รายงานวิจัยฉบับสมบูรณ์

สมบัติเชิงหน้าที่และการใช้ประโยชน์ในอาหารจากพืชสมุนไพร/เครื่องเทศและเครื่องแกง

Functional properties and using in food from spices/herbs and curry pastes

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(ภาษาอังกฤษ) Functional properties and using in food from spices/herbs and curry pastes

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กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณกองทุนวิจัย มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ ในการให้เงินสนับสนุนการทำวิจัยและให้โอกาสผลิตนักศึกษาปริญญาโท นอกจากนั้นขอขอบคุณคณะอุตสาหกรรมเกษตรที่ได้เอื้อเฟื้ออุปกรณ์และสถานที่ในการวิจัยจนแล้วเสร็จ ผลงานวิจัยนี้แสดงให้เห็นว่า พืชสมุนไพร/เครื่องเทศและเครื่องแกงซึ่งใช้เป็นวัตถุดิบที่สำคัญในการประกอบอาหารไทย มีสมบัติเชิงหน้าที่ที่สำคัญ ซึ่งเป็นข้อมูลช่วยสนับสนุนและส่งเสริมการบริโภคอาหารไทย ที่นอกจากมีจะมีรสชาติดี มีลักษณะจำเพาะ แล้วยังมีสมบัติเสริมสุขภาพ ซึ่งแตกต่างจากการบริโภคอาหารตะวันตกหลายชนิดที่บริโภคแล้วส่งผลเสียต่อสุขภาพ

สุนิสา ศิริพงศ์วุฒิกร

หัวหน้าโครงการ

บทคัดย่อ

แกงเหลือง หรือ แกงส้มภาคใต้เป็นอาหารท้องถิ่นที่ได้รับความนิยมอย่างแพร่หลาย โดยเฉพาะภาคใต้ เครื่องปรุงที่เป็นส่วนประกอบหลักที่สำคัญ ได้แก่ ขมิ้น กระเทียม หอมแดง และพริกชี้ฟ้าแห้ง ซึ่งมีรายงานว่าเป็นแหล่งของสารต้านจุลินทรีย์ และต้านออกซิเดชัน ในระหว่างการปรุงเป็นแกง จะมีการเติมน้ำมะนาว น้ำมะขามหรือส้มแขก เพื่อเป็นสารให้รสเปรี้ยว ส้มแขก เป็นพืชประจำท้องถิ่นของหลายจังหวัดในภาคใต้ เช่น ยะลา ปัตตานี นราธิวาสและสงขลา ผลของส้มแขกสามารถใช้เป็นสารให้รสเปรี้ยวในอาหารหลายชนิดโดยไม่ก่อให้เกิดรสขม และมีบริโภคได้ทั้งปี ดังนั้น การศึกษาครั้งนี้ประกอบด้วย 3 หัวข้อหลัก คือ (1) ผลของการเติมส้มแขกแห้งต่อคุณภาพโดยรวมของเครื่องแกงและน้ำแกง (2) ผลของการเติมส้มแขกแห้งต่อปริมาณ ฟีนอลิกทั้งหมด สมบัติการต้านออกซิเดชันและการเปลี่ยนแปลงคุณภาพของเครื่องแกงส้มภาคใต้ ระหว่างการเก็บรักษา (3) การศึกษาสมบัติการเป็นพรีไบโอติกของเครื่องแกงส้มภาคใต้ ที่เติมและไม่เติมส้มแขก

ผลการเติมส้มแขกแห้งในเครื่องแกงส้มภาคใต้ในระดับร้อยละ 0, 3, 5, 7, 10, 13, 15, 17, 20 และ 25 พบว่า ค่าสีของเครื่องแกงไม่มีความสัมพันธ์กับปริมาณการเติมส้มแขกแห้ง อย่างไรก็ตามพบว่า เมื่อมีการเติมส้มแขกปริมาณมากขึ้น มีผลให้ค่าพีเอชลดลงและค่าความเป็นกรดเพิ่มขึ้น เช่นเดียวกับการนำเครื่องแกงมาปรุงเป็นน้ำแกง ส่วนค่า A_w และค่าความชื้นของเครื่อแกงมีแนวโน้มเพิ่มขึ้น เมื่อมีการเติมส้มแขกแห้งเพิ่มขึ้น ผลทางจุลินทรีย์ของเครื่องแกงทุกชุดการทดลอง พบว่าปริมาณจุลินทรีย์ทั้งหมดอยู่ระหว่าง 10^2-10^3 cfu/g นอกจากนั้นพบว่า ชุดการทดลองเครื่องแกงที่มีการเติมส้มแขกแห้ง 15% จะมีคะแนนความชอบสูงสุด

