meal; Physicochemical properties

1. Introduction

The physicochemical properties of various feed ingredients are related to their *in vitro* digestibility. Starch characteristics that relate to carbohydrate digestibility include gelatinization, amylose content, granule diameter, crystallinity and degradation (Sadeghi and Shawrang, 2006; Chung and Liu, 2009). Similarly, the digestibility of proteins is related to disulphide bonds, aspartic acid racemization (Rungruangsak-Torrissen et al., 2002), and subunit degradation (Ebrahimi et al., 2009). However, the relationships between the physicochemical properties and feed utilization have been investigated in only a few trials. Improvement of growth in Siamese fighting fish (*Betta splendens*) with modified feeds varying in the degree of gelatinization and water solubility, have been reported (Thongprajukaew et al., 2011). Peres and Oliva-Teles (2002) reported differences in growth and feed efficiency of European sea bass (*Dicentrarchus labrax*) that consumed three isonitrogenous and isolipidic feeds containing raw or gelatinized starch, despite a similar chemical composition of the feeds. This indicates that the physicochemical characteristics of the feed are practically important to the *in vivo* nutrient response in reared animals.

Physical modifications of palm kernel meal (PKM) by water soaking and microwave irradiation can improve its physicochemical characteristics and *in vitro* carbohydrate digestibility in Nile tilapia (*Oreochromis niloticus*) and striped snakehead (*Channa striata*) (Thongprajukaew et al., 2013). However, predictions of *in vivo* PKM digestibility based on the *in vitro* data are not necessarily accurate (Castagna et al., 1984; O' Mara et al., 1999). Typical underestimates are partly due to the presence of galactomannans, which are not easily hydrolyzed by the *in vitro* prepared enzymes. The use of this feedstuff at 200 g/kg or even higher inclusion levels has been optimal in feeds for Nile tilapia, *O. niloticus* (Ng et al., 2002), and hybrid Asian–African catfish, *Clarias macrocephalus* × *C. gariepinus* (Ng and Chen, 2002). Nevertheless, little information is currently available on the use of PKM in

aquafeed because its large amount of indigestible non-starch polysaccharides (from cell wall constituents), low protein content and amino acid deficiencies limit its use as a feed constituent (Ng, 2004). The destruction of cell wall barriers might help increase the use of PKM in aquafeeds.

The objective of this study was to investigate alternative physicochemical modifications of PKM, and whether the feed physicochemical properties can be related to growth and feed utilization of Nile tilapia (*O. niloticus*). Such physicochemical properties of feed that relate to the degree of *in vitro* hydrolysis were investigated, namely turbidity (Jacobson et al., 1997; Perera and Hoover, 1999), pH, microstructure, lignocellulosic constituents, diffraction pattern, and thermal transition properties (Chung and Liu, 2009, 2010; Kaur et al., 2010; Chumwaengwapee et al., 2013; Thongprajukaew et al., 2013). These analyses were done concurrently and compared to the responses in fish growth and feed utilization. The findings support the use of PKM as an aquafeed ingredient, and more generally support the physicochemical assessments of feed quality for improving animal nutrition.

2. Materials and methods

2.1. Preparation of PKM-based feeds

The physical modifications of PKM, which alter its physicochemical properties, were those described by Thongprajukaew et al. (2013). Ingredients and inclusion levels in the feeds are shown in Table 1, deviating slightly from the design used by Ng and Chen (2002). The 200 g/kg PKM was used in each dietary treatment, as unprocessed (UPKM), water-soaked (SPKM), microwave-irradiated (MPKM), or water-soaked and microwave-irradiated (SMPKM). The SPKM was prepared by soaking raw PKM in distilled water (1: 2 *w*/*v*) at room temperature for 12 h. The MPKM was prepared by placing 100 g of raw PKM in a

plastic box (20 cm diameter × 10 cm height), mixed with distilled water (1: 2 *w*/*v*) and then irradiating at 800 W in a microwave oven (MW 71B, Samsung, Malaysia) for 4 min. For SMPKM, the combination of both soaking and microwave irradiation was done in this order. The PKM was mixed with other feedstuffs (fish meal, soybean meal, alpha starch, corn flour and rice hull) along with additives, and 300 g/kg of water added for appropriate moisture content. The glutinous mixtures were passed through a hand pelletizer, dried at 60°C for 48 h, and then stored at 4°C until use in feeding.

2.2. Analysis of chemical composition

Samples of the experimental feeds were dried at 105^{o}C for 24 h before analyzing their chemical composition. The proximate composition, including crude protein, crude lipid, crude ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre, were determined according to standard methods of AOAC (2005). The nitrogen free extract (NFE, g/kg) was calculated from 1000 – (crude protein + crude lipid + crude ash + crude fibre). All the chemical analyses were done in triplicate and reported on a dry matter basis.

2.3. Physicochemical properties

2.3.1. Preparation of samples

The experimental feed samples were ground, dried using a freeze dryer (Delta 2-24 LSC, Germany) for 24 h, and then kept in a desiccator for later analysis of the physicochemical properties.

2.3.2. pH

One gram of an experimental feed was mixed with 25 mL of water at 25°C and agitated for 10 min (Sokhey and Chinnaswamy, 1993). The sample suspension was then probed with a pH meter (CyberScan 510, Eutech Instrument, Singapore).

2.3.3. Turbidity

The turbidity of each experimental feed was analyzed as described by Perera and Hoover (1999), with minor modifications. Briefly, an aqueous suspension (1% *w/v*) of the experimental feed was kept at 90°C for 1 h, under 100 rpm agitation. Subsequently, the suspension was cooled to 30°C and held for 1 h, and then stored at 4°C for 48 h. The light transmittance of the supernatant was then measured spectrophotometrically at 640 nm against a water blank.

2.3.4. Microstructure

Freeze-dried pellets were mounted with double-sided adhesive tape on an aluminum stub and coated with gold. Microscopic imaging was carried out by scanning electron microscopy (Quanta 400, FEI, Czech Republic) at 100, 1000 and 10000× magnifications. The accelerating voltage of the SEM was set at 20 kV.

2.3.5. Fourier transform infrared (FT-IR)

The lignocellulosic constituents were analyzed using an FT-IR spectrometer (Equinox 55, Bruker, Germany), based on the KBr technique. One milligram of dried experimental feed was mixed with 100 mg of KBr in a mortar, and the mixture was pressed at 10 MPa for 5 min to obtain a sample disc. The FT-IR spectra were taken from 4000 to 400 cm^{-1}, and the main alterations in lignocellulosic spectra were examined from 1800 to 900 cm^{-1}. The spectra were

comparable to those previously reported by Saikia et al. (1995), Fang et al. (2000), Szeghalmi et al. (2007), Rangel-Vázquez and Leal-García (2010), Yang et al. (2010), Fu et al. (2012), Watanabe et al. (2012) and Liu et al. (2013).

2.3.6. X-ray diffraction patterns

The diffraction patterns of experimental feeds were determined with an x-ray diffractometer (X' Pert MPD, Philips, Netherlands), operated at 40 kV voltage and 40 mA current. The diffractograms were recorded for 4 to 35° (2θ), with a scanning rate of 2^{o}/min.

2.3.7. Thermal transition properties

Onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and the transition enthalpy (ΔH), were measured with a differential scanning calorimeter (DSC7, Perkin Elmer, USA), and the calculated melting temperature range was defined as T_c–T_o. A three milligram feed sample was placed in an aluminum pan, sealed, allowed to equilibrate at room temperature for 1 h, and then heated from 40 to 400^{o}C at a rate of 10^{o}C/min.

2.4. Growth trial of Nile tilapia

Sixty-days-old Nile tilapia were obtained from Trang Agriculture and Technology College, Trang province. The fish were acclimatized for 10 days in fibreglass tanks (1 × 1 × 1 m) with 20-cm water levels, and fed twice daily to satiation with UPKM feed. Subsequently, the fish (20.61 ± 0.15 g initial weight and 10.45 ± 0.03 cm initial length) were randomly distributed into 12 aquaria (36 × 18 × 40 cm containing 20 L) at a density of 8 fish per aquarium. All the fish were fed with 7% of their body weight per day, twice daily (07.00 and 16.00 h), under a 12-h light/12-h dark cycle. Stocked water (25.0 ± 0.4^{o}C and 7.53 ± 0.10 mg/L dissolved oxygen) was used and the water was changed weekly, and the water quality

was measured on day 6 of each cycle. The average water temperature during the experiments was 24.78 ± 0.97^{o}C, and the dissolved oxygen was 7.28 ± 0.11 mg/L. Growth and feed utilization were recorded weekly during the experimental 8-week period.

2.5. Statistical analyses and calculations

A completely randomized design of experiments was used with four treatments and three replicates. Statistical analysis was performed using SPSS Version 14 (SPSS Inc., Chicago, USA). The results are summarized as mean and SEM. Significant differences between means were tested with Duncan's multiple range test, requiring $P < 0.05$. The growth parameters, feed conversion ratio and visceral organ indices were computed as follows:

$$\text{Condition factor (CF, g/cm}^3\text{)} = 100\ [\text{live body weight (g)/total body length (cm)}^3]$$

$$\text{Average daily gain (ADG, g/day)} = [W_t\ (g) - W_0\ (g)]/[t - t_0]$$

$$\text{Specific growth rate (SGR, \%/day)} = 100\ [\ln W_t - \ln W_0]/[t - t_0]$$

Here W_t = mean weight at day t, W_0 = mean weight at day t_0.

$$\text{Feed conversion ratio (FCR, g feed/g gain)} = \text{dry feed fed/wet weight gain}$$

$$\text{Viscerosomatic index (VSI, \%)} = 100\ (\text{wet weight of visceral organ/wet body weight})$$

$$\text{Stomasomatic index (SSI, \%)} = 100\ (\text{wet weight of stomach/wet body weight})$$

$$\text{Intestosomatic index (ISI, \%)} = 100\ (\text{wet weight of intestine/wet body weight})$$

3. Results

3.1. Chemical composition of experimental feeds

The chemical composition in terms of crude protein, lipid, ash, nitrogen free extract and gross energy was similar among the PKM-based feeds (Table 1). Significant increases in ADF and NDF were observed in modified PKM-based feeds, except for NDF in MPKM,

when compared with the control dietary treatment. Within the modified dietary groups, SPKM-based feed was lower in ADF but higher in NDF.

3.2. Physicochemical properties

3.2.1. pH

The pH was significantly lower for the feeds with processed PKMs than with UPKM ($P < 0.05$, Table 2).

3.2.2. Turbidity

Turbidity was significantly lower for the feeds containing processed PKMs than for the UPKM ($P < 0.05$, Table 2).

3.2.3. Microstructure

Significant differences were observed in the general morphology and surface roughness among the four experimental feeds (Fig. 1). The swelling and fusion of gelatinized starch granules was most prominent in SMPKM (Figs. 1j–l) followed by SPKM (Figs. 1d–f), whereas it was nearly absent in UPKM (Figs. 1a–c) and MPKM (Figs. 1g–i).

3.2.4. Lignocellulosic constituents

The spectral bands at 1078, 1416 and 1657 cm^{-1} represent the presence of lignin as indicated by β(1-3) polysaccharide (Szeghalmi et al., 2007), benzene ring stretching vibrations (Liu et al., 2013) and C=O in conjugated carbonyl groups (Yang et al., 2010), respectively. The 1461 and 1548 cm^{-1} bands are related to the skeleton stretching vibration of the aromatic rings and C–H deformation in lignin (Fu et al., 2012). Spectral bands at 1154 and 1381 cm^{-1} are designated as C–O symmetric stretching (Rangel-Vázquez and Leal-García,

2010) and C–H absorption (Saikia et al., 1995) of cellulose and hemicelluloses, respectively. Both lignin and hemicellulose constituents could be indicated by C–O stretching at 1028 cm^{-1} (Watanabe et al., 2012), and the band at 1244 cm^{-1} by syringyl ring and C–O stretching (Fang et al., 2000). These lignocellulosic (cellulose, hemicelluloses and lignin) spectra were different among the four experimental feeds (Fig. 2). Moreover, an analysis of the peak ratio between the crystalline and amorphous starch regions, namely 1047/1022 cm^{-1}, indicates a significant improvement of available constituents (amorphous) in SMPKM and SPKM relative to UPKM and MPKM (Table 2).

3.2.5. Diffraction patterns

Differences in the diffraction patterns were observed among the four PKM-based feeds (Fig. 3). Significant differences were observed in the two peaks at 15.9^{o} and 26.6^{o} (arrows in Fig. 3) between the processed PKMs and the UPKM. Both these peaks are found in the PKM constituent used in feeds, as reported by Thongprajukaew et al. (2013). Some other nearby peaks come from the other constituents (29.2^{o} and 31.5^{o}), while the peak at 28.0^{o} was prominent in SPKM but weak in UPKM and SMPKM.

3.2.6. Thermal transition properties

The modifications of PKM significantly affected the onset (T_o), peak (T_p) and conclusion temperatures (T_c) of the experimental feeds (Table 2). These thermal transition temperatures were very similar for UPKM and SPKM, while higher values were generally observed for MPKM and lower for SMPKM. A broad melting temperature range (T_c–T_o) was found in SMPKM, relative to UPKM and MPKM, whereas a narrower range was observed for SPKM. The thermal enthalpy (ΔH) was significantly lower for the three processed PKM-based feeds, especially for SMPKM and SPKM, relative to UPKM.

3.3. Growth and feed utilization of Nile tilapia

The growth and feed utilization of Nile tilapia are shown in Table 3. Body weight, total length, average daily gain (ADG), and specific growth rate (SGR) were significantly higher in tilapia fed with SMPKM or SPKM, relative to MPKM and UPKM. The body morphometry, as indicated by the condition factor (CF), showed faster skeletal growth in proportion to body weight for the fish fed with UPKM, and slower with MPKM. The feed conversion ratio (FCR) was significantly reduced by SMPKM and SPKM. Moreover, both treatments with soaked PKM correlated with increased visceral organ and gastrointestinal tract weights relative to UPKM and MPKM (Table 3).

4. Discussion

4.1. Chemical composition

Thongprajukaew et al. (2013) reported no differences in crude protein and significant reductions of ash, whereas the nitrogen free extract was increased by microwave irradiation and water soaking of PKM. This may suggest that with only 200 g/kg modified PKM in the feed, the overall nutritional composition is not significantly impacted by the changes in PKM. On the contrary, significant changes in the ADF and NDF content between modified and unmodified PKM-based feeds, as well as within the modified groups, indicate the complex physicochemical interactions between PKM and the other ingredients in the feed environments during the processing procedure. This presumption is strongly supported by the changed physical properties of feeds affected by water addition of the mash feed ingredients at 45^{o}C, in comparison to the untreated feed (Kraugerud and Svihus, 2011). The changes in our PKM-based feeds are similar to the increase of ADF and NDF in microwave-irradiated rice straw when compared with its untreated group (Ma et al., 2009). Ng and Chen (2002) and Ng et al. (2002) reported no significant differences in growth and feed utilization of Nile

tilapia when fed diets containing 400 g/kg of raw, fungal fermented, or enzyme-supplemented PKM, in comparison to the soybean meal-based feed (control). Effects on growth and feed utilization have also been reported in Siamese fighting fish, despite the similar chemical compositions of various modified feeds (Thongprajukaew et al., 2011). Similar findings are reported with the supplementation of soaked Sesbania seeds to improve the growth performance and feed utilization of common carp, *Cyprinus carpio* (Hossain et al., 2001), as well as those using soaked Leucaena seeds in African catfish, *Clarias gariepinus* (Sotolu and Faturoti, 2008). These findings indicate that the nutritional quality of a feed is not fully determined by its chemical composition, and can be manipulated with physicochemical alterations.

4.2. Physicochemical properties in relation to feed utilization

A significant reduction of pH in processed PKMs could be due to the breakdown of carbohydrates into smaller molecules, and the formation of carboxyl groups (Chung and Liu, 2010). This suggests that SPKM, MPKM and SMPKM might be better digestible than UPKM, due to their cleaved molecules. An increase of pH by water soaking and microwave irradiation of PKM has been previously observed (Thongprajukaew et al., 2013). The current finding is the opposite of that previously reported for PKM alone, indicating that the pH of the feed mixture is not a simple weighted sum of contributions from ingredients, but instead there may be complicated interactions.

Jacobson et al. (1997) reported that starch turbidity relates to granule swelling, granule remnants, leached amylose, and amylopectin chain length. The changes of turbidity are caused by interactions between leached amylose and amylopectin chains, which reflect or scatter light significantly (Perera and Hoover, 1999). The high turbidity of UPKM indicates strong aggregation of molecules, while the comparatively lower turbidity of processed PKMs

might be due to the molecules are smaller in size or a reduction in the number of leached molecules. Therefore, the turbidity results suggest that processed PKM might be hydrolyzed in the alimentary tract of an animal more easily than UPKM. The leached amylose and amylopectin are particularly easy to access and digest, so processed PKM should have a nutritional advantage.

Prominent swelling and fusion of starch granules in SMPKM and SPKM was due to water absorption during soaking, and then processing by the extruder for pelleting and drying. Water saturation of the soaked PKMs prior to pelleting improved the gelatinization of starch in the feed mixtures. Surface roughness was highest for SPKM. Thongprajukaew et al. (2013) reported that an increase in surface roughness of water-soaked PKM enhances the *in vitro* digestibility of carbohydrate with enzymes from Nile tilapia and striped snakehead. Similar findings have been reported in water-soaked coconut meal for Nile tilapia and silver barb, *Barbonymus gonionotus* (Chumwaengwapee et al., 2013). Surface roughness promotes the efficiency of enzymatic hydrolysis by increasing the surface-to-volume ratio and enabling a high enzyme loading volume (Ji et al., 2008). Overall, the microscopic observations suggested that SMPKM and SPKM should perform comparatively well in *in vivo* hydrolysis by fish digestive enzymes.