ผลของการเปลี่ยนแปลงคุณภาพ ปริมาณฟีนอลิกทั้งหมด และสมบัติการต้านอนุมูลอิสระ ของเครื่องแกงที่เติมส้มแขกแห้ง ระหว่างการเก็บรักษานาน 6 เดือน พบว่า ปริมาณฟีนอลิกทั้งหมดของเครื่องแกงสูตรพื้นฐานซึ่งมีการเติมเกลือ (P1) เครื่องแกงที่มีการเติมส้มแขกและเกลือ (P2) และเครื่องแกงที่เติมส้มแขกแต่ไม่เติมเกลือ (P3) มีปริมาณฟีนอลิกลดลง เมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น นอกจากนี้คุณสมบัติในการต้านอนุมูลอิสระด้วยวิธี DPPH และ FRAP ของเครื่องแกงสูตรพื้นฐานไม่มีการเติมส้มแขก (P1) ลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น อย่างไรก็ตามคุณสมบัติในการต้านอนุมูลอิสระของเครื่องแกง P2 และ P3 เพิ่มขึ้นในเดือนที่ 2 ของการเก็บรักษา หลังจากนั้นจะค่อยๆลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น ปริมาณจุลินทรีย์ทั้งหมดของเครื่องแกงทุกชุดการทดลองอยู่ระหว่าง 10^2-10^3 cfu/g ปริมาณยีสต์และราของเครื่องแกง P1 และ P2 มีปริมาณน้อยกว่า 30

cfu/g ตลอดระยะเวลา 6 เดือนในการเก็บรักษา ส่วนปริมาณยีสต์และราของเครื่องแกง P3 มีปริมาณน้อยกว่า 10^2cfu/g จนสิ้นสุดการเก็บรักษา ปริมาณของแบคทีเรียแลกติค ของเครื่องแกง P2 มีปริมาณน้อยกว่าของเครื่องแกง P1 และ P3 จนสิ้นสุดการเก็บรักษา อย่างไรก็ตามไม่พบเชื้อ *Staphylococcus aureus*, *Bacilluus cereus, Clostridium perfringens, Salmonella* spp., *Escherichia coli* และ coliforms ตลอดการเก็บรักษาในทุกชุดการทดลอง ส่วนผลการทดลองทางประสาทสัมผัส พบว่าเครื่องแกงที่มีการเติมส้มแขกแห้งและเกลือ (P2) มีคะแนนความชอบมากกว่าเมื่อเปรียบเทียบกับเครื่องแกงที่เติมส้มแขก ไม่เติมเกลือ (P3) อายุการเก็บรักษาของเครื่องแกงจะขึ้นอยู่กับการยอมรับของผู้บริโภคเป็นหลัก โดยคะแนนความชอบของน้ำแกงไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างคะแนนความชอบวันแรกและวันสุดท้ายของการเก็บรักษาในทุกคุณลักษณะ โดยอายุการเก็บรักษาของเครื่องแกง P1, P2 และ P3 อยู่ที่ 6, 6 และ 4 เดือนตามลำดับ

ผลการศึกษาคุณสมบัติการเป็นพรีไบโอติกของเครื่องแกงเหลืองที่เติมส้มแขก (P2) และไม่เติมส้มแขก (P1) พบว่า เครื่องแกงทั้ง 2 ชุดการทดลองมีคุณสมบัติการต้านการย่อยในระบบจำลองทางเดินอาหารส่วนบน ในการหมักในระบบจำลองลำไส้ใหญ่แบบกะ นำเครื่องแกงที่ผ่านการย่อยในระบบจำลองในปาก กระเพาะและลำไส้เล็กมาหมักด้วยอุจจาระมนุษย์พบว่าจำนวนของ bifidobacteria, lactobacilli และ eubacteria เพิ่มขึ้น และสามารถลดจำนวนของ bacteroides และ clostridia ในการหมักที่ 24 ชั่วโมง ส่งผลให้สมบัติความเป็นพรีไบโอติก (prebiotic index (PI)) ของเครื่องแกงที่มีการเติมส้มแขก (P2) (prebiotic index (PI) = 2.75) มากกว่า เครื่องแกงที่ไม่เติมส้มแขก (P1) (prebiotic index (PI) = 1.19) อย่างไรก็ตามพบว่าปริมาณกรดไขมันสายสั้น เช่น กรดแลกติก กรดอะซิติก กรดโพพิโอนิก และ กรดบิวทีริก ของเครื่องแกงที่ไม่เติมส้มแขก (P1) มีแนวโน้มสูงกว่าเครื่องแกงที่เติมส้มแขก (P2) นอกจากนี้เครื่องแกงที่ไม่เติมส้มแขก (P1) สามารถผลิตวิตามิน B1 (18.38±0.10 μg/ml) และ B2 (18.38±0.10 μg/ml) แต่ไม่ผลิต folic acid ส่วนเครื่องแกงที่เติมส้มแขกสามารถผลิตวิตามิน B1 (5.99±0.48 μg/ml) แต่ไม่ผลิตวิตามิน B2 และ folic acid ใน 24 ชั่วโมงของการหมัก

ABSTRACT

Southern sour curry soup or Keang-Hleung is a traditional popular spicy-sour curry consumed particularly in southern part of Thailand. The ingredients used in the paste are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds. Lime juice, tamarind pulp and garcinia fruit or any sour fruits available will be used in the soup for making the sour taste. This study was divided into three main sections: 1. Effect of added garcinia fruit on total quality of Keang-Hleung paste and soup 2. Effect of added garcinia fruit on total phenolic compound content, antioxidant properties and quality changes of the southern sour curry paste, Keang-Hleung, during storage 3. Study of prebiotic properties of the southern sour curry paste, Keang-Hleung, with and without garcinia.

Effect of added dried garcinia fruit at 0, 3, 5, 7, 10, 13, 15, 17, 20 and 25% garcinia on total quality of southern curry paste showed that L^*, a^*, b^* of the paste did not have any relationship with amount of added dried garcinia. However, it was found that the more added dried garcinia the less pH value and the higher acidity were found. When the paste was taken to cook, it was found that pH values and acidity (%) decreased and increased, respectively as amount of added garcinia fruit increased. The A_w and moisture content tended to increase when more added garcinia. Total viable counts, TVC, of all treatments were in the range of 10^2-10^3 cfu/g. In addition, it was found that the paste containing 15% garcinia fruit gave the highest sensory score of the soup and comparable with the control soup using lime juice.

Quality changes, total phenolic compound and antioxidant properties of the pastes were studied during 6 months storage. It was found that the total phenolic compound content of basic paste without the garcinia (P1), garcinia Keang-hleung paste (P2) and garcinia Keang-hleung paste without salt (P3) decreased as increased storage time. Moreover, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and the ferric reducing power (FRAP) activity of the basic paste (P1) decreased as increased storage time. However, the DPPH radical scavenging activity and the FRAP activity of the P2 and P3 increased during 2 months of storage and then decreased as increased storage

time. TVC of all paste samples (P1, P2 and P3) were in the range of 10^2-10^3 cfu/g. Yeast and mold counts of P1 and P2 were less than 30 cfu/g during storage. While, yeast and mold counts of P3 were less than 10^2 cfu/g at the end of storage. Lactic acid bacteria counts of P2 were lesser than that of P1 and P3 at end of storage. However, *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Salmonella* spp., *Escherichia coli* and coliforms were not detected in all treatments throughout the storage period. Moreover, the paste added with garcinia and salt (P2) had higher sensory score compared with the paste added with garcinia but no salt added (P3). The shelf-life of the paste mainly depended on consumer acceptability. Sensory score of the soup was not significantly different between score of day 0 and the end of storage in all attributes. Therefore, P1, P2 and P3 had shelf-life as at least 6, 6 and 4 mo, respectively.

Prebiotic properties showed that both the basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) were partially resistance to simulated gastrointestinal conditions hydrolysis. When the partially hydrolyzed pastes were fermented with human feces it was found that the numbers of bifidobacteria, lactobacilli and eubacteria were slightly increased whereas the numbers of bacteroids and clostridia were decreased in batch culture by fecal slurry fermentation for 24 h. Garcinia Keang-hleung paste (P2) showed prebiotic index (PI) of 2.75 which was higher than basic Keang-hleung paste without garcinia (P1) with PI of 1.19. However, the concentrations of short-chain fatty acids including lactic acid, acetic acid, propionic acid and butyric acid produced by fecal fermentation of basic Keang-hleung paste without garcinia (P1) was higher than garcinia Keang-hleung paste (P2). Moreover, basic Keang-hleung paste (P1) could produce vitamin B1 (18.38±0.10 µg/ml) and B2 (45.28±2.02 µg/ml) but not folic acid meanwhile garcinia Keang-hleung paste (P2) produced only vitamin B1 (5.99±0.48 µg/ml) at 24 h fermentation.

บทสรุปผู้บริหาร

การวิจัยในครั้งนี้เริ่มต้นจากการทบทวนวรรณกรรมด้านสมบัติเชิงหน้าที่ของพืชสมุนไพร/เครื่องเทศ ชนิดต่างๆ พบว่ามีสมบัติของการส่งเสริมสุขภาพของผู้บริโภคได้ อย่างไรก็ตาม งานวิจัยที่ผ่านมาเป็นการศึกษาพืชชนิดเดี่ยวๆ และใช้สารเคมีเป็นตัวทำละลายในขั้นตอนการสกัด ในขณะที่อาหารไทยซึ่งใช้พืชสมุนไพร/เครื่องเทศ ผสมหรือทำเป็นเครื่องแกงเป็นส่วนประกอบในอาหารที่ไม่สามารถใช้ตัวทำละลายที่เป็นสารเคมีที่อาจก่อให้เกิดความเป็นพิษได้ ดังนั้นงานวิจัยเรื่องสมบัติเชิงหน้าที่และการใช้ประโยชน์ในอาหารจากพืชสมุนไพร/เครื่องเทศและเครื่องแกง ซึ่งในภาคใต้นิยมแกงส้ม จึงมีวัตถุประสงค์ของโครงการวิจัยดังนี้