The data from diffraction patterns suggest changes in starch crystallinity, which may affect the overall feed utilization of tilapia. This finding is consistent with the change in 1047/1022 cm^{-1} peak ratio of the FT-IR spectra, which reflects the degree of order at the surfaces of starch granules (van Soest et al., 1995). The two diffraction peaks observed at 15.9^{o} and 26.6^{o} in this study are similar to a prior report on PKM (Thongprajukaew et al., 2013), whereas the other peaks observed were either due to the other ingredients in the feed formulations (29.2^{o} and 31.5^{o}), or potentially due to the interaction of feed ingredients induced by the water-soaking pretreatment (28.0^{o} for SPKM and SMPKM). Kaur et al. (2010)

reported a negative relationship between the *in vitro* digestibility of rapidly and slowly digestible starches and their relative crystallinity, based on digestion with amyloglucosidase and pancreatic α-amylase. This indicates a disruption of the crystalline barriers in the modified feeds, which might promote enzymatic hydrolysis. Similar findings on improved *in vitro* digestibility, by water-soaking and microwave-irradiating feedstuff, have been reported in Chumwaengwapee et al. (2013) and Thongprajukaew et al. (2013).

The observed effects on the thermal transition parameters (T_o, T_p, T_c, T_c–T_o and ΔH) suggested that the PKM modifications affected the overall molecular properties of feeds, although only 200 g/kg of PKM was used. A decrease in T_p could result from the weakening of starch granules affected by the modification (Tomasik and Zaranyika, 1995), which is consistent with our microscopic observations of SMPKM. The structure of starch granules is undesirable, and its destruction would facilitate enzymatic hydrolysis of the nutritional constituents. Bao and Corke (2002) suggested that the T_c–T_o might be increased by the heterogeneity of crystallites and reduced co-operative melting of the amorphous and crystalline regions in foodstuff. The broad T_c–T_o range of SMPKM, relative to UPKM, indicates heterogeneity of the polymer chain lengths due to cleavage by pretreatment. On the other hand, the narrower range in SPKM might indicate a narrow distribution of chain lengths. The changes in ΔH primarily reflect the loss of double helical order in starch (Cooke and Gidley, 1992), which would require more energy for structural transformation. Therefore, a low ΔH indicates a high portion of partially transformed macromolecules in the feed. This characteristic has correlated well with the degree of gelatinization, as observed in γ-irradiated RS_4 waxy maize starches (Chung et al., 2010). The findings in XRD, FT-IR and DSC profiles suggest a disruption of crystalline architecture concurrently with expansion of the amorphous region, in the SPKM and SMPKM feeds. These changes could effectively enhance *in vitro* hydrolysis of carbohydrates, and probably also the *in vivo* digestibility in animals.

4.3. Feed utilization of Nile tilapia in relation to feed physicochemical properties

The FCR of Nile tilapia correlates well with the overall feed physicochemical properties that are expected to affect the rate of enzymatic digestion, as discussed above. The PKMs that were soaked before extrusion exhibited superior physicochemical traits supporting feed utilization efficiency, when compared to feed with untreated PKM. Laboratory-based physicochemical characterization is less time consuming than an actual growth trial, and it is at least indicative, though not yet predictive, of growth performance. Induced increased weights of visceral organs and the gastrointestinal tract in both treatments with soaked PKM suggest an increased capacity for digestion and absorption of nutrients along the alimentary tract (Thongprajukaew et al., 2011). These changes are important for improving nutrient utilization, and also indicate the health of the fish gut, confirmed by a radical scavenging activity assay of gastrointestinal extracts (present work). The modified PKMs incorporated in feeds affected the nutritional status, and analyses of their effects on digestive enzyme activity, radical scavenging activity, carcass composition, and muscle quality is underway. The results overall support the significance of physicochemical feed modifications, and that these may be essential in future developments of aquafeed.

5. Conclusions

Both pH and turbidity distinguished between unprocessed and processed PKM-based feeds, whereas microstructure, FT-IR spectra, diffraction pattern and thermal transition parameters indicated the most suitable processing for maximizing growth and successful feed utilization of Nile tilapia. The type of processing of PKM affected various growth characteristics by about 200 g/kg or more, therefore, the effects were not only statistically but also practically significant. These findings suggest that modifying feed physicochemical properties provides a significant opportunity to improve feed quality for aquatic animals, and

that physicochemical characteristics may be useful in quality control of the feed. Our experiments also support a high inclusion level of processed PKMs (by water soaking alone or in combination with microwave irradiation) in Nile tilapia feed. In summary, physicochemical analyses of feed and the engineering of its physicochemical characteristics for improved nutritional quality have the potential to positively impact future aquaculture.

Acknowledgements

This research was funded by the Beginner Research Scholarship (Contract No. SCI5502895), Research and Development Office, Prince of Songkla University, Songkhla, Thailand. We would like to thank Dr. Seppo Karrila and the Publication Clinic, Prince of Songkla University, for their help in manuscript preparation.

References

AOAC, 2005. Official Methods of Analysis, Eighteenth ed. Association of Official Analytical Chemists, Washington, DC.

Bao, J., Corke, H., 2002. Pasting properties of γ-irradiated rice starches as affected by pH. J. Agric. Food Chem. 50, 336–341.

Castagna, A., Giger, S., Sauvant, D., 1984. Study of enzymatic degradability of concentrate feeds and by-products. Annales de Zootechnie 33, 265–270.

Chumwaengwapee, S., Soontornchai, S., Thongprajukeaw, K., 2013. Improving chemical composition, physicochemical properties, and *in vitro* carbohydrate digestibility of fish coconut meal. ScienceAsia 39, 636–642.

Chung, H.J., Lee, S.Y., Kim, J.H., Lee, J.W., Byun, M.W., Lim, S.T., 2010. Pasting characteristics and *in vitro* digestibility of γ-irradiated RS_4 waxy maize starches. J. Cereal Sci. 52, 53–58.

399 Chung, H.J., Liu, Q., 2009. Effect of gamma irradiation on molecular structure and
400 physicochemical properties of corn starch. J. Food Sci. 74, 353–361.
401 Chung, H.J., Liu, Q., 2010. Molecular structure and physicochemical properties of potato and
402 bean starches as affected by gamma-irradiation. Int. J. Biol. Macromol. 47, 214–222.
403 Cooke, D., Gidley, M.J., 1992. Loss of crystalline and molecular order during starch
404 gelatinization: origin of the enthalpic transition. Carbohydr. Res. 227, 103–112.
405 Ebrahimi, S.R., Nikkhah, A., Sadeghi, A.A., Raisali, G., 2009. Chemical composition,
406 secondary compounds, ruminal degradation and *in vitro* crude protein digestibility of
407 gamma irradiated canola seed. Anim. Feed Sci. Technol. 151, 184–193.
408 Fang, J.M., Sun, R.C., Tomkinson, J., 2000. Isolation and characterization of hemicelluloses
409 and cellulose from rye straw by alkaline peroxide extraction. Cellulose 7, 87–107.
410 Fu, J., Zhang, X., Yu, C., Guebitz, G.M., Cavaco-Paulo, A., 2012. Bioprocessing of bamboo
411 materials. Fibres Text. East. Eur. 20, 13–19.
412 Hossain, M.A., Focken, U., Becker, K., 2001. Effect of soaking and soaking followed by
413 autoclaving of Sesbania seeds on growth and feed utilisation in common carp, *Cyprinus*
414 *carpio* L. Aquaculture 203, 133–148.
415 Jacobson, M.R., Obanni, M., BeMiller, J.N., 1997. Retrogradation of starches from different
416 botanical sources. Cereal Chem. 79, 183–192.
417 Ji, J., Zhang, Y., Zhou, X., Kong, J., Tang, Y., Liu, B., 2008. Enhanced protein digestion
418 through the confinement of nanozeolite-assembled microchip reactors. Anal. Chem. 80,
419 2457–2463.
420 Kaur, M., Sandhu, K.S., Lim, S.T., 2010. Microstructure, physicochemical properties and *in*
421 *vitro* digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars.
422 Carbohydr. Polym. 79, 349–355.

423 Kraugerud, O.F., Svihus, B., 2011. Effects of online pretreatment of plant ingredients on
424 processing response and physical properties in extruded fish feed. Anim. Feed Sci.
425 Technol. 168, 250–256.
426 Liu, H.M., Li, M.F., Yang, S., Sun, R.C., 2013. Understanding the mechanism of cypress
427 liquefaction in hot-compressed water through characterization of solid residues.
428 Energies 6, 1590–1603.
429 Ma, H., Liu, W.W., Chen, X., Wu, Y.J., Yu, Z.L., 2009. Enhanced enzymatic saccharification
430 of rice straw by microwave pretreatment. Biores. Technol. 100, 1279–1284.
431 Ng, W.K., 2004. Researching the use of palm kernel cake in aquaculture feeds. Palm Oil Dev.
432 41, 19–21.
433 Ng, W.K., Chen, M.L., 2002. Replacement of soybean meal with palm kernel meal in
434 practical diets for hybrid Asian–African catfish, *Clarias macrocephalus* × *C.*
435 *gariepinus*. J. Appl. Aquacult. 12, 67–76.
436 Ng, W.K., Lim, H.A., Lim, S.L., Ibrahim, C.O., 2002. Nutritive value of palm kernel meal
437 pretreated with enzymes or fermented with *Trichoderma koningii* (Oudemans) as a
438 dietary ingredient for red hybrid tilapia (*Oreochromis* sp.). Aquacult. Res. 33, 1199–
439 1207.
440 O' Mara, F.P., Mulligan, F.J., Cronin, E.J., Rath, M., Caffrey, P.J., 1999. The nutritive value
441 of palm kernel meal measured *in vivo* using rumen fluid and enzymatic techniques.
442 Livest. Prod. Sci. 60, 305–316.
443 Perera, C., Hoover, R., 1999. Influence of hydroxypropylation on retrogradation properties of
444 native, defatted and heat-moisture treated potato starch. Food Chem. 64, 361–375.
445 Peres, H., Oliva-Teles, A., 2002. Utilization of raw and gelatinized starch by European sea
446 bass (*Dicentrarchus labrax*) juveniles. Aquaculture 205, 287–299.

447 Rangel-Vázquez, N.A., Leal-García, T., 2010. Spectroscopy analysis of chemical
448 modification of cellulose fibers. J. Mex. Chem. Soc. 54, 192–197.
449 Rungruangsak-Torrissen, K., Rustad, A., Sunde, J., Eiane, S.A., Jensen, H.B., Opstvedt, J.,
450 Nygård, E., Samuelsen, T.A., Mundheim, H., Luzzana, U., Venturini, G., 2002. *In vitro*
451 digestibility based on fish crude enzyme extract for prediction of feed quality in growth
452 trials. J. Sci. Food Agric. 82, 644–654.
453 Sadeghi, A.A., Shawrang, P., 2006. Effects of microwave irradiation on ruminal protein and
454 starch degradation of corn grain. Anim. Feed Sci. Technol. 127, 113–123.
455 Saikia, C.N., Ali, F., Goswami, T., Ghosh, A.C., 1995. Esterification of high α-cellulose
456 extracted from *Hibiscus cannabinus* L. Ind. Crop. Prod. 4, 233–239.
457 Sokhey, A.S., Chinnaswamy, R., 1993. Chemical and molecular properties of irradiated starch
458 extrudates. Cereal Chem. 70, 260–268.
459 Sotolu, A.O., Faturoti, E.O., 2008. Digestibility and nutritional values of differently processed
460 *Leucaena leucocephala* (Lam. deWit) seed meals in the diet of African catfish (*Clarias*
461 *gariepinus*). Middle East J. Sci. Res. 3, 190–199.
462 Szeghalmi, A., Kaminskyj, S., Gough, K.M., 2007. A synchrotron FT-IR microspectroscopy
463 investigation of fungal hyphae grown under optimal and stressed conditions. Anal.
464 Bioanal. Chem. 387, 1779–1789.
465 Thongprajukaew, K., Kovitvadhi, U., Kovitvadhi, S., Somsueb, P., Rungruangsak-Torrissen,
466 K., 2011. Effects of different modified diets on growth, digestive enzyme activities and
467 muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910).
468 Aquaculture 322–323, 1–9.
469 Thongprajukaew, K., Yawang, P., Dudae, L., Bilanglod, H., Dumrongrittamatt, T., Tantikitti,
470 C., Kovitvadhi, U., 2013. Physical modification of palm kernel meal improved available

carbohydrate, physicochemical properties and *in vitro* digestibility in economic freshwater fish. J. Sci. Food Agric. 93, 3832–3840.

Tomasik, P., Zaranyika, M.F., 1995. Nonconventional methods of modification of starch. Adv. Carbohydr. Chem. Biochem. 51, 243–318.

van Soest, J.J.G., Tournois, H., de Wit, D., Vliegenthart, J.F.G., 1995. Short-range structure in (partially) crystalline potato starch determined by Fourier-transform IR spectroscopy. Carbohydr. Res. 279, 201–214.

Watanabe, T., Shino, A., Akashi, K., Kikuchi, J., 2012. Spectroscopic investigation of tissue-specific biomass profiling for *Jatropha curcas* L. Plant Biotechnol. 29, 163–170.

Yang, X., Zeng, Y., Zhang, X., 2010. Influence of biopretreatment on the character of corn stover lignin as shown by thermogravimetric and chemical structural analyses. BioRes. 5, 488–498.

Figure captions

Fig. 1. The microstructures of PKM-based feeds incorporating UPKM (a–c), SPKM (d–f), MPKM (g–i) and SMPKM (j–l). SEM images at magnifications 100× (left panel), 1000× (middle panel) and 10000× (right panel).

Fig. 2. The FT-IR spectra of PKM-based feeds containing UPKM, SPKM, MPKM and SMPKM. The spectra cover the wave number range 1800–900 cm^{-1}. The arrows indicate specific spectral bands (cm^{-1}) of each lignocellulosic constituent.

Fig. 3. The diffractograms of PKM-based feeds containing UPKM, SPKM, MPKM and SMPKM. The diffraction angles are in the range of 4–35^{o} (2θ). The arrows indicate significant variation in the peaks at 15.9^{o} and 26.6^{o}.

Table 1 Formulations and chemical compositions of PKM-based feeds used for rearing Nile tilapia.

Ingredients and composition	UPKM	SPKM	MPKM	SMPKM
Ingredient (g/kg)				
Fish meal	305	305	305	305
Soybean meal	195	195	195	195
Unprocessed palm kernel meal	200	–	–	–
Water-soaked palm kernel meal	–	200	–	–
Microwave-irradiated palm kernel meal	–	–	200	–
Water-soaked and microwave-irradiated palm kernel meal	–	–	–	200
Alpha starch	50	50	50	50
Corn flour	120	120	120	120
Cod liver oil	20	20	20	20
Palm oil	30	30	30	30
Vitamin premix*	30	30	30	30
Mineral premix**	30	30	30	30
Rice hull	20	20	20	20
Chemical composition (g/kg on dry matter)				
Crude protein	272	271	278	268
Crude lipid	89	85	84	92
Acid detergent fibre	94	117	157	146
Neutral detergent fibre	262	277	266	272
Crude ash	101	104	101	104
Nitrogen free extract	448	444	434	441
Gross energy (kJ/g)	17.62	17.38	17.33	17.53

* Vitamin premix, 1 kg of feed contained 1000 mg vitamin B_1, 1000 mg vitamin B_2, 2 mg vitamin B_{12}, 55 g vitamin C, 400 mg vitamin K_3, 1000 mg inositol and 1000 mg choline chloride.

** Mineral premix, 1 kg of feed contained 5000 mg calcium oxide, 11430 mg alumina, 1000 mg ferric oxide, 50 mg manganese oxide, 700 mg magnesium, 60000 mg silica, 5000 mg potassium oxide, 20 mg phosphorus pentoxide, 30 mg nitrogen, 2000 mg sodium oxide, 700 mg zinc, 50 mg iron, 70 mg selenium, 120 mg copper, 200 mg iodine, 20 mg cobalt, 260 mg molybdenum and 70 mg vanadium.

UPKM, unprocessed palm kernel meal feed; SPKM, water-soaked palm kernel meal feed; MPKM, microwave-irradiated palm kernel meal feed; SMPKM, water-soaked and microwave-irradiated palm kernel meal feed.

Table 2 Some physicochemical properties of PKM-based feeds used for rearing Nile tilapia.

Physicochemical property	UPKM	SPKM	MPKM	SMPKM	SEM	*P* value
pH	6.32^{a}	6.25^{b}	6.27^{b}	6.27^{b}	< 0.01	0.019
Turbidity (A_{640})	0.367^{a}	0.144^{b}	0.133^{b}	0.160^{b}	0.03	< 0.001
FT-IR intensity ratio (1047/1022 cm^{-1})	0.9647^{b}	0.9637^{c}	0.9875^{a}	0.9647^{b}	< 0.01	< 0.001
Thermal transition properties						
1. T_o (^oC)	260.78^{c}	262.44^{b}	266.02^{a}	259.56^{d}	0.81	< 0.001
2. T_p (^oC)	267.25^{a}	267.00^{a}	267.75^{a}	265.08^{b}	0.34	< 0.001
3. T_c (^oC)	269.27^{c}	270.46^{b}	274.41^{a}	270.74^{b}	0.64	< 0.001
4. T_c–T_o (^oC)	8.49^{b}	8.02^{c}	8.39^{b}	11.18^{a}	0.42	< 0.001
5. ΔH (J/g)	6.73^{a}	2.87^{d}	3.70^{b}	3.22^{c}	0.51	< 0.001

UPKM, unprocessed palm kernel meal feed; SPKM, water-soaked palm kernel meal feed; MPKM, microwave-irradiated palm kernel meal feed; SMPKM, water-soaked and microwave-irradiated palm kernel meal feed.
Data are expressed as mean and SEM from triplicate determinations.
Differences between means were tested with Duncan's multiple range test.
Different superscripts in the same row indicate a significant difference ($P < 0.05$).