1. เพื่อศึกษาการยืดอายุการเก็บรักษาเครื่องแกงส้มโดยการใช้เทคโนโลยีเฮอร์เดิล
2. เพื่อศึกษาปริมาณฟีนอลิก สมบัติการต้านออกซิเดชัน และสมบัติการต้านจุลินทรีย์ในเครื่องแกงส้ม
3. เพื่อเป็นหลักฐานทางวิทยาศาสตร์ผลของการบริโภคแกงต่อจุลินทรีย์ที่เกี่ยวข้องกับสุขภาพลำไส้

โดยการศึกษาสมบัติการต้านอนุมูลอิสระ การเป็นพรีไบโอติกและการยอมรับของผู้บริโภคของเครื่องแกงส้มภาคใต้ (แกงเหลือง) ที่มีและไม่มีส้มแขก ผลการทดลองแสดงให้เห็นว่าปริมาณฟีนอลิกทั้งหมดของเครื่องแกงสูตรพื้นฐานซึ่งมีการเติมเกลือ (P1) เครื่องแกงที่มีการเติมส้มแขกและเกลือ (P2) และเครื่องแกงที่เติมส้มแขกแต่ไม่เติมเกลือ (P3) มีปริมาณฟีนอลิกลดลง เมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น (6 เดือน) คุณสมบัติในการต้านอนุมูลอิสระด้วยวิธี DPPH และ FRAP ของเครื่องแกงสูตรพื้นฐานไม่มีการเติมส้มแขก (P1) ลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น อย่างไรก็ตาม คุณสมบัติในการต้านอนุมูลอิสระของเครื่องแกง P2 และ P3 เพิ่มขึ้นในเดือนที่ 2 ของการเก็บรักษา หลังจากนั้นจะค่อยๆลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น ปริมาณจุลินทรีย์ทั้งหมดของเครื่องแกงทุกชุดการทดลองอยู่ระหว่าง 10^2-10^3 cfu/g ปริมาณยีสต์และราของเครื่องแกง P1 และ P2 มีปริมาณน้อยกว่า 30 cfu/g ตลอดระยะเวลา 6 เดือนในการเก็บรักษา ส่วนปริมาณยีสต์และราของเครื่องแกง P3 มีปริมาณน้อยกว่า 10^2cfu/g จนสิ้นสุดการเก็บรักษา ปริมาณของแบคทีเรียแลกติค ของเครื่องแกง P2 มีปริมาณน้อยกว่าของเครื่องแกง P1 และ P3 จนสิ้นสุดการเก็บรักษา อย่างไรก็ตามไม่พบเชื้อ *Staphylococcus aureus*, *Bacilluus cereus, Clostridium perfringens, Salmonella* spp., *Escherichia coli*

และ coliforms ตลอดการเก็บรักษาในทุกชุดการทดลอง ส่วนผลการทดลองทางประสาทสัมผัส พบว่าเครื่องแกงที่มีการเติมส้มแขกแห้งและเกลือ (P2) มีคะแนนความชอบมากกว่าเมื่อเปรียบเทียบกับเครื่องแกงที่เติมส้มแขก ไม่เติมเกลือ (P3) อายุการเก็บรักษาของเครื่องแกงจะขึ้นอยู่กับการยอมรับของผู้บริโภคเป็นหลัก โดยคะแนนความชอบของน้ำแกงไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างคะแนนความชอบวันแรกและวันสุดท้ายของการเก็บรักษาในทุกคุณลักษณะ โดยอายุการเก็บรักษาของเครื่องแกง P1, P2 และ P3 อยู่ที่ 6, 6 และ 4 เดือนตามลำดับ

ผลการศึกษาคุณสมบัติการเป็นพรีไบโอติกของเครื่องแกงเหลืองที่เติมส้มแขก (P2) และไม่เติมส้มแขก (P1) ในการหมักในระบบจำลองลำไส้ใหญ่แบบกะ นำเครื่องแกงที่ผ่านการย่อยในระบบจำลองในปาก กระเพาะและลำไส้เล็กมาหมักด้วยอุจจาระมนุษย์พบว่าจำนวนของ bifidobacteria, lactobacilli และ eubacteria เพิ่มขึ้น และสามารถลดจำนวนของ bacteroides และ clostridia ในการหมักที่ 24 ชั่วโมง ส่งผลให้สมบัติความเป็นพรีไบโอติก (prebiotic index (PI)) ของเครื่องแกงที่มีการเติมส้มแขก (P2) (prebiotic index (PI) = 2.75) มากกว่า เครื่องแกงที่ไม่เติมส้มแขก (P1) (prebiotic index (PI) = 1.19) อย่างไรก็ตามพบว่าปริมาณกรดไขมันสายสั้น เช่น กรดแลกติก กรดอะซิติก กรดโพพิโอนิก และกรดบิวทีริก ของเครื่องแกงที่ไม่เติมส้มแขก (P1) มีแนวโน้มสูงกว่าเครื่องแกงที่เติมส้มแขก (P2) นอกจากนี้เครื่องแกงที่ไม่เติมส้มแขก (P1) สามารถผลิตวิตามิน B1 และ B2 แต่ไม่ผลิต folic acid ส่วนเครื่องแกงที่เติมส้มแขกสามารถผลิตวิตามิน B1 แต่ไม่ผลิตวิตามิน B2 และ folic acid ใน 24 ชั่วโมงของการหมัก

งานวิจัยครั้งนี้ได้มีการตีพิมพ์แล้ว 1 ฉบับและอยู่ในระหว่างเตรียม manuscript 2 ฉบับ นอกจากนี้ผลการได้รับทุนสนับสนุนงานวิจัยครั้งนี้ยังช่วยทำให้ได้เกิดการผลิตบัณฑิตศึกษาระดับปริญญาโท 1 คน คือ นางสาวปรียาพร พรหมเจียม ซึ่งได้สอบปกป้องวิทยานิพนธ์ผ่าน และดำเนินการส่งวิทยานิพนธ์เมื่อวันที่ 8 มกราคม พ.ศ. 2558 เป็นที่เรียบร้อยแล้ว

สุนิสา ศิริพงศ์วุฒิกร

หัวหน้าโครงการ

ภาคผนวก

บทความที่ได้รับการตีพิมพ์แล้ว

Effect of Added Garcinia Fruit on Total Phenolic Compound Content, Antioxidant Properties and Quality Changes of the Southern Sour Curry Paste, Keang-hleung, During Storage

ผลงานวิจัยที่ยังไม่ได้ตีพิมพ์แต่อยู่ในวัตถุประสงค์ของการวิจัย และอยู่ในระหว่างการเตรียม manuscript จำนวน 2 ฉบับ คือ Effect of Added Garcinia Fruit on Total Quality of Keang-Hleung Paste and Soup **และ** Study of Prebiotic Properties of the Southern Sour Curry Paste, Keang-hleung, with and without Garcinia

1. Effect of Added Garcinia Fruit on Total Quality of Keang-Hleung Paste and Soup

Abstract

Southern sour curry or Keang-hleung soup is a traditional popular spicy-sour curry consumed particularly in southern part of Thailand. The ingredients used in the paste are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds. Lime juice, tamarind pulp and garcinia fruit or any sour fruits available will be used in the soup for making the sour taste. This study aimed to find out the suitable amount of garcinia fruit added into the paste for making the highest sensory score when the paste was taken to cook as spicy-sour soup. It was found that *L**, *a**, *b** of the paste did not have any relationship with amount of added dried garcinia (0, 3, 5, 7, 10, 13, 15, 17, 20 and 25%). However, it was found that the more added dried garcinia the less pH value and the higher acidity were found. When the paste was taken to cook, it was found that pH values and acidity (%) decreased and increased, respectively as amount of added garcinia fruit increased. The A_w and moisture content of the paste tended to increase when more added garcinia. Total viable count,TVC, of all

treatments were in the range of 10^2-10^3 cfu/g. In addition,it was found that the paste containing 15% garcinia fruit gave the highest sensory score (>7/9) of the soup and comparable with the control soup using lime juice (7/9).

Key words: Keang-hleung paste, Southern sour curry, garcinia, quality, consumer acceptability

Introduction

One of traditional popular spicy-sour curries of Southern Thais is Keang-hleung or southern sour curry. Due to less fat content but high in proportion of vegetables containing dietary fiber makes this curry soup low calories and turns to be healthy food. Therefore, it is recommented by physician to the diabetic person or even people who concern about their weight. Moreover, the ingredients used in the paste normally are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds (Ruby *et al*., 1995; Ahsan *et al*. 1999; Cousin *et al*. 2006; Siripongvutikorn *et al*., 2005; Jayaprakasha *et al*. 2006). For making the sour curry soup, acid fruits such as lime juice, tamarind pulp juice and garcinia fruit as well as any fruit giving the sour taste will be used if available.

Garcinia, *Garcinia atroviridis* Griff. exT. Anders, is a local plant found in southern part of Thailand particularly Pattani, Yala the province connect to Malaysia. This fruit is normally harvested and subjected to slice and dry and use as a dried form in various southern dishes for all year round. Moreover, it was noticed that using dried garcinia fruit in the soup did not only cause any bitter taste but also did control sourness better compared with lime juice. Nowadays, it is proved that hydroxy citric acid, an important organic compound in the garcinia fruit responsible for inhibitor of lipogensis with commercial and clinical applications (McCarty, 1995; Moffeff *et al*., 1996).

As busy of life and small family of people around the world then convenient food or ready to cook food is more interesting. However, Thais still prefer hot and spicy food. In addition, issue of healthy or function food is very interesting therefore the ready to cook paste as garcinia sour curry paste was attempted to produce and evaluate its quality during storage.

Materials and Methods

Materials

Turmeric rhizomes (*Curcuma longa* Linn.), garlic bulb (*Allium sativum* Linn.), dried chili (*Capsicum annuum* Linn.), shallot (*Allium ascalonicum* Linn.) and garcinia (*Garcinia atroviridis* Griff. Ex T. Anderson) were purchased from a local market in Hat-Yai, Thailand.