Table 3 Growth performance, organ indices and feed conversion ratio of Nile tilapia reared using PKM-based feeds.

Parameter	UPKM	SPKM	MPKM	SMPKM	SEM	*P* value
Body weight (g)	69.16^{bc}	77.45^{ab}	65.84^{c}	82.02^{a}	2.15	0.013
Total length (cm)	13.80^{b}	14.61^{a}	14.12^{ab}	14.87^{a}	0.14	0.043
Visceral organ weight (g)	7.68^{a}	8.21^{a}	4.51^{b}	8.13^{a}	0.51	0.004
Viscerosomatic index (VSI, %)	7.88^{a}	8.97^{a}	6.00^{b}	9.23^{a}	0.42	0.003
Stomach weight (g)	1.73^{a}	2.04^{a}	0.44^{b}	2.10^{a}	0.22	0.001
Stomasomatic index (SSI, %)	1.75^{a}	2.27^{a}	0.91^{b}	2.42^{a}	0.22	0.006
Intestinal weight (g)	5.06^{a}	4.99^{a}	3.33^{b}	4.57^{a}	0.23	0.002
Intestosomatic index (ISI, %)	5.21^{a}	5.41^{a}	4.37^{b}	6.04^{a}	0.20	0.053
Condition factor (CF, g/cm^3)	2.63^{a}	2.48^{ab}	2.33^{b}	2.50^{ab}	0.03	0.127
Average daily gain (ADG, g/day)	0.82^{b}	0.96^{a}	0.77^{b}	1.04^{a}	0.04	0.008
Specific growth rate (SGR, %/day)	2.01^{b}	2.25^{a}	1.98^{b}	2.36^{a}	0.05	0.003
Feed conversion ratio (FCR, g feed/g gain)	1.89^{ab}	1.63^{b}	2.10^{a}	1.50^{b}	0.08	0.047

UPKM, unprocessed palm kernel meal feed; SPKM, water-soaked palm kernel meal feed; MPKM, microwave-irradiated palm kernel meal feed; SMPKM, water-soaked and microwave-irradiated palm kernel meal feed.
Data are expressed as mean and SEM from triplicate determinations.
Differences between means were tested with Duncan's multiple range test.
Different superscripts in the same row indicate a significant difference ($P < 0.05$).

Figure
Click here to download high resolution image

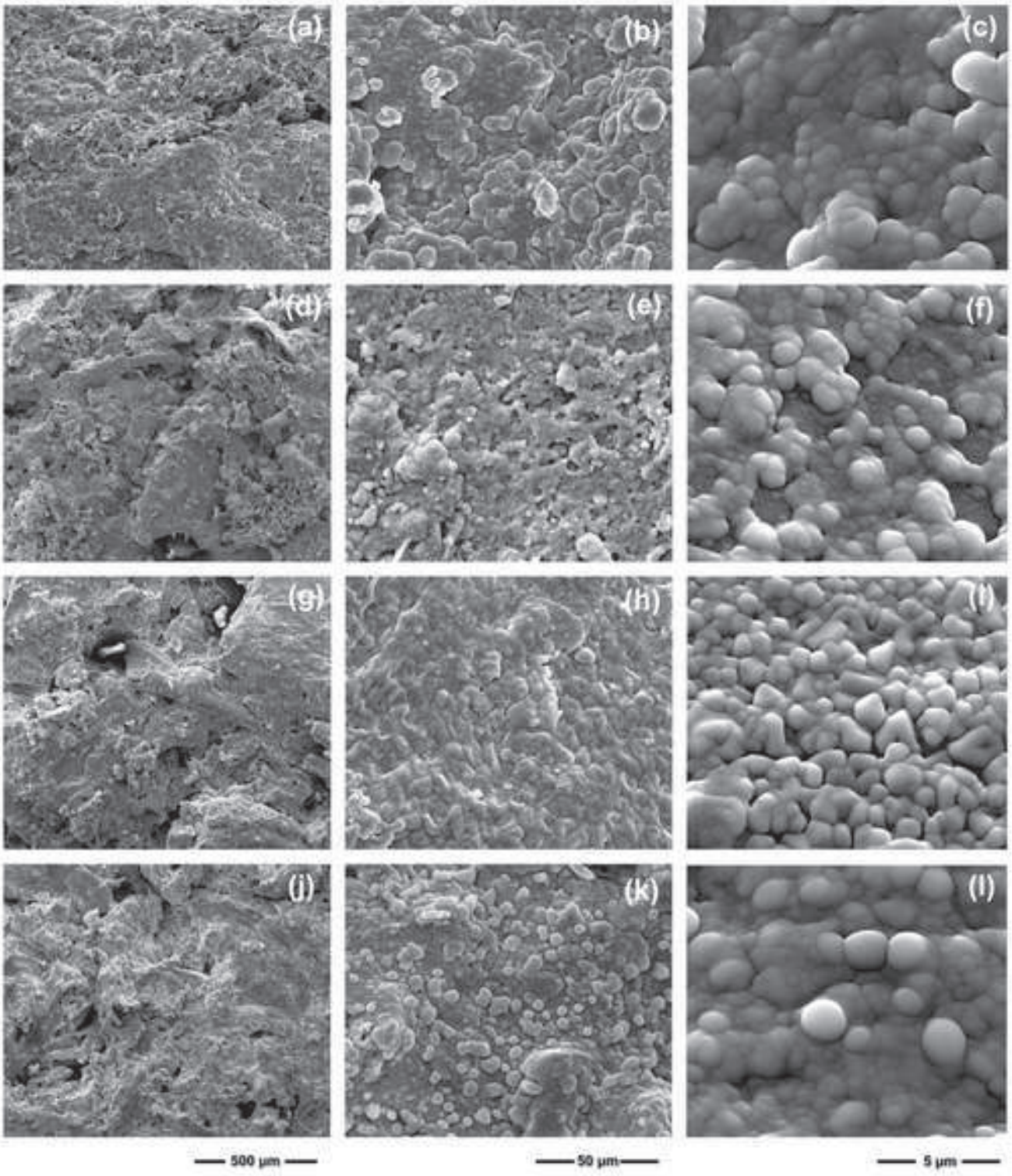
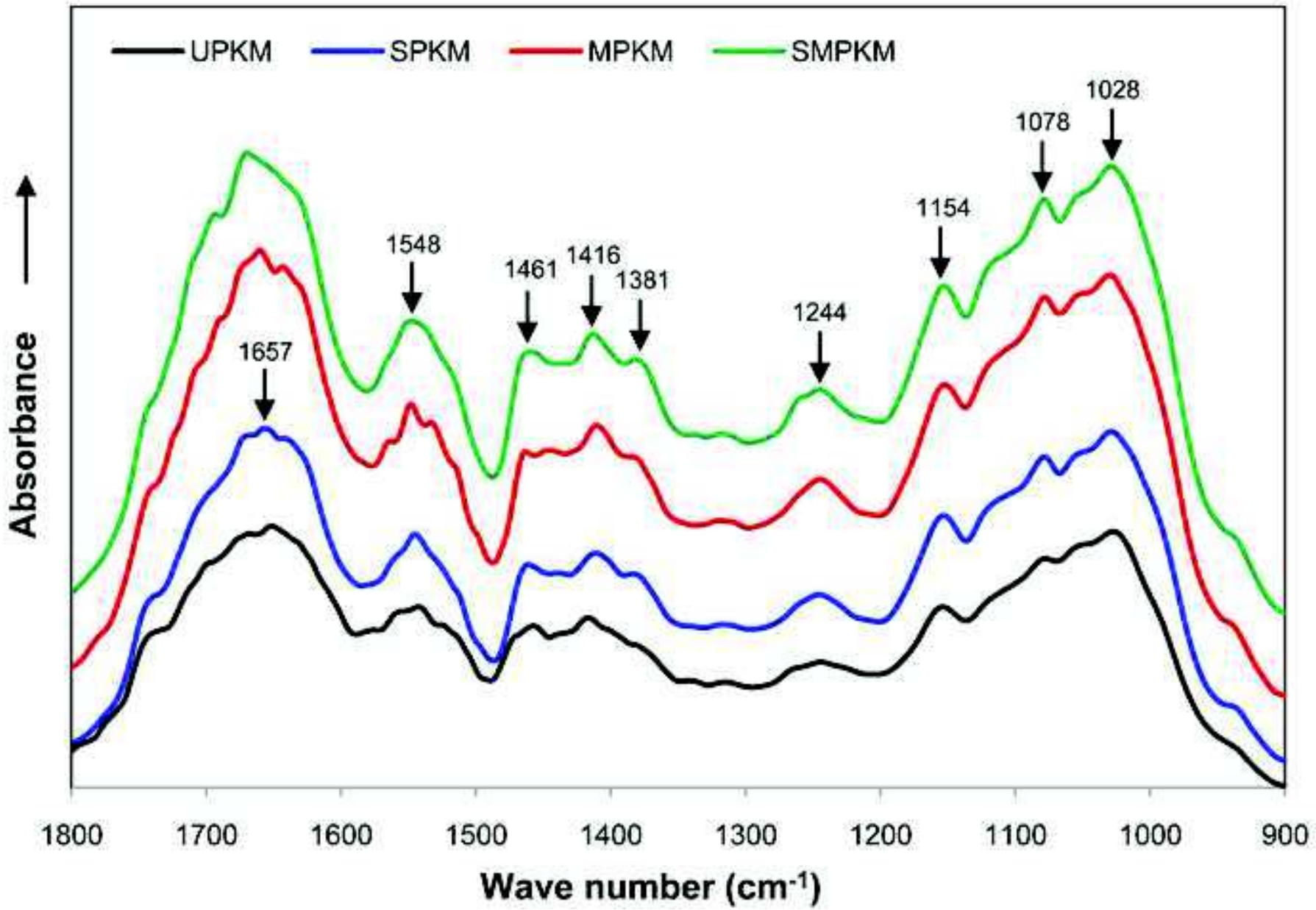
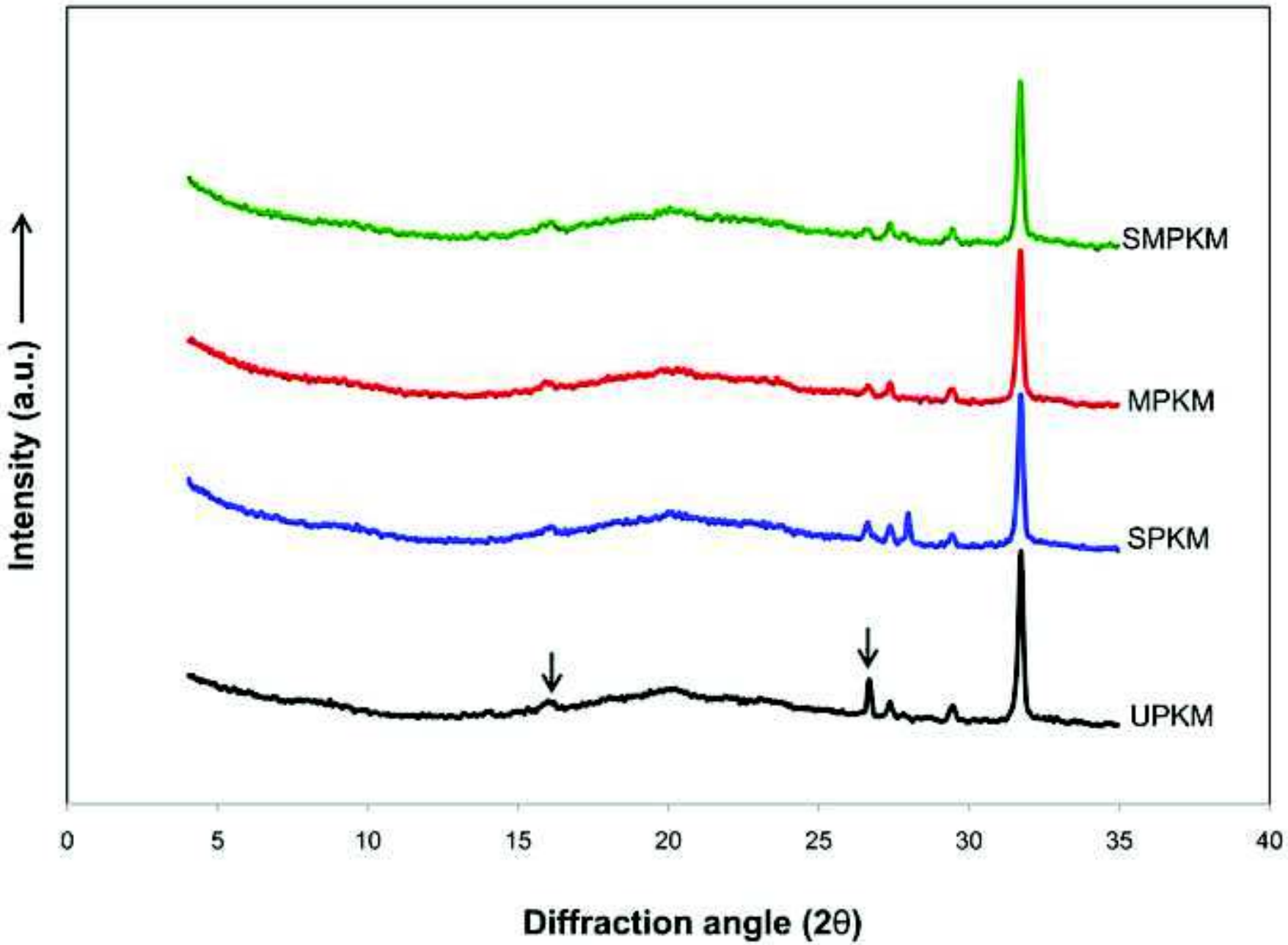


Figure
Click here to download high resolution image



Figure

Click here to download high resolution image



Effects of dietary modified palm kernel meal on growth, feed utilization, radical scavenging activity, carcass composition and muscle quality in sex reversed Nile tilapia (*Oreochromis niloticus*)

Karun Thongprajukaew[a, b, *], Somrak Rodjaroen[c], Piyanuch Sornthong[a], Nittaya Hutcha[a], Krueawan Yoonram[a], Chutima Tantikitti[d], Uthaiwan Kovitvadhi[b, e]

[a] *Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand*

[b] *Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

[c] *Department of Bioscience, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang 92150, Thailand*

[d] *Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112, Thailand*

[e] *Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

* Corresponding author: Tel: +66 74288562; Fax: +66 74446681.

E-mail address: karun.t@psu.ac.th (K. Thongprajukaew).

Abstract

Effects of dietary modified palm kernel meal (PKM) were investigated on growth performance, feed utilization, digestive enzyme specific activities, radical scavenging activity, carcass and muscle compositions, and muscle quality in sex reversed Nile tilapia (*Oreochromis niloticus*). The experiment was conducted in a completely randomized design with triplicate observations (4 treatments × 3 replicates × 8 fish per aquarium) during ten weeks of duration. The Nile tilapia (20.61 ± 0.15 g initial body weight and 10.45 ± 0.03 cm initial length) were fed with four isonitrogenous, isolipidic and isoenergetic diets containing 20% PKM, the treatments differing only in the nature of the PKM. It was either unprocessed (UPKM), water-soaked (SPKM), microwave-irradiated (MPKM), or water-soaked and microwave-irradiated (SMPKM). The fish fed with SPKM and SMPKM diets were superior in specific growth rate ($P < 0.05$) and feed conversion ratio ($P > 0.05$) when compared with the UPKM treatment. The digestion capacity of fish also slightly improved with both these dietary treatments, as indicated by the specific activities of trypsin and amylase ($P > 0.05$). Only the fish fed with SPKM were adapted to digest lipids as compared to the control. Significant improvements in the radical scavenging activity of stomach were observed with MPKM and SMPKM treatments, but the levels were unaffected in liver and intestine. No negative effects were found on scavenging activity in the three organs, in fish fed SPKM diet as compared to the control. The carcass and muscle compositions were closely similar across the four dietary treatments, with only small improvements from the SPKM and the SMPKM diets in terms of carcass moisture and ash, and muscle protein and RNA/protein ratio. No differences were observed in the enthalpy responses of muscle actin and myosin between the four dietary treatments, but an inducible protein peak was found only in the fish fed with SPKM. Overall, these findings indicate that water soaking or soaking followed by microwave irradiation improved the nutritional quality of the PKM-containing diet. Practically, the

simple low-cost modification by water soaking has wide application potential in the administration of aquafeed.

Keywords: Digestive enzyme; Muscle; Nile tilapia; Palm kernel meal; Radical scavenging activity; Soaking

1. Introduction

Palm kernel meal (PKM) is a common by-product from palm oil production, and is generally used as fodder for terrestrial animals (Esuga et al., 2008; Khadijat et al., 2012). In South-East Asian and African countries that are global leaders in the oil palm industry, PKM offers a cheap and readily available feed resource for use in fish feed formulations (Ng and Chen, 2002; Ng et al., 2002). Over 2 million tonnes of PKM is produced annually, of which 99% is exported mainly to the European Union (Zahari et al., 2012). Among aquatic animals, a 20% or even higher PKM fraction in the diet has been optimal in Nile tilapia, *Oreochromis niloticus* (Ng et al., 2002) and hybrid Asian-African catfish, *Clarias macrocephalus* × *C. gariepinus* (Ng and Chen, 2002). The limited nutritional benefits from this feed constituent are due to its large content of cell wall materials, its' low protein content, and amino acid deficiencies (Ng and Chen, 2002; Ng et al., 2002). Recently, modifications of PKM by water soaking or microwave irradiation have been successful in the destruction of cell wall barriers, which has improved the nutritional quality in terms of *in vitro* physicochemical properties and digestibility (Thongprajukaew et al., 2013b). However, the *in vivo* effects of these modified PKMs on growth and feed utilization need to be assessed by feeding trials.

To assess such response by physiological acclimatization, the digestive enzyme activities in the alimentary tract may be helpful. Thongprajukaew et al. (2011) reported responses in the digestive enzymes of Siamese fighting fish (*Betta splendens*) to diets differing in product form and quality, while these diets were similar in their proximate chemical compositions. Similarly, significant improvements in the protein utilization, the amylase activity, and the carbohydrate digestibility of rohu (*Labeo rohita*) correlated positively with gelatinized starch content in the diet (Mohapatra et al., 2002). Both these prior findings indicate the important role of form and quality, as opposed to only chemical composition.

For sex reversed Nile tilapia, the supplementation of PKM at 15–30% did not have any negative effects on survival, hematological parameters, or liver histopathology over ten weeks of rearing (Sukasem and Ruangsri, 2007). Similarly, African carp (*Labeo senegalensis*) fed with a fish meal-based diet containing 10% PKM showed no evidence of nutritional pathology (Omoregie, 2001). Recently, the assaying of radical scavenging activities has been used to assess feed ingredient quality (Kim et al., 2008; Behgar et al., 2011). This biochemical technique is sensitive and reliable, and could be applied to antioxidant activities affected by high-fat feedstuffs (Behgar et al., 2009) such as PKM. Moreover, while rapid growth of reared fish is desired, negative impacts on their carcass and muscle compositions (Thongprajukaew et al., 2011) or the muscle quality of consumed fish should be avoided.

Enzyme supplementation (Ng and Chen, 2002) and fermentation by cellulolytic (Ng et al., 2002) or cocktail enzymes (Esuga et al., 2008) have been used to improve qualities of PKM. The first method requires expensive commercially available enzymes, while the other methods are time consuming and include the risk of contamination by undesirable microbes. Alternative ways to improve PKM are water soaking, microwave irradiation (Thongprajukaew et al., 2013b), and possibly their combination. The objective of this study was to investigate *in vivo* whether these types of PKM modifications could improve the growth and the feed utilization efficiency of Nile tilapia (*O. niloticus*), relative to native PKM. The responses in the fish to these dietary treatments were further assessed by observing: 1) specific activities of carbohydrate-, protein- and lipid-digesting enzymes, 2) radical scavenging activities, 3) compositions of carcass and muscle, and 4) quality of muscle actin and myosin. The findings from this study could help improve the quality of diets using PKM, in addition to providing data for future comparisons in studies of other feedstuff ingredients.

2. Materials and methods

2.1. Preparation of PKM-containing diets

The PKM was physically modified as described by Thongprajukaew et al. (2013b). The ingredients and their inclusion levels in the experimental diets are shown in Table 1. Unprocessed PKM (UPKM) was used as the baseline in the control treatment. The water-soaked PKM (SPKM) was prepared by soaking the raw PKM in distilled water (1: 2 *w*/*v*) at room temperature for 12 h. The microwave-irradiated PKM (MPKM) was prepared by placing 100 g of raw PKM in a plastic box (20 cm diameter × 10 cm height), mixed with distilled water (1: 2 *w*/*v*) and then irradiated at 800 W in a microwave oven (MW 71B, Samsung, Malaysia) for 4 min. The water-soaked and microwave-irradiated PKM (SMPKM) was treated in this sequence with the steps described. The 20% UPKM in control treatment was substituted by SPKM, MPKM, or SMPKM in the actual dietary treatments. The PKM ingredient was mixed with the other feedstuffs (fish meal, soybean meal, alpha starch, corn flour and rice hull) along with additives, adding water for appropriate moisture content. The glutinous mixtures were formed to pellets with a hand pelletizer, dried at 60°C for 48 h, and then stored at 4°C until use in feeding.

2.2. Determination of diet chemical compositions

Three samples of each experimental diet were subjected to determination of the moisture content, after oven drying at 105^{o}C for 24 h. The proximate compositions of the diets, including crude protein, crude lipid, crude ash, crude fiber and nitrogen free extract, were determined according to standard methods of AOAC (2005). Gross energy values were calculated from the amounts of crude protein, crude lipid and nitrogen free extract. All the chemical analyses were done in triplicates, and the values are reported on % dry matter basis.

146 **2.3. Dietary treatment trials on sex reversed Nile tilapia**

147 Sex reversed Nile tilapia (20.61 ± 0.15 g initial body weight and 10.45 ± 0.03 cm
148 initial length) were obtained from Trang Agriculture and Technology College, Trang
149 province. The fish were acclimatized for 10 days in fiberglass tanks (1 × 1 × 1 m) with 20 cm
150 water level, and were fed twice daily to satiation with the UPKM diet. Subsequently, eight
151 fish were randomly assigned to each aquarium (36 × 18 × 30 cm). All the fish were fed
152 initially with 7% of their body weight per day and then the level was adjusted weekly
153 according to the actual feeding performance, with feeding twice daily (07.00 and 16.00 h),
154 under 12-h light/12-h dark cycle, for ten weeks. The water was refreshed weekly by 80%
155 replacement, and continuous aeration was supplied by air compressor pumps. The quality of
156 water was maintained by immediately siphoning leftover food and wastes after feeding, as
157 well as monitored daily by qualitative ammonia detection with test paper. The averaged water
158 characteristics, measured on day 6 of each week during the whole test period, were 24.78 ±
159 0.97^{o}C and 7.28 ± 0.11 mg L^{-1} dissolved oxygen. Growth performances were recorded
160 weekly during the experimental period. Feed conversion ratio (FCR) was measured from the
161 dry weight of diet consumed and was calculated per individual fish. At the end of the
162 experiment, all the fish were starved for 24 h and then killed by chilling in ice. Visceral
163 organs (including liver, stomach and intestine), white muscle (epaxial muscle under dorsal
164 fin) and carcass (without visceral organs) were collected and then kept at –20^{o}C until used.
165
166 **2.4. Digestive enzyme assays**
167 ***2.4.1. Digestive enzyme extraction***
168 The frozen small intestine was dissected by scissors and then the tissue was
169 homogenized using a micro-homogenizer (THP-220; Omni International, Kennesaw GA,
170 USA) in the presence of 1 mM HCl (1: 3 *w*/*v*). The homogenate was centrifuged at 15000 × *g*,

at 4°C for 30 min, the lipid layer on the surface was removed, and the supernatant was kept at –20°C until the assaying of digestive enzymes.

2.4.2. Assaying of protein-digesting enzymes

The optimal conditions used for assaying trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were pH 9 at 50°C, and pH 9 at 60°C, respectively (Engkagul et al., 2010). The 1.25 mM *N*-benzoyl-*L*-arginine-*p*-nitroanilide (BAPNA) and 0.10 M *N*-succinyl-ala-ala-pro-phe-*p*-nitroanilide (SAPNA) were used as substrates, and the assays were run according to Rungruangsak-Torrissen et al. (2006). The activities of both these enzymes were determined spectrophotometrically at 410 nm, by comparison to linear response concentration range of standard *p*-nitroanilide.

2.4.3. Assaying of carbohydrate-digesting enzymes

The activities of α-amylase (EC 3.2.1.1) and cellulase (EC 3.2.1.4) were determined based on Areekijseree et al. (2004) and Mendels and Weber (1969), using soluble starch and carboxymethylcellulose (CMC) as the substrates, respectively. The optimal conditions used were pH 7 at 50°C, both for α-amylase and for cellulase. The activities of both these enzymes were determined spectrophotometrically at 540 nm, by comparison to linear response concentration ranges of standard maltose and glucose, respectively.

2.4.4. Assaying of lipid-digesting enzyme

The optimal conditions for assaying lipase (EC 3.1.1.3) activity in Nile tilapia were pH 8 and 60°C (Engkagul et al., 2010). The activity was analyzed based on Winkler and Stuckmann (1979) using *p*-nitrophenyl palmitate as the substrate. The activity of lipase was

determined spectrophotometrically at 410 nm, by comparison to linear response concentration range of standard *p*-nitrophenol.

2.4.5. Determination of protein concentration in crude enzyme extracts

Protein concentration (mg mL^{-1}) was determined using the method of Lowry et al. (1951). Bovine serum albumin (BSA) was used as the protein standard. The protein concentrations in the crude enzyme extracts were used for normalization when quantifying digestive enzyme specific activities (U mg $protein^{-1}$).

2.5. Radical scavenging activity assay

Intestinal extracts were obtained as described in section 2.4.1 above, and the stomach and liver were extracted similarly. The 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity was determined according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and was stored at –20^{o}C. The working solution was obtained by diluting the stock solution with methanol to obtain an absorbance of 1.0 ± 0.5 units at 517 nm. Then, 3 mL of this working solution were mixed with 100 μL of a 15-fold diluted sample, and allowed to react in the dark for 30 min, after which the absorbance was measured at 517 nm. The control sample was extraction buffer in equal volume to the actual sample. The radical scavenging activity (% inhibition) was calculated from $[(A_o–A_i)/A_o] \times 100$ where A_o and A_i are the absorbances of the control sample and the extract, respectively.

2.6. Determination of carcass and muscle compositions

The moisture and ash contents were determined according to the standard method of AOAC (2005). The concentrations of RNA and protein were determined using Trizol reagent

as described in Rungruangsak-Torrissen (2007). The extinction coefficients used in the calculations were E_{260} = 40 µg RNA mL^{-1} and E_{280} = 2.1 mg protein mL^{-1}, for RNA and protein respectively. The lipid content was extracted using ethyl acetate as described in Supannapong et al. (2008). The values in this report on RNA, protein, RNA/protein ratio and lipid are given on wet weight basis.

2.7. Analysis of muscle quality

The quality of fresh white muscle was quantified based on thermal transition properties, determined with a differential scanning calorimeter (DSC7, Perkin Elmer, USA). The onset (T_o), peak (T_p) and conclusion (T_c) temperatures of protein denaturation, and the transition enthalpy (ΔH), were detected in the temperature range from 20 to 120°C, scanned at a rate of 5°C min^{-1}. Approximately twenty milligrams of a muscle sample was placed in an aluminum pan, sealed, allowed to equilibrate at room temperature, and then heated with comparison against an empty reference pan. The identification of muscle actin and myosin was based on their thermal transition properties according to Schubring (2009) and Matos et al. (2011). These transition properties were determined for temperatures from 35 to 95°C.

2.8. Statistical analysis and calculations

The experiment was conducted in a completely randomized design, with the four dietary treatments each in triplicates (4 treatments × 3 replicates × 8 fish per replication). The data were analyzed using SPSS Version 14 (SPSS Inc., Chicago, USA), and the results are summarized as mean ± SEM (n = 3). Significances of differences between means were tested with Duncan's multiple range test, with significance equated to $P < 0.05$. The calculation of growth and feed utilization parameters was as follows:

Viscerosomatic index (VSI, %) = 100 (wet weight of visceral organ/wet body weight)

Stomasomatic index (SSI, %) = 100 (wet weight of stomach/wet body weight)

Intestosomatic index (ISI, %) =100 (wet weight of intestine/wet body weight)

Hepatosomatic index (HSI, %) =100 (wet weight of liver/wet body weight)

Condition factor (CF, g cm^{-3}) = 100 [live body weight (g)/total body length $(cm)^3$]

Specific growth rate (SGR, % day^{-1}) = 100 $[(\ln W_t - \ln W_0)/(t-t_0)]$

Where W_t = mean weight (g) at day t, W_0 = mean weight (g) at day t_0

Feed conversion ratio (FCR, g feed g $gain^{-1}$) = Dry feed consumed (g)/wet weight gain (g)

3. Results

3.1. Chemical compositions of the diets

Diet proximate chemical compositions in terms of crude protein, lipid, fiber, nitrogen free extract and gross energy are given in Table 1. These constituents had similar levels in the four PKM-containing diets, so that the replacement of baseline UPKM with a modified PKM had no effect on the overall nutritional composition of the diet. Thus, the four PKM-containing diets were isonitrogenous, isolipidic and isoenergetic.

3.2. Growth and feed utilization of sex reversed Nile tilapia

No mortality of fish was detected during the ten weeks duration, and all dietary treatments had 100% survival. The growth and feed utilization parameters of sex reversed Nile tilapia are shown in Table 2. Final body weight was highest in the fish fed SMPKM diet, followed by SPKM, relative to MPKM and UPKM. Total length and specific growth rate (SGR) were significantly increased in tilapia fed with the SMPKM diet, and also with the SPKM, relative to MPKM and UPKM. The condition factor (CF) showed faster skeletal growth in proportion to body weight for the fish fed with UPKM, SPKM or SMPKM diet in comparison to the MPKM diet. The feed conversion ratio (FCR) of the fish was improved in

the treatments with SMPKM and SPKM when compared with UPKM ($P > 0.05$) and MPKM ($P < 0.05$). Both these treatments with soaked PKM induced increased viscerosomatic index (VSI), hepatosomatic index (HSI), stomasomatic index (SSI) and intestosomatic index (ISI), relative to UPKM and MPKM (Table 2).

3.3. Specific activities of digestive enzymes

The specific activity of trypsin was similar in the fish fed with SMPKM ($P < 0.05$), UPKM ($P > 0.05$) and SPKM ($P > 0.05$) diets, and this activity was higher than in the fish fed with the MPKM diet (Table 3). There were no significant differences in the chymotrypsin specific activity between the four dietary treatments. The activity ratio of trypsin to chymotrypsin (T/C ratio) was comparatively high in the fish fed with SMPKM and SPKM, but without statistical significance between any dietary groups. These dietary treatments gave also high amylase specific activities and low specific activities of cellulase, when compared with UPKM ($P > 0.05$) and MPKM ($P < 0.05$). For the lipid-digesting enzyme, the modifications of PKM tended to decrease the specific activity of lipase, but not SPKM ($P > 0.05$), significantly in the fish fed with MPKM and SMPKM relative to UPKM ($P < 0.05$).

3.4. Radical scavenging activity

There were no significant differences in the radical scavenging activity of liver (23.79 ± 0.26% inhibition on average) or the intestinal extracts (20.90 ± 0.16% inhibition on average) between the dietary treatments. However, in stomach samples the scavenging activity was highest in the fish fed with MPKM (59.93 ± 0.46% inhibition) and SMPKM (59.09 ± 1.16% inhibition), the significances being for comparisons with the baseline UPKM diet (55.23 ± 0.85% inhibition) that had the lowest activity. However, no differences in

294 stomach scavenging activity were observed between fish fed SPKM diet (56.87 ± 0.35%
295 inhibition) when compared with the three dietary groups.
296
297 **3.5. Carcass compositions**
298 The fish fed with modified PKM diets had significantly lower moisture contents than
299 those fed with the UPKM diet (Table 4). On the other hand, their ash contents were
300 significantly higher than with the control UPKM diet, except for SPKM. No significant
301 differences between the dietary treatments were observed in RNA, protein, RNA/protein ratio
302 and lipid.
303
304 **3.6. Muscle composition and quality**
305 There were no significant differences between the treatments in moisture, ash, RNA or
306 lipid of the muscle samples (Table 4). However, the protein concentration was highest in the
307 fish fed with SPKM and SMPKM, and the RNA/protein ratio was lowest with these
308 treatments, when compared with the other dietary groups. In the thermal transition properties,
309 muscle samples of the fish fed with UPKM (Fig. 1a), MPKM and SMPKM (data not
310 presented) had mutually similar patterns with two transition peaks, while fish fed with SPKM
311 had three transition peaks (Fig. 1b). The first peak of denaturation, assigned to myosin, had
312 T_o, T_p and T_c at 43.0, 46.5 and 49.9^oC on an average, respectively. The second peak was
313 assigned to actin, and it had T_o, T_p and T_c at 66.6, 71.6 and 74.6^oC on an average,
314 respectively. These peaks were also observed for the fish fed with SPKM diet (Fig. 1b),
315 whose muscles also showed a minor peak at T_o = 76.3^oC, T_p = 80.1^oC, and T_c = 84.2^oC
316 (matching protein not assigned). The values of ΔH_{Myosin} (1.14 ± 0.09 J g^{-1}), ΔH_{Actin} (0.45 ±
317 0.03 J g^{-1}) and $\Sigma\Delta H$ (1.62 ± 0.09 J g^{-1}), on average, did not differ significantly between the
318 dietary treatments.