Chemicals and media

All chemicals and reagents as media for microbiological analysis were of analytical grade purchased from Sigma-Aldrich Chemical Co. St. Louis (USA) or Merck Darmstadt (Germany).

The paste preparation

The materials as above mentioned were sorted, trimmed and washed with tap water to remove dust, dirt and foreign matter then soaked in 150 ppm chlorine solution (solution: material as 3:1) 1 min, 10 ppm chlorine solution 1 min and tap water for 1 min and drained on the sieve for 60 min. Then the paste was formulated based on basic recipe obtaining from small and medium enterprise (SME) in Chumporn province. Actually, the basic recipe consisted of dried chili, shallot, turmeric rhizomes, garlic and salt as 30, 30, 10, 10 and 20 %, respectively. Thereafter, 0, 3, 5, 7, 10, 13, 15, 17, 20 and 25 g of dried garcinia fruit was added into the basic recipe 100g and coded as P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, respectively. All ingredients in each formula were blended together until its became a fine paste as 40-20 mesh.

Soup preparation

Ten-g of each paste was boiled with 100 ml drinking water for 2 min then 25 g white shrimp meat and 2.5 g sugar were added and further boiled for 4 min. The soup of basic recipe (without added dried garcinia fruit, P1) was added with 10 ml of fresh lime juice.

Analyses

Physicochemical properties

Color values

Color values of the sample were measured using a color meter (Hunter Lab Universal Software).The color values were express as CIE Lab* coordinates where L^* represents the luminosity (0 = black; 100 = white), a^* the redness ($a^* > 0$) or ($a^* < 0$) and b^* the blueness ($b^* > 0$) or yellowness ($b^* < 0$).

Water activity

Water activity of the curry paste was determined using the Novasina water activity meter (Thermoconstanter Novasina TH200, Switzerland).

pH values

Ten-g of the pastes was homogenized in 40 ml distilled water for 1 min with the homogenizer (Wiggen Hauser D500, Germany) (Bartolome *et al*., 1995) and measured for pH at room temperature with a SatoriusDocu-pH Meter (Germany).

Titratable acidity (AOAC, 1999)

Ten-g of the pastes was homogenized in 40 ml distilled water for 1 min with the homogenizer (Wiggen Hauser D500, Germany) and five ml of the soup was diluted with 45 ml distilled water and titrated with 0.1 N sodium hydroxide to reach pH 8.1. The results were expressed as percentage of citric acid (g citric acid/ 100g) as equation below.

% Total acidity (citric acid) = (vol. of NaOH x Normality of NaOH x 0.064 x 100)/wt. of sample

Moisture content (AOAC, 2000)

The empty dish and lid were dried in the oven at 105 ºC for 3 h and transfered to a desiccator to cool then weighed the empty dish and lid. Three-g of the paste was put into the dish and spread the paste to the uniformity before taken to place in the oven and dried at 105 ºC until the weight was constant. The dish contained the dried sample partially covered with the lid was taken to the desiccator to cool down the temperature before brought to check the weight and calculate as equation below.

Calculation

Moisture (%) = (W1-W2)/W1 x 100

W1= weight (g) of sample before drying

W2= weight (g) of sample after drying

Microbiological quality

Total Viable Count (BAM, 2001)

Twenty-five grams of Keang-hleung paste was blended with 225 ml of 0.1% peptone water. Serial dilution was made at 10^{-1} to 10^{-6} by using the 0.1% peptone water. Appropriate dilution was plated using Plate Count Agar (PCA, Merck, Germany). The plates were incubated at 35±2 ºC for 48 hours. Total viable count was recorded as colony forming unit/gram of sample (cfu/g).

Consumer acceptability

Thirty-panelists who familiar with the paste and the soup were asked for sensory determination. The panelists were asked to evaluate the paste for appearance, color, flavor and overall liking. Thereafter, the paste was brought to make a soup then the warmed soup temperature around 50-60 ºC was served to the panelists to evaluate the attributes as asked in the paste section and viscosity as well as taste also checked. The plain milk temperature at 25-30 ºC followed by drinking water were served to the panelists during evaluation to reduce hot sensation.

Statistical analyses

The experiment was run by using completely randomized design (CRD). Data were subjected to Analysis of Variance (ANOVA) and mean comparisons were performed using the Duncan's new multiple range test (DMRT). Statistical analyses were carried out using the SPSS statistical (SPSS, Inc., Chicago,IL).

Results and Discussion

Physical and chemical properties

The color value (*L**, *a**, *b**), pH and acidity (g/100g) of the paste are shown in Table 1. As a whole, it was found that *L**, *a**, *b** of the paste did not have any changing trend meant that there was no relationship between color value and amount of added dried garcinia. However, it was found that the more added dried garcinia the less pH value and the higher acidity explained that the organic acid derived from the fruit played the important role for lower pH of the system. Therefore, the garcinia paste was classified as high acid food since its pH lower than 4.6. In addition, when the paste was taken to cook, it was found that pH values and acidity (g/100g) decreased and increased, respectively as amount of added garcinia fruit increased (Table 2).