4. Discussion

Sex reversed Nile tilapia fed with four PKM-containing diets exhibited differences in their growth and feed utilization characteristics. This indicates significant effects of the PKM modifications on the overall quality of the diets, when 20% PKM inclusion level was used. The diet chemical compositions showed no modification effects, while changed physicochemical properties in terms of pH, water solubility, turbidity, microstructure, gelatinization, lignocellulosic profiles and crystallinity were observed (Thongprajukaew et al., in press). In the current study, water soaking of the PKM in tilapia diet (SPKM and SMPKM) gave superior responses relative to other forms of PKM. There are prior reports on feed modification by water soaking, on Sesbania seeds for common carp, *Cyprinus carpio* (Hossain et al., 2001) and on leucaena seeds for African catfish, *Clarias gariepinus* (Sotolu and Faturoti, 2008), with improved growth and feed utilization. Similar findings were obtained in goat fed with water-soaked fodder (Wina et al., 2005). Thongprajukaew et al. (2011) and Sansuwan (2014) found superior growth and feed consumption in juvenile Siamese fighting fish and in sex reversed Nile tilapia (*O. niloticus*), when fed with microwave-irradiated diets. On the other hand, in the current study, the growth of sex reversed Nile tilapia fed with SMPKM was better than with MPKM. The PKM contains a large amount of unavailable lignocellulosic constituents (lignin, cellulose and hemicellulose), and possibly the long 12 h water soaking followed by irradiation disrupted the PKM structure better than microwave irradiation alone.

The gastrointestinal weight tends to correlate positively with the growth rate of fish (Thongprajukaew et al., 2011, 2014). This suggests superior feed utilization in the tilapia fed with SPKM or SMPKM, relative to the UPKM and the MPKM diets. This is corroborated by the physiochemical properties of soaked or microwave-irradiated PKM, namely effects on pH, solubility, microstructure, lignocellulosic constituents and the x-ray diffraction pattern

suggest enhancements of enzymatic reaction, as do observations of *in vitro* digestibility (Thongprajukaew et al., 2013b). Similar improvements of *in vitro* digestibility have been reported with soaked black gram and chick pea (Rehman, 2007), soaked moth bean (Negi et al., 2001) and soaked coconut meal (Chumwaengwapee et al., 2013). From an *in vivo* dietary treatment trial, Kumar et al. (2006) report significant positive effects of corn modifications (gelatinized and non-gelatinized corns) on apparent digestibility coefficient, SSI and HSI, in juvenile rohu. These findings agree with the earlier ones by Mohapatra et al. (2002). Therefore, an increase in the gelatinization degree of a diet, accomplished by water soaking or its combination with microwave irradiation, can improve the feed utilization efficiency.

The dietary proteins are mainly digested (40–50%), in carnivorous fish, by trypsin activity (Eshel et al., 1993). This enzyme regulates the activation of many zymogens and also its own activity. Therefore, the intestinal protein digestion appears to be controlled by the activity of this enzyme. The specific activity of trypsin and the T/C ratio have been used as indicators related to the growth and the feed conversion efficiency in aquatic animals (Sunde et al., 2004; Rungruangsak-Torrissen et al., 2006; Thongprajukaew et al., 2013a). In our case, these indicators increased, though without statistical significance, in the tilapia fed with SPKM and SMPKM diets. Based on the match with prior observations, we may assume that these indicators would have shown significant differences with either larger treatment groups or with a prolonged treatment. No treatment effects were observed on chymotrypsin activity. The two serine proteases differ in their active sites and respond to different dietary proteins, so the observations are still consistent without any discrepancy.

Adaptability of the carbohydrate digestion was clear in our observations of sex reversed Nile tilapia fed with different diets. The ability to digest carbohydrates is indicated by amylase specific activity, and this improved in the tilapia fed with SPKM and SMPKM. This enzyme is mainly produced in the pancreas, and then secreted into the small intestine

where it cleaves glycosidic bonds. The amylase specific activity tends to correlate positively with the degree of starch gelatinization in the diet (Mohapatra et al., 2002; Kumar et al., 2006; Thongprajukaew et al., 2011). On the other hand, the dietary treatments including soaked PKM significantly decreased cellulase specific activity. This enzyme is chiefly produced by the fish intestinal microflora, and it breaks cellulose molecules down to monosaccharides or short polysaccharides and oligosaccharides. Therefore, this activity response to a diet indicates a reduction in the indigestible constituents, in other words the diet has become easier to digest. The nitrogen free extract and cellulose quality in PKM are affected by water soaking and microwave irradiation (Thongprajukaew et al., 2013b), as they are also in coconut meal (Chumwaengwapee et al., 2013). However, in the current study the low 20% inclusion level of PKM may have allowed the other 80% of diet components to mask any changes in the proximate chemical compositions.

Palm-originated oil has significant benefits as a replacement in diets of Atlantic salmon, being comparable in fish feed to equivalent levels of fish oil (Rosenlund, 2001; Caballero et al., 2002). Although the four experimental diets in the current study were isolipidic, the fish fed with UPKM and SPKM diets had comparatively high lipase specific activities, suggesting that microwave irradiation had a negative effect on lipid digestion in Nile tilapia. The findings in lipase expression of fish fed either of these diets were matched by increased HSI, relative to MPKM and SMPKM treatment groups. These findings indicate an improved ability to utilize the lipids in UPKM and SPKM, and suggest that microwave irradiation reduced lipid quality. Such quality changes would occur mainly at the double bonds of unsaturated fatty acids, releasing hydroperoxides and secondary oxidation products, and these reactions are also promoted by the presence of water, and affected by processing time and temperature (Stewart et al., 2003). However, the three modifications of PKM did not affect the radical scavenging activity in liver or intestine of sex reversed Nile tilapia, while an

improvement in the stomach was observed. However, the significantly decreased SSI in tilapia fed with the MPKM diet, when compared with the others, was counteracted by the radical scavenging activity. This is in agreement with the negative response of digestive functionality of stomach, as indicated by pepsin specific activity in this dietary group (135.82 ± 5.73 mU mg protein^{-1}) when compared with the fish fed SPKM diet (100.17 ± 2.94 mU mg protein^{-1}) (unpublished data). A further study of stomach histopathology effects of these dietary treatments would be warranted. No negative effects on the gastrointestinal and the liver functionalities of the reared tilapia in the current study supported the use of modified PKM (SPKM) as aquafeed. This agrees with a prior study, where 15–30% (diets containing 24% protein and 7% lipid) inclusion level of PKM had no negative effects on hematological parameters or liver histopathology of sex reversed Nile tilapia (Sukasem and Ruangsri, 2007).

A pre-gelatinized diet has no effects on the carcass and the muscle compositions in juvenile European sea bass, *Dicentrarchus labrax* (Peres and Oliva-Teles, 2002) or in yellowfin seabream, *Sparus latus* (Wu et al., 2007). Similarly, rapid growth of Siamese fighting fish on a microwave-irradiated diet has been reported without effects on protein synthesis capacity (RNA concentration) or lipid (Thongprajukaew et al., 2011). In the current study, some improvements in the carcass and the muscle compositions were observed in the fish fed with SPKM and SMPKM diets. Decreased carcass moisture probably indicates increased body strength of the fish fed with modified PKMs in the diet. This characteristic tends to correlate negatively with the ash, although the ash contents did not significantly differ between the treatments. Ash content indicates the relative skeletal contribution in the body, quantified by the index CF. Ng et al. (2002) reported no differences in carcass moisture and ash of red tilapia (*Oreochromis* sp.) fed with diets containing 20% PKM, either as raw, as enzyme-treated (Allzyme Vegpro^{TM}), or as fungal-fermented (*Trichoderma koningii*). On comparison to this prior work, our physical modification by water soaking or by its

419 combination with microwave irradiation appears to be more effective than biological
420 modifications, in improving the utilization of PKM in fish. Moreover, water soaking is an
421 effective method to reduce the amount of phytic acid (Wina et al., 2005; Esmaeilipour et al.,
422 2013), and such reduction probably improves the availability of phosphorous in PKM.

423 White muscle is a reservoir for metabolism and protein growth (Carter et al., 1995).
424 Therefore, faster protein metabolism is better observable in the muscle rather than in the
425 carcass. The accumulation of protein and its turnover rate (RNA/protein ratio) in muscle
426 indicated a significant response in the fish to the dietary protein quality, although the four
427 experimental diets were isonitrogenous. A high protein concentration correlates positively
428 with fillet quality, while a low turnover rate indicates superior growth performance of the fish
429 (Thongprajukaew et al., 2013a). The DSC data indicated the presence of myosin and actin, in
430 the respective denaturation temperature ranges 43.0–49.9^{o}C and 66.6–74.6^{o}C. In addition, the
431 undefined peak from the fish fed with SPKM diet could be attributed to the denaturation of
432 collagen and sarcoplasmic proteins, which might be induced by the diet. Based on enthalpy
433 responses, the amounts of major proteins (myosin and actin) did not differ between the four
434 dietary treatments. This measurement result, the enthalpy, relates to the amount of protein left
435 in its native state (Matos et al., 2011) in which it full denaturing requires more energy.
436 Therefore, the high total enthalpy of muscle proteins with SPKM treatment may indicate the
437 fish in this dietary group had physical exercise, although their growth was rapid.
438

439 **5. Conclusions**

440 The fish fed with SPKM and SMPKM diets were superior in specific growth rate
441 and feed conversion ratio. The digestion capacity of fish also improved with both these
442 dietary treatments, as indicated by the specific activities of trypsin and amylase, while only
443 the fish fed with SPKM were well acclimatized to digest lipids. In conclusion, the water

444 soaking, or soaking followed by microwave irradiation, improved the PKM nutritional quality
445 in the diet. Practically, the pre-soaking of fresh PKM (without drying) can be incorporated in
446 the feed preparation, and this reduces the amount of additional water required for mixing and
447 pelleting processes. This simple low-cost feed modification by water soaking alone has wide
448 application potential in the administration of aquafeed.
449

450 **Acknowledgements**

451 This research was funded by the Beginner Research Scholarship (Contract No.
452 SCI5502895), Research and Development Office, Prince of Songkla University, Songkhla,
453 Thailand. We would like to thank the Publication Clinic, Prince of Songkla University, for
454 their help in manuscript preparation.
455

456 **References**

457 AOAC, 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical
458 Chemists, Washington, DC.
459 Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P.,
460 Rungruangsak-Torrissen, K., 2004. Temperature and pH characteristics of amylase and
461 proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson
462 1900. Aquaculture 234, 575–587.
463 Behgar, M., Ghasemi, S., Naserian, A., Borzoie, A., Fatollahi, H., 2011. Gamma radiation
464 effects on phenolics, antioxidants activity and *in vitro* digestion of pistachio (*Pistachia*
465 *vera*) hull. Radiat. Phys. Chem. 80, 963–967.
466 Behgar, M., Valizadeh, R., Mirzaee, M., Naserian, A.A., Nasiri, M.R., 2009. Correlation
467 between the physical and chemical properties of some forages and non-forage fiber
468 sources. J. Anim. Vet. Adv. 8, 2280–2285.

469 Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate
470 antioxidant activity. Lebensm. Wiss. Technol. 28, 25–30.
471 Carter, C.G., He, Z.Y., Houlihan, D.F., McCarthy, I.D., Davidson, I., 1995. Effects of feeding
472 on the tissue free amino acid concentrations in rainbow trout (*Oncorhynchus mykiss*
473 Walbaum). Fish Physiol. Biochem. 14, 153–164.
474 Chumwaengwapee, S., Soontornchai, S., Thongprajukeaw, K., 2013. Improving chemical
475 composition, physicochemical properties, and *in vitro* carbohydrate digestibility of fish
476 coconut meal. ScienceAsia 39, 636–642.
477 Engkagul, A., Rungruangsak-Torrissen, K., Aeidnoie, Y., Kovitvadhi, S., Kovitvadhi, U.,
478 Siruntawineti, J., Choowongkomon, K., Unajak, S., Preprame, P., Trenet, E., Meeswad,
479 P., Sunthornchot, J., 2010. Development of suitable feed for commercial production of
480 Nile tilapia (*Oreochromis niloticus*). Kasetsart University Technical Report,
481 Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science,
482 Kasetsart University, Bangkok (in Thai).
483 Eshel, A., Lindner, P., Smirnoff, P., Newton, S., Harpaz, S., 1993. Comparative study of
484 proteolytic enzymes in the digestive tracts of the European sea bass and hybrid striped
485 bass reared in freshwater. Comp. Biochem. Physiol. 106A, 627– 634.
486 Esmaeilipour, O., Van Krimpen, M.M., Jongbloed, A.W., De Jonge, L.H., Bikker, P., 2013.
487 The effects of temperature, moisture, duration of incubation time, calcium level,
488 and soaking with water or citric acid on *in vitro* phytate degradation in a wheat–
489 barley–rye–soybean meal-based diet. Anim. Feed Sci. Technol. 183, 168–174.
490 Esuga, P.M., Sekoni, A.A., Omage, J.J., Bawa, G.S., 2008. Evaluation of enzyme
491 (Maxigrain®) supplementation of graded levels of palm kernel meal (PKM) on the
492 performance of broiler chickens. Pakistan J. Nutr. 7, 60–613.

493 Zahari, M.W., Alimon, A.R., Wong, H.K., 2012. Utilization of oil palm co-products as feeds
494 for livestock in Malaysia, in: Makkar, H.P.S. (Ed.), Biofuel Co-Products as Livestock
495 Feed–Opportunities and Challenges. FAO, Rome, pp. 243–262.
496 Hossain, M.A., Focken, U., Becker, K., 2001. Effect of soaking and soaking followed by
497 autoclaving of Sesbania seeds on growth and feed utilisation in common carp, *Cyprinus*
498 *carpio* L. Aquaculture 203, 133–148.
499 Khadijat, B.M., Enoch, O.O., Suleiman, B.E., 2012. Performance, carcass characteristics and
500 blood composition of broilers fed varying levels of palm kernel meal (*Elaise guinensis*)
501 supplemented with different levels of fish meal. Int. J. Poult. Sci. 11, 73–77.
502 Kim, J., Lee, B.C., Lee, J., Nam, K., Lee, S., 2008. Effect of electron-beam irradiation on the
503 antioxidant activity of extracts from *Citrus unshiu* pomaces. Radiat. Phys. Chem. 77,
504 87–91.
505 Kumar, S., Sahu, N.P., Pal, A.K., Choudhury, D., Mukherjee, S.C., 2006. Studies on
506 digestibility and digestive enzyme activities in *Labeo rohita* (Hamilton) juveniles: effect
507 of microbial α-amylase supplementation in non-gelatinized or gelatinized corn-based
508 diet at two protein levels. Fish Physiol. Biochem. 32, 209–220.
509 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with
510 the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
511 Matos, E., Silva, T.S., Tiago, T., Aureliano, M., Dinis M.T., Dias, J., 2011. Effect of
512 harvesting stress and storage conditions on protein degradation in fillets of farmed
513 gilthead seabream (*Sparus aurata*): A differential scanning calorimetry study. Food
514 Chem. 126, 270–276.
515 Mendels, M., Weber, J., 1969. The product of cellulose in cellulase and their application.
516 Adv. Chem. Ser. 95, 39–443.

517 Mohapatra, M., Sahu, N.P., Chaudhari, A., 2002. Utilization of gelatinized carbohydrate in
518 diets of *Labeo rohita* fry. Aquacult. Nutr. 9, 189–196.
519 Negi, A., Boora, P., Khetarpaul, N., 2001. Effect of microwave cooking on the starch and
520 protein digestibility of some newly released moth bean (*Phaseolus aconitifolius* Jacq.)
521 cultivars. J. Food Com. Anal. 14, 541–546.
522 Ng, W.K., Chen, M.L., 2002. Replacement of soybean meal with palm kernel meal in
523 practical diets for hybrid Asian–African catfish, *Clarias macrocephalus* × *C.*
524 *gariepinus*. J. Appl. Aquacult. 12, 67–76.
525 Ng, W.K., Lim, H.A., Lim, S.L., Ibrahim, C.O., 2002. Nutritive value of palm kernel meal
526 pretreated with enzymes or fermented with *Trichoderma koningii* (Oudemans) as a
527 dietary ingredient for red hybrid tilapia (*Oreochromis* sp.). Aquacult. Res. 33, 1199–
528 1207.
529 Omoregie, E., 2001. Utilization and nutrient digestibility of mango seeds and palm kernel
530 meal by juvenile *Labeo senegalensis* (Antheriniformes: Cyprinidae). Aquacult. Res. 32,
531 681–687.
532 Peres, H., Oliva-Teles, A., 2002. Utilization of raw and gelatinized starch by European sea
533 bass (*Dicentrarchus labrax*) juveniles. Aquaculture 205, 287–299.
534 Rehman, Z.U., 2007. Domestic processing effects on available carbohydrate content and
535 starch digestibility of black grams (*Vigna mungo*) and chick peas (*Cicer arietium*). Food
536 Chem. 100, 764–767.
537 Rosenlund, G., 2001. Effect of alternative lipid sources on long-term growth performance and
538 quality of Atlantic salmon (*Salmo salar* L.). Aquacult. Res. 32 (Suppl. 1), 323–328.
539 Rungruangsak-Torrissen, K., 2007. Digestive efficiency, growth and qualities of muscle and
540 oocyte in Atlantic salmon (*Salmo salar* L.) fed on diets with krill meal as an alternative
541 protein source. J. Food Biochem. 31, 509–540.