Adding of 10 g lime juice in to the basic soup made pH value of it closed to 20% garcinia paste, however the acidity of the soup added lime juice was highest compared with other soups (Table 2). It pointed out that organic acid containing mainly ascorbic acid and citric acid in the lime juice have a higher pK_a (Tripathi *et al*., 2009) compared with hydroxy citric acid, major organic found in the garcinia fruit. However, the more garcinia fruit added the less pH and more acidity occurred.

Table 1. Color, pH and acidity (g/100g) of the Keang-Hleung paste

Sample	Color			pH	Acidity (g/100g)
	L*	a*	b*		
P1 (no garcinia)	29.37 ± 0.37^{cd}	32.39 ± 0.44^{e}	39.89 ± 0.59^{e}	5.25±0.01^{a}	0.46±0.04^{f}
P2 (3% garcinia)	30.99 ± 0.07^{b}	34.22 ± 0.23^{bc}	41.25 ± 0.44^{d}	4.05±0.02^{b}	0.84±0.01^{ef}
P3(5% garcinia)	31.33 ± 0.63^{b}	34.51 ± 0.72^{b}	38.58 ± 0.58^{f}	3.73±0.02^{bc}	1.02±0.01^{e}
P4 (7% garcinia)	32.96 ± 0.04^{a}	33.74 ± 0.06^{cd}	43.76 ± 0.06^{b}	3.43±0.01^{bc}	1.34±0.03^{de}
P5 (10%garcinia)	28.82 ± 0.33^{de}	34.79 ± 0.46^{b}	41.05 ± 1.06^{d}	3.46±0.05^{bc}	1.58±0.04^{d}
P6 (13%garcinia)	28.04 ± 1.68^{e}	30.97 ± 0.06^{f}	36.36 ± 0.25^{g}	3.39±0.01^{c}	2.32±0.01^{c}
P7 (15%garcinia)	27.75 ± 0.02^{e}	35.64 ± 0.03^{a}	47.04 ± 0.05^{a}	3.25±0.03^{c}	2.52±0.02^{bc}
P8 (17%garcinia)	26.04 ± 0.20^{f}	33.18 ± 0.42^{d}	41.88 ± 0.46^{cd}	3.08±0.01^{cd}	2.66±0.01^{bc}
P9 (20%garcinia)	28.22 ± 0.01^{e}	33.49 ± 0.03^{d}	42.68 ± 0.23^{c}	2.96±0.01^{d}	2.86±0.05^{b}
P10 (25garcinia)	30.37 ± 0.04^{bc}	30.07 ± 0.04^{g}	39.42 ± 0.77^{ef}	2.86±0.01^{d}	3.59±0.02^{a}

Different letters within a column mean significantly difference ($p<0.05$)

Table 2. Color, pH and acidity (g/100g) of the Keang-Hleung soup

Sample	Color			pH	Acidity (g/100g)
	L*	a*	b*		
S1(no garcinia and used 10% limejuice)	41.40±0.04[a]	33.64±0.05[a]	59.33±0.39[b]	4.02±0.01[d]	5.76±0.02[a]
S2 (3% garcinia)	35.65±0.23[d]	29.86±0.23[c]	51.42±0.53[d]	5.93±0.02[a]	1.10±0.03[f]
S3(5% garcinia)	34.57±0.07[e]	26.68±0.30[e]	48.70±0.97[f]	5.67±0.01[a]	1.28±0.00[f]
S4 (7% garcinia)	38.03±0.26[b]	27.41±0.24[d]	48.02±1.37[f]	5.50±0.01[a]	1.58±0.01[e]
S5 (10% garcinia)	37.16±0.19[bc]	29.16±0.29[c]	50.26±1.86[e]	5.27±0.01[b]	1.83±0.01[e]
S6 (13% garcinia)	37.70±0.02[bc]	29.91±0.01[c]	58.78±0.26[b]	4.73±0.01[c]	3.52±0.01[d]
S7 (15% garcinia)	38.35±0.05[b]	30.63±0.01[bc]	61.45±0.41[a]	4.61±0.02[c]	3.73±0.00[cd]
S8 (17% garcinia)	33.84±0.01[f]	31.96±0.67[b]	56.30±0.35[c]	4.32±0.02[cd]	3.94±0.00[cd]
S9 (20% garcinia)	36.70±0.02[c]	29.50±0.10[c]	58.89±0.18[b]	4.08±0.01[d]	4.10±0.00[c]
S10 (25% garcinia)	36.70±0.02[c]	27.11±0.03[d]	58.36±0.33[b]	3.97±0.01[e]	5.06±0.01[b]

Different letters within a column mean significantly difference ($p<0.05$)

The A_w and moisture content of all pastes are shown in Table 3. The results showed that A_w and moisture increased when added more garcinia. The lowest A_w was found in the basic paste (P1) while added garcinia in the paste particularly the paste containing 25% (P10) garcinia had higher A_w. This evident pointed out that washing step brought to rehydrated stage as free water leading to higher A_w. It is the reason why draining step needs to be property applied otherwise shorten shelf-life would be occur afterward. As well-known food products having A_w less than 0.85 are quite safe for pathogenic or spoilage bacteria and have more shelf-life compared with another product having A_w higher than 0.85 (Sautour *et al*., 2000).