542 Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg, A., Waagbo, R., 2006.
543 Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic
544 salmon (*Salmo salar* L.). Fish Physiol. Biochem. 32, 7–23.
545 Sansuwan, K., 2014. Modification of feedstuffs using microwave processing for feed
546 development in culturing sex reversed Nile tilapia (*Oreochromis niloticus*). Master
547 thesis, Kasetsart University, Bangkok, Thailand.
548 Schubring, R., 2009. DSC studies on deep frozen fishery products. Thermochimica Acta 337,
549 89–95.
550 Sotolu, A.O., Faturoti, E.O., 2008. Digestibility and nutritional values of differently processed
551 *Leucaena leucocephala* (Lam. deWit) seed meals in the diet of African catfish (*Clarias*
552 *gariepinus*). Middle East J. Sci. Res. 3, 190–199.
553 Stewart, O.J., Raghavan, G.S.V., Orsat, V., Golden, K.D., 2003. The effect of drying on
554 unsaturated fatty acids and trypsin inhibitor activity in soybean. Proc. Biochem. 39,
555 483–489.
556 Sukasem, N., Ruangsri, J., 2007. Effects of palm kernel cake (PKC) on growth performance,
557 blood components and liver histopathology of sex reversed red tilapia (*Oreochromis*
558 *niloticus*). Songklanakarin J. Sci. Technol. 29, 1283–1299.
559 Sunde, J., Eiane, S.A., Rustad, A., Jensen, H.B., Opstvedt, J., Nygard, E., Venturini, G.,
560 Rungruangsak-Torrissen, K., 2004. Effect of fish feed processing conditions on
561 digestive protease activities, free amino acid pools, feed conversion efficiency and
562 growth in Atlantic salmon (*Salmo salar* L.). Aquacult. Nutr. 10, 261–277.
563 Supannapong, P., Pimsalee, T., A-komol, T., Engkakul, A., Kovitvadhi, U., Kovitvadhi, S.,
564 Rungruangsak-Torrissen, K., 2008. Digestive enzymes and *in vitro* digestibility of
565 different species of phytoplankton for culture of the freshwater pearl mussel, *Hyriopsis*
566 (*Hyriopsis*) *bialatus*. Aquacult. Int. 16, 437–453.

567 Thongprajukaew, K., Kovitvadhi, U., Kovitvadhi, S., Engkagul, A., Rungruangsak-Torrissen,
568 K., 2013a. Evaluation of growth performance and nutritional quality of diets using
569 enzymatic markers and *in vitro* digestibility in Siamese fighting fish (*Betta splendens*
570 Regan, 1910). Afr. J. Biotechnol. 12, 1689–1702.
571 Thongprajukaew, K., Kovitvadhi, U., Kovitvadhi, S., Somsueb, P., Rungruangsak-Torrissen,
572 K., 2011. Effects of different modified diets on growth, digestive enzyme activities and
573 muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910).
574 Aquaculture 322–323, 1–9.
575 Thongprajukaew, K., Rodjaroen, S., Tantikitti, C., Kovitvadhi, U., Physicochemical
576 modifications of dietary palm kernel meal affect growth and feed utilization of Nile
577 tilapia (*Oreochromis niloticus*). Anim. Feed Sci. Technol. (in press).
578 Thongprajukaew, K., Sutthinon, P., Ketmanee, R., Yawang, P., 2014. Characterisation of
579 digestive enzymes and *in vitro* screening of feed ingredients for the new hybrid grouper,
580 *Epinephelus coioides* (Hamilton 1822) × *Epinephelus lanceolatus* (Bloch 1790). Asian
581 Fish. Sci. 27, 1–15.
582 Thongprajukaew, K., Yawang, P., Dudae, L., Bilanglod, H., Dumrongrittamatt, T., Tantikitti,
583 C., Kovitvadhi, U., 2013b. Physical modification of palm kernel meal improved
584 available carbohydrate, physicochemical properties and *in vitro* digestibility in
585 economic freshwater fish. J. Sci. Food Agric. 93, 3832–3840.
586 Wina, E., Tangendjaja, B., Susana, I.W.R., 2005. Effects of chopping, and soaking in water,
587 hydrochloric acidic and calcium hydroxide solutions on the nutritional value of *Acacia*
588 *villosa* for goats. Anim. Feed Sci. Technol. 122, 79–92.
589 Winkler, U.K., Stuckmann, M., 1979. Glycogen, hyaluronate and some other polysaccharides
590 greatly enhance the formation of exolipase by *Serratia marcescens*. J. Bacteriol. 138,
591 663–670.

592 Wu, X.Y., Liu, Y.J., Tian, L.X., Mai, K.S., Gou, R., Jin, S.J., 2007. Effect of different dietary
593 raw to pre-gelatinized starch ratios on growth performance, feed utilization and body
594 composition of juvenile yellowfin seabream (*Sparus latus*). Aquacult. Int. 15, 467–477.

Figure caption

Figure 1 The thermal transition characteristics of proteins in white muscles of sex reversed Nile tilapia when fed with diets containing UPKM (a) and SPKM (b).

Table 1 The formulations by weight of ingredients and the chemical compositions of the PKM-containing diets used for rearing sex reversed Nile tilapia. The unprocessed and processed PKMs are given as dry matter pretreatment amounts.

Ingredient* and composition	UPKM	SPKM	MPKM	SMPKM
Ingredient (%)				
Fish meal	30.5	30.5	30.5	30.5
Soybean meal	19.5	19.5	19.5	19.5
Unprocessed palm kernel meal (UPKM)	20	–	–	–
Water-soaked palm kernel meal (SPKM)	–	20	–	–
Microwave-irradiated palm kernel meal (MPKM)	–	–	20	–
Water-soaked and microwave-irradiated palm kernel meal (SMPKM)	–	–	–	20
Alpha starch	5	5	5	5
Corn flour	12	12	12	12
Cod liver oil	2	2	2	2
Refined palm oil	3	3	3	3
Vitamin premix**	3	3	3	3
Mineral premix***	3	3	3	3
Rice hull	2	2	2	2
Chemical composition (% on dry matter)				
Crude protein	27.23	27.07	27.76	26.77
Crude lipid	8.86	8.53	8.43	9.21
Crude fiber	9.05	9.63	10.34	9.55
Crude ash	10.09	10.42	10.13	10.40
Nitrogen free extract	44.77	44.35	43.35	44.08
Gross energy (kJ g^{-1})	17.62	17.38	17.33	17.53

* Ingredients of experimental diets and its compositions as previously reported in Thongprajukaew et al. (in press). The feedstuffs including fish meal, soybean meal, palm kernel meal, corn flour and rice hull were purchased from Phatthalung Livestock CO., LTD, Phatthalung, Thailand. All other ingredients were purchased from Mastertec CO., LTD, Bangkok, Thailand.

** Vitamin premix, 1 kg contained 1000 mg vitamin B_1, 1000 mg vitamin B_2, 2 mg vitamin B_{12}, 55 g vitamin C, 400 mg vitamin K_3, 1000 mg inositol and 1000 mg choline chloride.

*** Mineral premix, 1 kg contained 5000 mg calcium oxide, 11430 mg alumina, 1000 mg ferric oxide, 50 mg manganese oxide, 700 mg magnesium, 60000 mg silica, 5000 mg potassium oxide, 20 mg phosphorus pentoxide, 30 mg nitrogen, 2000 mg sodium oxide, 700 mg zinc, 50 mg iron, 70 mg selenium, 120 mg copper, 200 mg iodine, 20 mg cobalt, 260 mg molybdenum and 70 mg vanadium.

Table 2 The growth performance parameters of sex reversed Nile tilapia when fed with various PKM-containing diets for ten weeks.

Growth parameter	UPKM	SPKM	MPKM	SMPKM
Final body weight (g)	86.45 ± 2.67^{bc}	96.81 ± 3.89^{ab}	82.30 ± 4.92^{c}	102.53 ± 1.90^{a}
Viscerosomatic index (VSI, %)	9.84 ± 0.55^{a}	11.21 ± 0.25^{a}	7.50 ± 0.70^{b}	11.54 ± 0.57^{a}
Hepatosomatic index (HSI, %)	0.88 ± 0.03^{ab}	1.41 ± 0.14^{a}	0.91 ± 0.06^{b}	0.81 ± 0.06^{b}
Stomasomatic index (SSI, %)	2.19 ± 0.42^{a}	2.84 ± 0.06^{a}	1.14 ± 0.25^{b}	3.02 ± 0.27^{a}
Intestosomatic index (ISI, %)	6.52 ± 0.36^{ab}	6.76 ± 0.35^{ab}	5.46 ± 0.46^{b}	7.54 ± 0.45^{a}
Total length (cm)	18.49 ± 0.22^{b}	19.57 ± 0.35^{a}	18.92 ± 0.44^{ab}	19.92 ± 0.11^{a}
Condition factor (CF, g cm^{-3})	2.17 ± 0.04^{a}	2.05 ± 0.09^{ab}	1.93 ± 0.05^{b}	2.06 ± 0.06^{ab}
Specific growth rate (SGR, % day^{-1})	2.39 ± 0.03^{b}	2.62 ± 0.06^{a}	2.35 ± 0.08^{b}	2.74 ± 0.03^{a}
Feed conversion ratio (FCR, g feed g $gain^{-1}$)	1.63 ± 0.07^{ab}	1.40 ± 0.07^{b}	1.80 ± 0.20^{a}	1.29 ± 0.04^{b}

UPKM, unprocessed palm kernel meal diet; SPKM, water-soaked palm kernel meal diet; MPKM, microwave-irradiated palm kernel meal diet; SMPKM, water-soaked and microwave-irradiated palm kernel meal diet.
Data are expressed as mean ± SEM ($n = 3$).
Different superscripts in the same row indicate significant difference ($P < 0.05$).

Table 3 Specific activities of digestive enzymes in sex reversed Nile tilapia, when fed with various PKM-containing diets for ten weeks.

Digestive enzyme	UPKM	SPKM	MPKM	SMPKM
Trypsin (U mg protein^{-1})	1.09 ± 0.05^{ab}	1.25 ± 0.11^{ab}	0.94 ± 0.09^{b}	1.35 ±0.14^{a}
Chymotrypsin (U mg protein^{-1})	0.83 ± 0.04	0.83 ± 0.03	0.73 ± 0.02	0.81 ± 0.04
Activity ratio of trypsin to chymotrypsin (T/C ratio)	1.34 ± 0.12	1.54 ± 0.18	1.26 ± 0.12	1.63 ± 0.10
Amylase (U mg protein^{-1})	776.32 ± 11.78^{ab}	884.49 ± 40.33^{a}	656.97 ± 56.45^{b}	872.66 ± 41.82^{a}
Cellulase (U mg protein^{-1})	34.22 ± 3.23^{ab}	24.05 ± 2.87^{b}	39.07 ± 3.53^{a}	25.92 ± 3.23^{b}
Lipase (U mg protein^{-1})	0.35 ± 0.05^{a}	0.28 ± 0.03^{ab}	0.21 ± 0.01^{b}	0.23 ± 0.01^{b}

UPKM, unprocessed palm kernel meal diet; SPKM, water-soaked palm kernel meal diet; MPKM, microwave-irradiated palm kernel meal diet; SMPKM, water-soaked and microwave-irradiated palm kernel meal diet.
Data are expressed as mean ± SEM (n = 3).
Different superscripts in the same row indicate significant difference ($P < 0.05$).

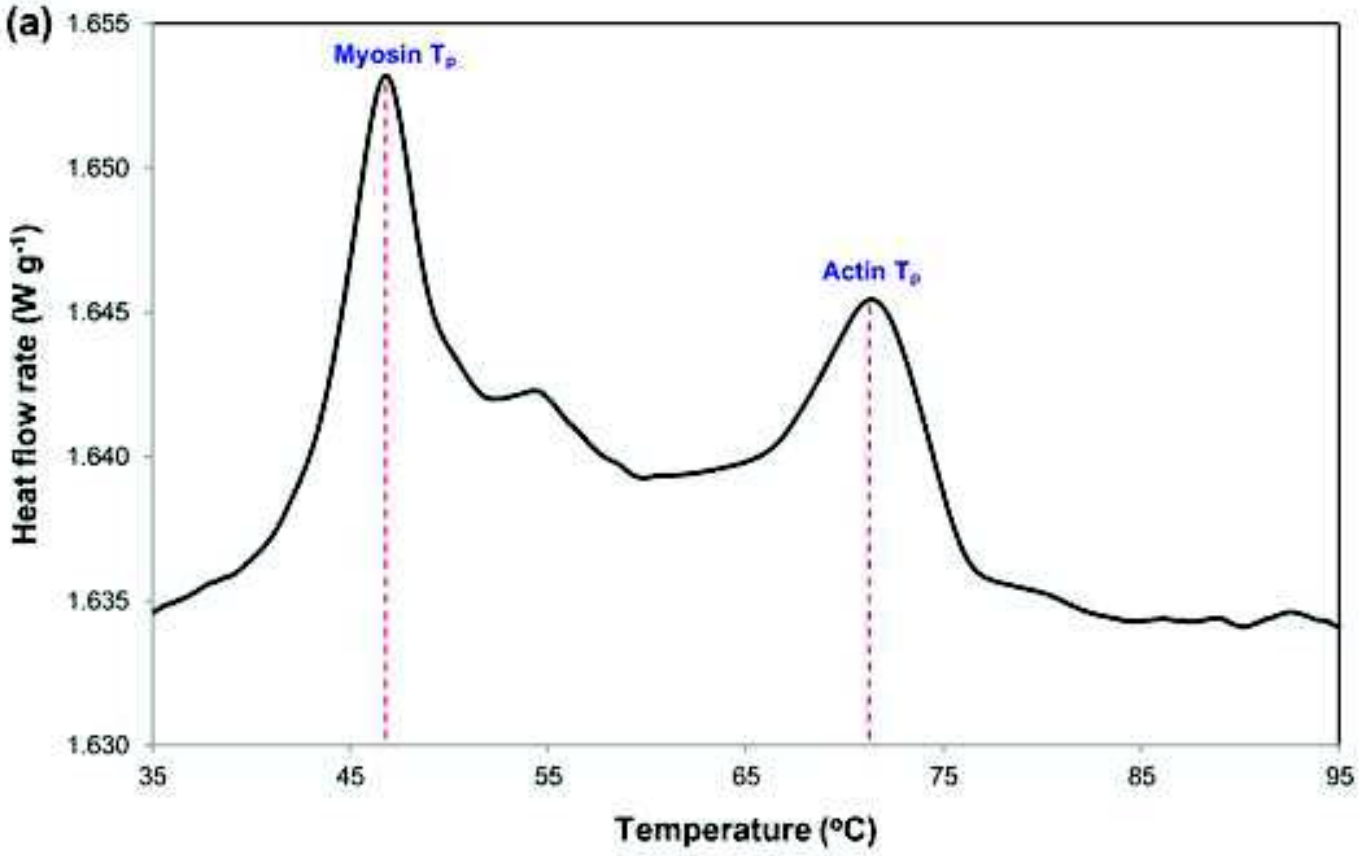
Table 4 The carcass (without visceral organs) and the muscle compositions (weight weight basis) of sex reversed Nile tilapia fed with various PKM-containing diets for ten weeks.

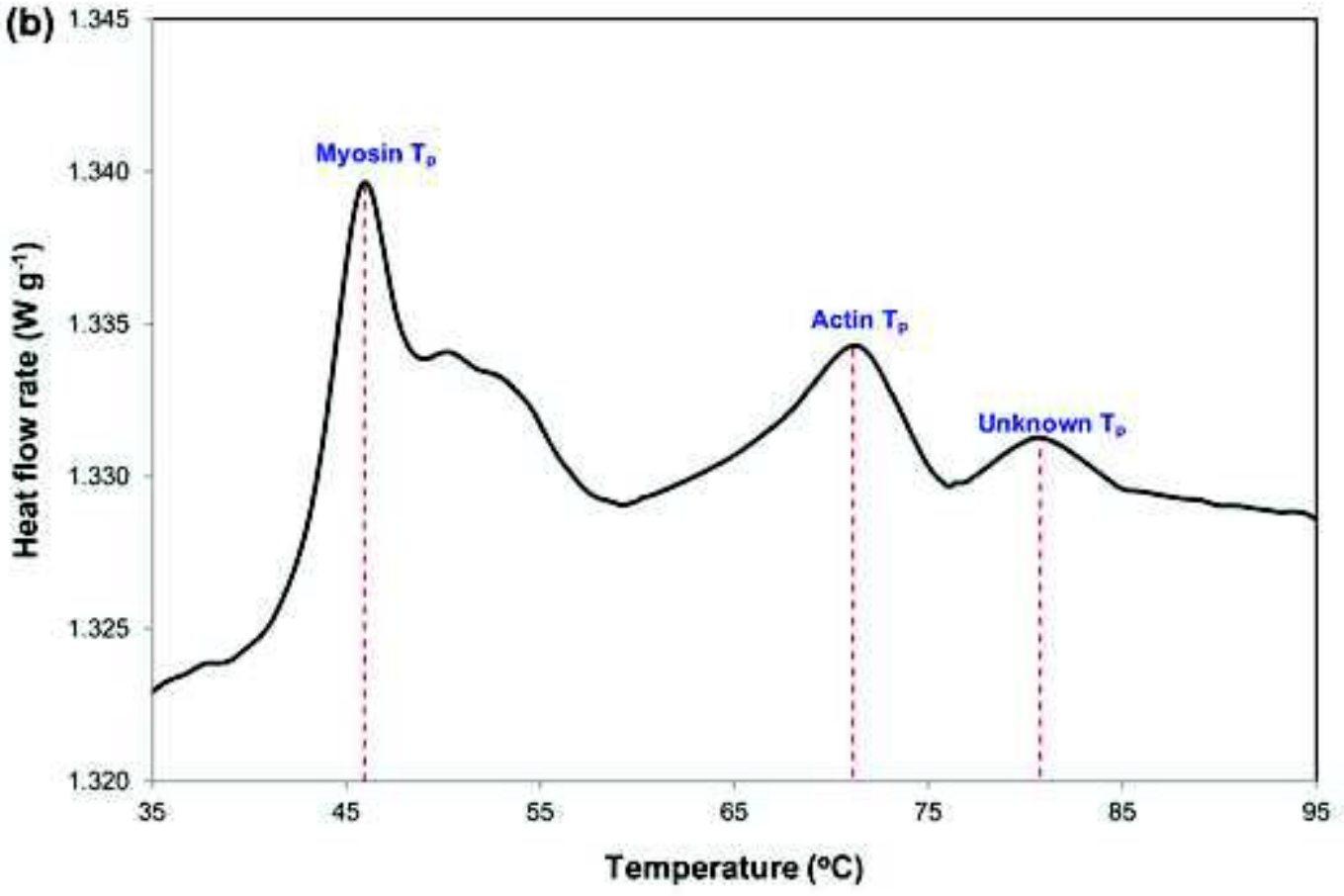
Composition	UPKM	SPKM	MPKM	SMPKM
Carcass				
1). Moisture (%)	74.11 ± 0.16^{a}	72.45 ± 0.47^{b}	72.71 ± 0.24^{b}	72.59 ± 0.43^{b}
2). Ash (%)	5.07 ± 0.24^{b}	6.17 ± 0.71^{ab}	7.42 ± 0.16^{a}	7.46 ± 0.16^{a}
3). RNA (mg g^{-1})	3.74 ± 0.13	3.76 ± 0.37	3.25 ± 0.16	3.39 ± 0.34
4). Protein (mg g^{-1})	208.96 ± 3.19	202.87 ± 4.91	207.38 ± 2.07	209.71 ± 6.74
5). Lipid (mg g^{-1})	20.95 ± 3.58	21.45 ± 2.72	23.64 ± 1.23	20.50 ± 2.71
6). RNA/protein ratio ($\times 10^{-3}$)	17.56 ± 2.29	16.13 ± 2.71	13.22 ± 1.98	16.19 ± 1.15
Muscle				
1). Moisture (%)	77.45 ± 0.41	77.48 ± 0.15	77.49 ± 0.31	77.26 ± 0.67
2). Ash (%)	1.14 ± 0.03	0.98 ± 0.04	0.94 ± 0.07	1.01 ± 0.13
3). RNA (mg g^{-1})	3.40 ± 0.44	3.43 ± 0.08	3.36 ± 0.34	3.19 ± 0.83
4). Protein (mg g^{-1})	180.87 ± 12.65^{b}	263.19 ± 14.62^{a}	185.73 ± 21.82^{b}	205.61 ± 34.64^{ab}
5). Lipid (mg g^{-1})	5.79 ± 0.35	7.24 ± 1.24	7.02 ± 1.00	6.32 ± 0.87
6). RNA/protein ratio ($\times 10^{-3}$)	18.84 ± 0.45^{a}	13.41 ± 0.74^{c}	16.55 ± 0.75^{b}	13.10 ± 0.54^{c}

UPKM, unprocessed palm kernel meal diet; SPKM, water-soaked palm kernel meal diet; MPKM, microwave-irradiated palm kernel meal diet; SMPKM, water-soaked and microwave-irradiated palm kernel meal diet.
Data are expressed as mean ± SEM ($n = 3$).
Different superscripts in the same row indicate significant difference ($P < 0.05$).

Figure(s)

Click here to download high resolution image





ภาคผนวก ค

ข้อคิดเห็นและข้อเสนอแนะสำหรับการวิจัยต่อไป

ข้อคิดเห็นและข้อเสนอแนะสำหรับการวิจัยต่อไป

1). ควรศึกษาสภาวะที่เหมาะสมเพิ่มเติมในการดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีการแช่น้ำ เช่น ระยะเวลาที่ใช้ อัตราส่วนของกากเนื้อในเมล็ดปาล์มต่อปริมาณน้ำ และอัตราการกวน เป็นต้น

2). ควรขยายผลในการทดสอบกับปลาเศรษฐกิจอื่นๆ รวมทั้งอาจปรับปริมาณของกากเนื้อในเมล็ดปาล์มให้อยู่ในระดับที่สูงขึ้น

3). อาจศึกษาเพิ่มเติมในผลพลอยได้อื่นๆ ที่ได้จากอุตสาหกรรมน้ำมันปาล์ม เช่น ช่อดอกเพศผู้ ทะลายปาล์มเปล่า เส้นใย กากปาล์มทั้งผล และกากเมล็ดปาล์ม เป็นต้น

ภาคผนวก ง

บทความวิจัยที่นำเสนอที่ประชุมวิชาการ

การดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีทางกายภาพเพื่อปรับปรุงการใช้ประโยชน์จากคาร์โบไฮเดรตในปลาเศรษฐกิจ

Physical Modification of Palm Kernel Meal for Improving Carbohydrate Utilization in Economic Fish

การุณ ทองประจุแก้ว[1] พิณญา ยาหวัง[1] ลาตีปะ ดูแด[1] เทิดทูน ดำรงค์ฤทธามาตย์[1] ชุติมา ตันติกิตติ[2] และ อุทัยวรรณ โกวิทวที[3,4]

Karun Thongprajukaew[1] Pinya Yawang[1] Lateepah Dudae[1] Terdtoon Dumrongrittamatt[1] Chutima Tantikitti[2] and Uthaiwan Kovitvadhi[3,4]

บทคัดย่อ

ตรวจสอบผลของการดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีทางด้านกายภาพที่ต่างกัน ได้แก่ การแช่น้ำ การใช้คลื่นไมโครเวฟ รังสีแกมมา และลำแสงอิเล็กตรอน ต่อองค์ประกอบทางเคมี สมบัติทางเคมีกายภาพ และประสิทธิภาพการย่อยคาร์โบไฮเดรตในหลอดทดลอง โดยใช้เอนไซม์ที่สกัดจากปลานิล (*Oreochromis niloticus*) ผลการศึกษาพบว่าการดัดแปรมีผลอย่างมีนัยสำคัญต่อองค์ประกอบทางเคมี ($P < 0.05$) โดยมีการลดลงของเยื่อใย และเพิ่มปริมาณของคาร์โบไฮเดรตที่ย่อยได้ การดัดแปรด้วยวิธีแช่น้ำช่วยปรับปรุงสมบัติทางเคมีกายภาพของวัตถุดิบได้ดีที่สุดเมื่อเปรียบเทียบกับการดัดแปรด้วยวิธีการอื่น สำหรับประสิทธิภาพการย่อยคาร์โบไฮเดรตพบว่าการแช่น้ำเป็นวิธีการดัดแปรที่เหมาะสมที่สุด รองลงมาคือการใช้ลำแสงอิเล็กตรอน และรังสีแกมมาตามลำดับ ผลการศึกษาจึงแสดงให้เห็นว่าการดัดแปรกากเนื้อในเมล็ดปาล์มโดยการแช่น้ำสามารถเพิ่มการใช้ประโยชน์จากคาร์โบไฮเดรตในปลาเศรษฐกิจได้

ABSTRACT

Effects of different physical modifications including water soaking, microwave irradiation, gamma irradiation and electron beam were investigated on chemical compositions, physicochemical property of palm kernel meal (PKM) and *in vitro* carbohydrate digestibility by digestive enzymes from

Key Words: palm kernel meal, soaking, physicochemical property, carbohydrate digestibility, economic fish

E-mail address: karun.t@psu.ac.th

[1]ภาควิชาวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ จ.สงขลา 90112

[1]Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112

[2]ภาควิชาวาริชศาสตร์ คณะทรัพยากรธรรมชาติ มหาวิทยาลัยสงขลานครินทร์ อ.หาดใหญ่ จ.สงขลา 90112

[2]Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90112

[3]ภาควิชาสัตวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ จ.กรุงเทพฯ 10900

[3]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900

[4]หน่วยปฏิบัติการวิจัยประเมินประสิทธิภาพอาหารสัตว์ทางชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ จ.กรุงเทพฯ 10900

[4]Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900

Nile tilapia (*Oreochromis niloticus*). Modified methods had significant effects on chemical compositions ($P < 0.05$) by decreasing crude fiber and increasing available carbohydrates. Improved physicochemical properties were mainly observed in soaked PKM when compared with other methods. For carbohydrate digestibility, soaking was the best modified method for increasing the digestion, followed by electron beam and gamma irradiation, respectively. These findings suggest that soaking could be practical method for increasing carbohydrate utilization in economic fish.

คำนำ

การเพิ่มขึ้นของอุตสาหกรรมปาล์มน้ำมัน (oil palm) เพื่อใช้เป็นแหล่งพลังงาน และวัตถุดิบในกระบวนการผลิตของอุตสาหกรรมต่อเนื่องหลายชนิด ก่อให้เกิดผลพลอยได้คือกากเนื้อในเมล็ดปาล์ม (palm kernel meal) เป็นจำนวนมาก ผลพลอยได้ดังกล่าวมีการใช้ประโยชน์ได้น้อย ส่วนใหญ่มักใช้เป็นส่วนผสมของอาหารสัตว์เคี้ยวเอื้อง รวมถึงสุกร และสัตว์ปีก (Agunbiade *et al*., 1999; Khadijat *et al*., 2012) สำหรับในสัตว์น้ำ การใช้กากเนื้อในเมล็ดปาล์มเพื่อเป็นวัตถุดิบอาหารพบว่ามีงานวิจัยค่อนข้างน้อย เนื่องจากมีข้อจำกัดในปริมาณเยื่อใย ซึ่งส่วนใหญ่เป็นกาแลกโตแมนแนน (galactomannan) ที่สัตว์ไม่สามารถย่อยได้ องค์ประกอบดังกล่าวจะห่อหุ้มสารอาหารและเพิ่มความหนืดในท่อทางเดินอาหาร ทำให้ประสิทธิภาพการย่อยและการใช้ประโยชน์จากวัตถุดิบเกิดขึ้นได้น้อย (Choct and Annison, 1992) อย่างไรก็ตาม กากเนื้อในเมล็ดปาล์มสามารถใช้เป็นส่วนผสมเพื่อเลี้ยงปลาเศรษฐกิจบางชนิดได้ในช่วง 10-30 เปอร์เซ็นต์ (Omoregie, 2001; Ng and Chong, 2002; Ng and Chen, 2002)

การศึกษาในวัตถุดิบอาหารหลายชนิดพบว่าการดัดแปรวัตถุดิบให้เหมาะสมจะทำให้สัตว์สามารถย่อย ดูดซึม และใช้ประโยชน์จากอาหารได้ดีขึ้น (Hossain *et al*., 2001; Olude *et al*., 2008; Thongprajukaew *et al*., 2011) เนื่องจากวัตถุดิบจะเปลี่ยนแปลงสมบัติทางเคมีกายภาพให้เหมาะสมต่อการไฮโดรไลส์ของเอนไซม์ย่อยอาหาร เช่น การละลายน้ำ (Chung *et al*., 2010; Kaur *et al*., 2010) โครงสร้างของพื้นผิว (Kristensen *et al*., 2008; Lopez-Rubio *et al*., 2008) ลิกโนเซลลูโลส (Al-Masri and Guenther, 1999; Kristensen *et al*., 2008; Chung and Liu, 2010) และความเป็นผลึก (Lopez-Rubio *et al*., 2008; Chung *et al*., 2010; Kaur *et al*., 2010) เป็นต้น ดังนั้น การดัดแปรกากเนื้อในเมล็ดปาล์มโดยวิธีทางกายภาพเพื่อเพิ่มประสิทธิภาพการย่อยและใช้ประโยชน์ในสัตว์จึงเป็นแนวทางหนึ่งที่จะช่วยลดต้นทุนการผลิต ลดปัญหากากของเสียที่ส่งผลกระทบต่อสิ่งแวดล้อม และเป็นการใช้ทรัพยากรได้อย่างคุ้มค่าและยั่งยืน

อุปกรณ์และวิธีการ

การดัดแปรกากเนื้อในเมล็ดปาล์ม

ดัดแปรกากเนื้อในเมล็ดปาล์มด้วยวิธีการต่างๆ ได้แก่ การแช่น้ำเป็นเวลา 12 ชั่วโมง (Negi *et al*., 2001) การใช้คลื่นไมโครเวฟเป็นเวลา 4 นาที (Alajaji and El-Adawy, 2006) และการใช้รังสีแกมมาและลำแสงอิเล็กตรอนที่ระดับ 30 กิโลเกรย์ (Shawrang *et al*., 2011)

การวิเคราะห์คาร์โบไฮเดรต

วิเคราะห์องค์ประกอบทางเคมีของกากเนื้อในเมล็ดปาล์มตามวิธีการของ AOAC (2005) และแสดงผลในรูปของเปอร์เซ็นต์น้ำหนักแห้ง

การวิเคราะห์สมบัติทางเคมีกายภาพ

นำตัวอย่างกากเนื้อในเมล็ดปาล์มมาทำให้แห้งโดยเครื่องทำแห้งภายใต้ความดันและอุณหภูมิต่ำ (Delta 2-24 LSC, Germany) และวิเคราะห์สมบัติทางเคมีกายภาพที่มีผลต่อการไฮโดรไลส์ของเอนไซม์ ได้แก่ การเปลี่ยนแปลงโครงสร้างทางจุลภาค โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (Quanta 400, FEI, Czech Republic) รูปแบบการเลี้ยวเบนรังสีเอกซ์และปริมาณของผลึก โดยใช้เอกซเรย์ดิฟแฟรกโตมิเตอร์ (X' Pert MPD, Philips, Netherlands) และการเปลี่ยนแปลงของลิกโนเซลลูโลส (เซลลูโลส เฮมิเซลลูโลส และลิกนิน) โดยใช้ฟูเรียทรานสฟอร์มอินฟราเรด (Equinox 55, Bruker, Germany) ที่แถบสเปกตรัมจำเพาะตามรายงานวิจัยของ Fang *et al*. (2000), Pandey and Pitman (2003) และ Rana *et al*. (2010) ซึ่งตรวจสอบเซลลูโลสที่ 1381, 1320 และ 1154 cm^{-1} เฮมิเซลลูโลสที่ 1745, 1738 และ 1090 cm^{-1} และลิกนินหรือเฮมิเซลลูโลสที่ 1244 cm^{-1}

การประเมินประสิทธิภาพการย่อยในหลอดทดลอง (*in vitro* digestibility)

ศึกษาประสิทธิภาพการย่อยของกากเนื้อในเมล็ดปาล์มในหลอดทดลองโดยใช้เอนไซม์ที่สกัดจากปลานิล โดยประเมินประสิทธิภาพการย่อยคาร์โบไฮเดรตจากปริมาณน้ำตาลมอลโทสที่เพิ่มขึ้นตามวิธีการของ Thongprajukaew *et al*. (2011)

การวิเคราะห์ข้อมูลทางสถิติ

รายงานผลข้อมูลในรูปค่าเฉลี่ย ± ความคลาดเคลื่อนมาตรฐาน (mean ± SEM) วิเคราะห์ความแปรปรวนของข้อมูลแบบทางเดียว และเปรียบข้อมูลโดยใช้ Duncan's Multiple Range Test (DMRT) ที่ระดับนัยสำคัญ 0.05

ผลและวิจารณ์ผลการทดลอง

องค์ประกอบทางเคมีของคาร์โบไฮเดรต

ปริมาณเยื่อใยของกากเนื้อในเมล็ดปาล์มที่ผ่านการดัดแปรมีการลดลงอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) โดยกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ ใช้คลื่นไมโครเวฟ รังสีแกมมา และลำแสงอิเล็กตรอน มีค่าเท่ากับ 17.91 ± 0.03, 14.36 ± 0.03, 13.54 ± 0.40 และ 12.42 ± 0.52 เปอร์เซ็นต์ ตามลำดับ เมื่อเปรียบเทียบกับกากเนื้อในเมล็ดปาล์มที่ไม่ผ่านการดัดแปร (21.74 ± 0.56 เปอร์เซ็นต์) การลดลงของเยื่อใยหลังจากดัดแปรมีรายงานในวัตถุดิบหลายชนิด เช่น เปลือกหุ้มเมล็ดฝ้าย เปลือกถั่วลิสง เปลือกถั่วเหลือง และเมล็ดทานตะวัน (Al-Masri and Guenther, 1999) และฟางข้าวสาลี (Kristensen *et al*., 2008) การเปลี่ยนแปลงดังกล่าวอาจเกิดขึ้นเนื่องจากการทำลายโครงสร้างของลิกโนเซลลูโลสหลังจากการดัดแปร ทำให้ปริมาณคาร์โบไฮเดรตที่ย่อยได้มีค่าเพิ่มขึ้นจาก 48.16 เปอร์เซ็นต์ (ชุดควบคุม) เป็น 52.65 (กากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ), 56.00

(กากเนื้อในเมล็ดปาล์มที่ผ่านคลื่นไมโครเวฟและรังสีแกมมา) และ 56.78 เปอร์เซ็นต์ (กากเนื้อในเมล็ดปาล์มที่ผ่านลำแสงอิเล็กตรอน)

สมบัติทางเคมีกายภาพ

โครงสร้างทางจุลภาค

โครงสร้างทางจุลภาคของกากเนื้อในเมล็ดปาล์มที่ผ่านการดัดแปรมีความแตกต่างจากชุดควบคุม (Figure 1) โดยกากเนื้อในเมล็ดปาล์มที่ผ่านการแช่น้ำ (Figures 1D-1F) มีพื้นผิวที่ขรุขระมากที่สุด รองลงมาคือการใช้คลื่นไมโครเวฟ (Figures 1G-1I) การเปลี่ยนแปลงดังกล่าวอาจมีผลต่อการย่อยของสัตว์ เนื่องจากการดัดแปรที่เหมาะสมควรทำให้พื้นที่ผิวเพิ่มขึ้นเพื่อให้เอนไซม์ไฮโดรไลส์วัตถุดิบอาหารได้ดี (Kristensen *et al*., 2008; Lopez-Rubio *et al*., 2008) สำหรับการดัดแปรด้วยรังสีแกมมา (Figures 1J-1L) และลำแสงอิเล็กตรอน (Figures 1M-1O) พบว่าทำให้พื้นผิวมีลักษณะเรียบมากขึ้น ซึ่งอาจทำให้อัตราการไฮโดรไลส์ลดลง

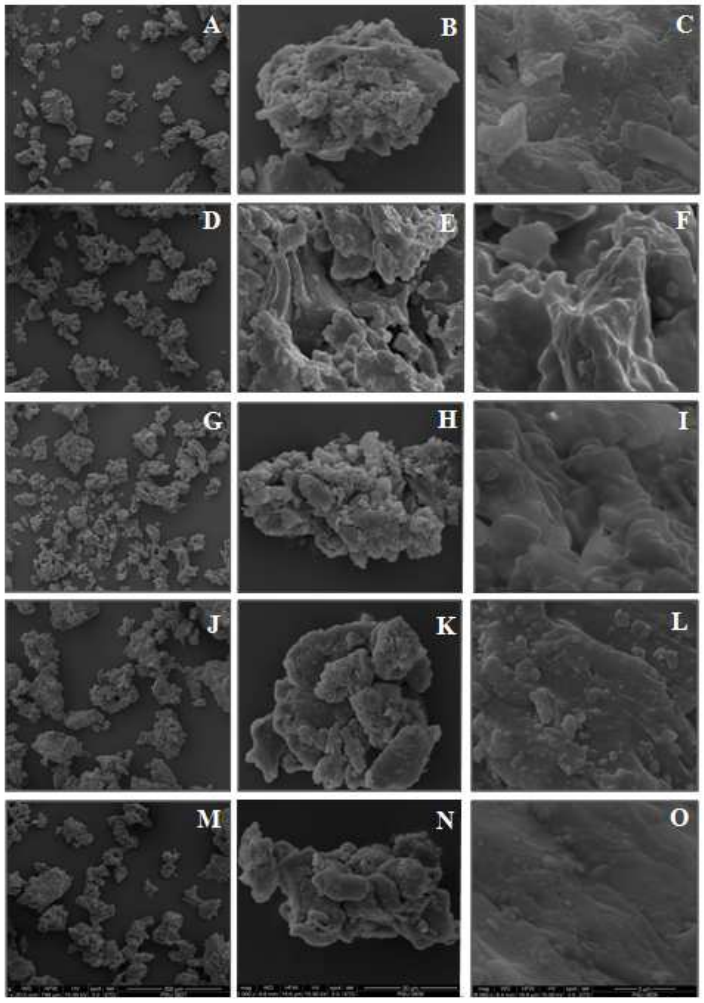


Figure 1 Microscopic structures of raw (A-C) and pretreated PKMs by soaking (D-F), microwave irradiation (G-I), gamma irradiation (J-L) and electron beam (M-O). Magnifications of photographs were recorded at 200× (left), 2000× (middle) and 8000× (right).

การเลี้ยวเบนรังสีเอกซ์

รูปแบบการเลี้ยวเบนรังสีเอกซ์ของกากเนื้อในเมล็ดปาล์มในชุดควบคุมและชุดทดลองมีความคล้ายคลึงกัน (Figure 2A) โดยพบพีคหลักที่มุมเลี้ยวเบน (2θ) 15.9, 19.9 และ 26.6 องศา เมื่อคำนวณความเป็นผลึกสัมพัทธ์พบว่าการดัดแปรโดยการแช่น้ำสามารถลดค่าผลึกสัมพัทธ์ได้อย่างมีนัยสำคัญ (33.52 ± 0.01 เปอร์เซ็นต์, $P < 0.05$) เมื่อเปรียบเทียบกับกากเนื้อในเมล็ดปาล์มที่ไม่ผ่านการดัดแปร (36.60 ± 0.01 เปอร์เซ็นต์) ส่วนการดัดแปรโดยการใช้คลื่นไมโครเวฟ (38.22 ± 0.02 เปอร์เซ็นต์) รังสีแกมมา (37.94 ± 0.01 เปอร์เซ็นต์) และลำแสงอิเล็กตรอน (41.24 ± 0.02 เปอร์เซ็นต์) พบว่าทำให้ค่าความเป็นผลึกสัมพัทธ์เพิ่มขึ้น การเปลี่ยนแปลงดังกล่าวอาจเกิดขึ้นเนื่องจากโครงสร้างผลึกบางส่วนถูกทำลายภายหลังการดัดแปร การลดลงของปริมาณผลึกสัมพัทธ์ทำให้โครงสร้างส่วนอสัณฐาน (amorphous) ของกากเนื้อในเมล็ดปาล์มที่แช่น้ำมีค่าเพิ่มขึ้น ซึ่งอาจส่งผลทำให้อัตราการไฮโดรไลส์ของเอนไซม์ย่อยอาหารสูงขึ้น เช่นเดียวกับการศึกษาของ Chung and Liu (2010) และ Kaur *et al* (2010) ซึ่งพบว่าแป้งที่มีความเป็นผลึกสัมพัทธ์ต่ำจะมีประสิทธิภาพการย่อยสูง

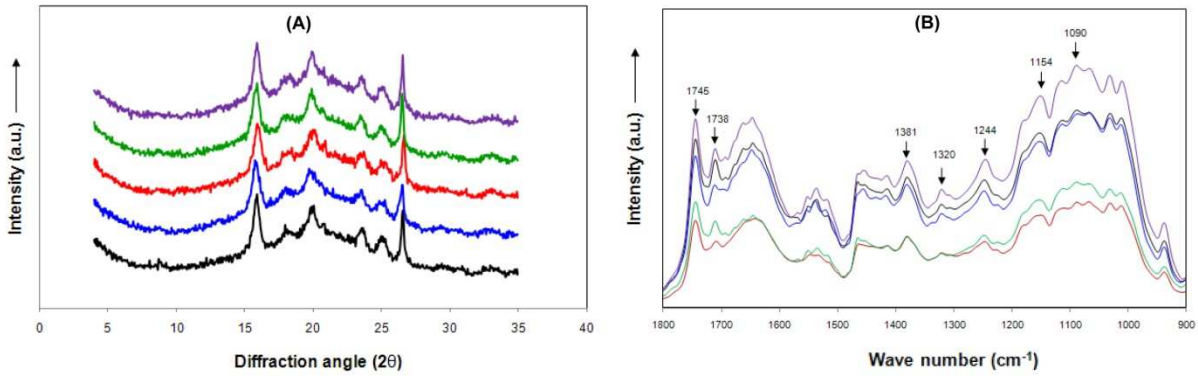


Figure 2 X-ray diffractograms (A) and FTIR spectra (B) of raw (—) and pretreated PKMs by soaking (—), microwave irradiation (—), gamma irradiation (—) and electron beam (—).

การเปลี่ยนแปลงของลิกโนเซลลูโลส

สเปกตรัมของลิกโนเซลลูโลสระหว่างกากเนื้อในเมล็ดปาล์มที่ผ่านและไม่ผ่านการดัดแปรมีความแตกต่างกัน (Figure 2B) การดัดแปรกากเนื้อในเมล็ดปาล์มโดยการแช่น้ำ ใช้คลื่นไมโครเวฟ และรังสีแกมมา พบว่าสามารถลดเซลลูโลส เฮมิเซลลูโลส และลิกนินได้ ซึ่งสอดคล้องกับอัตราส่วนสเปกตรัมที่ 1429 cm^{-1} (ส่วนผลึก) ต่อ 893 cm^{-1} (ส่วนอสัณฐาน) ที่มีค่าเท่ากับ 3.46, 3.13 และ 2.35 ตามลำดับ เมื่อเปรียบเทียบกับกากเนื้อในเมล็ดปาล์มในชุดควบคุม (3.67) อัตราส่วนดังกล่าวมีความสอดคล้องกับความเป็นผลึกสัมพัทธ์ที่คำนวณจากเอกซเรย์ดิฟแฟรกโตมิเตอร์ การลดลงของลิกโนเซลลูโลสหลังจากดัดแปรสอดคล้องกับการลดลงขององค์ประกอบผนังเซลล์ที่พบในผลพลอยทางทางการเกษตรหรือวัตถุดิบอาหารหลายชนิด (Al-Masri and Guenther, 1999; Kristensen *et al*., 2008; Chung and Liu, 2010)

ประสิทธิภาพการย่อยคาร์โบไฮเดรต

การดัดแปรกากเนื้อในเมล็ดปาล์มมีผลอย่างมีนัยสำคัญต่อประสิทธิภาพการย่อยคาร์โบไฮเดรต ($P < 0.05$, Figure 3) โดยการแช่น้ำทำให้ประสิทธิภาพการย่อยมีค่าสูงสุด รองลงมาคือการใช้ลำแสงอิเล็กตรอนและรังสีแกมมา ($P < 0.05$) สำหรับการผ่านไมโครเวฟพบว่าไม่มีผลอย่างมีนัยสำคัญต่อการเพิ่มประสิทธิภาพการย่อย ($P > 0.05$) เมื่อเปรียบเทียบกับชุดควบคุม การเพิ่มขึ้นของประสิทธิภาพการย่อยของวัตถุดิบอาหารหลังจากการแช่น้ำเกิดขึ้นเนื่องจากการมีองค์ประกอบของคาร์โบไฮเดรตที่ย่อยได้ในปริมาณที่มากขึ้น รวมทั้งวัตถุดิบมีสมบัติทางเคมีกายภาพที่เหมาะสมต่อการไฮโดรไลส์ ผลการศึกษานี้สอดคล้องกับการศึกษาใน moth bean (Negi *et al*., 2001), black gram และ chick pea (Rehman, 2007) ดังนั้น การแช่น้ำกากเนื้อในเมล็ดปาล์มก่อนการผลิตอาหารจึงอาจช่วยเพิ่มประสิทธิภาพการย่อยและอัตราการเจริญเติบโตของปลาได้เช่นเดียวกับการศึกษาในเมล็ดโสนคางคกป่น (Hossain *et al*., 2001) และกากมะพร้าว (Olude *et al*., 2008)

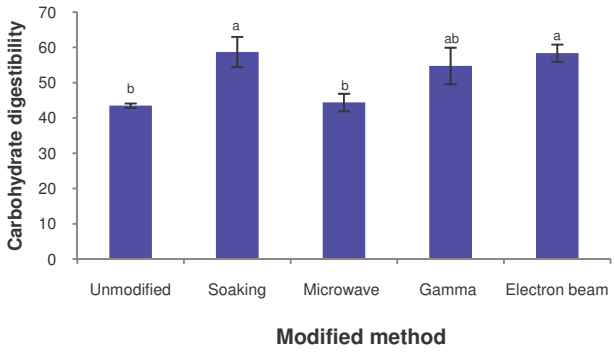


Figure 3 *In vitro* carbohydrate digestibility (μmol maltose g PKM^{-1}) of raw and pretreated PKMs using digestive enzyme extracts from Nile tilapia (amylase 2,000 U). Analysis was performed in quadruplicate. Data with different superscript indicate significant difference ($P < 0.05$).

สรุป

การแช่น้ำเป็นวิธีการที่เหมาะสมสำหรับดัดแปรกากเนื้อในเมล็ดปาล์มเพื่อการผลิตอาหารสัตว์น้ำ เนื่องจากทำให้ปริมาณคาร์โบไฮเดรตที่ย่อยได้มีค่าสูงขึ้น มีการเปลี่ยนแปลงสมบัติทางเคมีกายภาพที่เหมาะสม ได้แก่ โครงสร้างทางจุลภาคของวัตถุดิบมีพื้นผิวขรุขระ รูปแบบการกระจายรังสีเอกซ์คงเดิม แต่ความเป็นผลึกลดลง มีการเปลี่ยนแปลงของปริมาณของลิกโนเซลลูโลสในวัตถุดิบ ซึ่งมีผลทำให้ประสิทธิภาพการย่อยคาร์โบไฮเดรตมีค่าสูงขึ้น ดังนั้น การแช่น้ำจึงอาจเป็นทางเลือกหนึ่งที่เหมาะสมสำหรับเพิ่มคุณภาพ และความสามารถในการใช้ประโยชน์จากวัตถุดิบอาหารของสัตว์น้ำ

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณทุนสนับสนุนการวิจัยจากสำนักวิจัยและพัฒนาภายใต้โครงการพัฒนาศักยภาพการทำวิจัยของอาจารย์ใหม่ และโครงการทุนดรุณาจารย์ (PRPM ID 11908) และทุนวิจัยจากภาควิชาวิทยาศาสตร์ประยุกต์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่

เอกสารอ้างอิง

Agunbiade, J.A., J. Wiseman and D.J.A. Cole. 1999. Energy and nutrient use of palm kernels, palm kernel meal and palm kernel oil in diets for growing pigs. **Animal Feed Science and Technology** 80: 165-181.

Alajaji, S.A. and T.A. El-Adawy. 2006. Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. **Journal of Food Composition and Analysis** 19: 806-812.

Al-Masri, M.R. and K.D. Guenther. 1999. Changes in digestibility and cell-wall constituents of some agricultural by-products due to gamma irradiation and urea treatments. **Radiation Physics and Chemistry** 55: 323-329.

AOAC. 2005. **Official Methods of Analysis**. 18th ed. Association of Official Analytical Chemists, Washington, DC.

Choct, M. and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. **British Poultry Science** 33: 821-834.

Chung, H.J., S.Y. Lee, J.H. Kim, J.W. Lee, M.W. Byun and S.T. Lim. 2010. Pasting characteristics and *in vitro* digestibility of γ-irradiated RS_4 waxy maize starches. **Journal of Cereal Science** 52: 53-58.

Chung, H.J. and Q. Liu. 2010. Molecular structure and physicochemical properties of potato and bean starches as affected by gamma-irradiation. **International Journal of Biological Macromolecules 47**: 214-222.

Fang, J.M., R.C. Sun and J. Tomkinson. 2000. Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction. **Cellulose** 7: 87-107.

Hossain, M.A., U. Focken and K. Becker. 2001. Effect of soaking and soaking followed by autoclaving of Sesbania seeds on growth and feed utilization in common carp, *Cyprinus carpio* L. **Aquaculture** 203: 133-148.

Kaur, M., K.S. Sandhu and S.T. Lim. 2010. Microstructure, physicochemical properties and *in vitro* digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars. **Carbohydrate Polymer** 79: 349-355.

Khadijat, B.M., O.O. Enoch and B.E. Suleiman. 2012. Performance, carcass characteristics and blood composition of broilers fed varying levels of palm kernel meal (*Elaise guinensis*) supplemented with different levels of fish meal. **International Journal of Poultry Science** 11(1): 73-77.

Kristensen, J.B., L.G. Thygesen, C. Felby, H. Jørgensen and T. Elder. 2008. Cell-wall structural changes in wheat straw pretreated for bioethanol production. **Biotechnology for Biofuels** 1: 5-14.

Lopez-Rubio, A., B.M. Flanagan, A.K. Shrestha, M.J. Michael and E.P. Gilbert. 2008. Molecular rearrangement of starch during *in vitro* digestion: toward a better understanding of enzyme resistant starch formation in processed starches. **Biomacromolecules** 9: 1951-1958.

Negi, A., P. Boora and N. Khetarpaul. 2001. Effect of microwave cooking on the starch and protein digestibility of some newly released moth bean (*Phaseolus aconitifolius* Jacq.) cultivars. **Journal of Food Composition and Analysis** 14: 541-546.

Ng, W.K. and M.L. Chen. 2002. Replacement of soybean meal with palm kernel meal in practical diets for hybrid Asian-African catfish, *Clarias macrocephalus* × *C. gariepinus*. **Journal of Applied Aquaculture** 12 (4): 67-76.

Ng, W.K. and K.K. Chong. 2002. The nutritive value of palm kernel meal and the effect of enzyme supplementation in practical diets for red hybrid tilapia (*Oreochromis* sp.). **Asian Fisheries Society** 15: 167-176.

Olude, O.O., W.O.A. Alegbeleye and S.O. Obasa. 2008. The use of soaked copra meal as a partial substitute for soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*) fingerlings. **Livestock Research for Rural Development** 20(10). Article 169.

Omoregie, E. 2001. Utilization and digestibility of mango seeds and palm kernel meal by juvenile *Labeo senegalensis* (Antheriniformes: Cyprinidae). **Aquaculture Research** 32: 681-687.

Pandey, K.K. and A.J. Pitman. 2003. FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. **International Biodeterioration and Biodegradation** 52: 151-160.

Rana, R., R. Langenfeld-Heyser, R. Finkeldey and A. Polle. 2010. FTIR spectroscopy, chemical and histochemical characterisation of wood and lignin of five tropical timber wood species of the family of Dipterocarpaceae. **Wood Science and Technology** 44: 225-242.

Rehman, Z.U. 2007. Domestic processing effects on available carbohydrate content and starch digestibility of black grams (*Vigna mungo*) and chick peas (*Cicer arietium*). **Food Chemistry** 100: 764-767.

Shawrang, P., M.H. Mansouri, A.A. Sadeghi and F. Ziaie. 2011. Evaluation and comparison of gamma and electron beam irradiation effects on total and free gossypol of cottonseed meal. **Radiation Physics and Chemistry** 80: 761-762.

Thongprajukaew, K., U. Kovitvadhi, S. Kovitvadhi, P. Somsueb and K. Rungruangsak-Torrissen. 2011. Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910). **Aquaculture** 322-323: 1-9.