Table 3. A_w and moisture content of the Keang-Hleung paste

Sample	A_w	Moisture content (%)
P1 (no garcinia) (basic)	0.743 ± 0.001^{bc}	56.71 ± 0.07^{c}
P2 (3% garcinia)	0.750 ± 0.001^{b}	57.41 ± 0.06^{b}
P3 (5% garcinia)	0.748 ± 0.001^{b}	58.02 ± 0.18^{a}
P4 (7% garcinia)	0.745 ± 0.001^{b}	56.20 ± 0.13^{c}
P5 (10% garcinia)	0.750 ± 0.001^{b}	55.73 ± 0.16^{d}
P6 (13% garcinia)	0.754 ± 0.001^{b}	56.70 ± 0.08^{c}
P7 (15% garcinia)	0.743 ± 0.001^{bc}	57.51 ± 0.08^{ab}
P8 (17% garcinia)	0.735 ± 0.001^{c}	58.05 ± 0.19^{a}
P9 (20% garcinia)	0.740 ± 0.001^{b}	58.27 ± 0.13^{a}
P10 (25% garcinia)	0.794 ± 0.001^{a}	58.18 ± 0.16^{a}

Different letters within a column mean significantly difference ($p<0.05$)

Microbiological quality

The basic (P1) and garcinia Keang-hleung paste (P2-P10) monitored for TVC are shown in Table 4. Addition of garcinia fruit having high acid content seemed to not show a strong antimicrobial activity then TVC of all treatments were in the range of 10^2-10^3 cfu/g. This result explained that A_w played an important role even higher compared with pH or acidity. It confirmed that draining step would not be missed in washed sample particulary in dried form. Sautour *et al* (2000) stated that salt is an effective agent for A_w controlling. For example, 20% salt solution would reduce A_w from 1 to be 0.74 which control both pathogenic and spoilage organism.

Table 4. Total viable count in basic and garciniaKeang-hlueng paste

Sample	Total Viable Count (cfu/g)
P1 (no garcinia)	$1.80x10^{3e}$
P2 (3% garcinia)	$1.60x10^{3e}$
P3 (5% garcinia)	$1.02x10^{3e}$
P4 (7% garcinia)	$9.60x10^{2d}$
P5 (10%garcinia)	$9.26x10^{2d}$
P6 (13%garcinia)	$7.85x10^{2c}$
P7 (15%garcinia)	$6.60x10^{2b}$
P8 (17%garcinia)	$6.69x10^{2b}$
P9 (20%garcinia)	$5.30x10^{2a}$
P10 (25%garcinia)	$5.30x10^{2a}$

Different letters within a column mean significantly difference ($p<0.05$)

2.5.3 Sensory evaluation

In general, it was found that the more dried garcinia fruit added the less sensory score was found in the paste due to dark spot from dried garcinia retained in the paste (Table 5). The panelists also mentioned that added the dried garcinia into the paste led to unfamiliar smell since its generated the acid like dried fermented plum. However, after the soup making from various pastes were served, it was found that sensory score of the soup made from 15% dried garcinia fruit was comparable to the basic soup using lime juice as souring agent, and its score was more than 7/9 in every attribute (Table 6). Therefore, 15% dried garcinia fruit would be selected to produce the garciniaKeang-Hleung paste in the further experiment. Moreover, the panelists also stated that using dried garcinia fruit as souring agent made more body of the soup compared with using lime juice. And there was no problem in appearance, color and over all attributes in the soup though those were the problem which occurred in the paste. However, added the dried garcinia fruit more than 20% made the sensory score of every attribute lower. From the Table 5 and 6, it pointed out that if using only sensory score of the paste (Table 5) may not enough to judge the ultimate sensory quality of the soup.

Table 5. Sensory score of basic and garciniaKeang-Hleung paste

Formula	Attribute			
	Appearance	**Color**	**Flavor**	**Overall liking**
P1 (no garcinia)	7.28 ± 0.94^{a}	7.20 ± 0.70^{a}	7.32 ± 0.94^{a}	7.32 ± 0.85^{a}
P2 (3% garcinia)	7.16 ± 0.89^{ab}	7.28 ± 0.84^{a}	6.80 ± 0.76^{abc}	6.92 ± 0.76^{a}
P3 (5% garcinia)	7.32 ± 0.80^{a}	7.20 ± 0.76^{a}	6.84 ± 0.94^{abc}	6.96 ± 0.84^{a}
P4 (7% garcinia)	6.68 ± 1.24^{b}	7.04 ± 0.84^{a}	6.88 ± 0.83^{ab}	6.80 ± 0.70^{a}
P5 (10% garcinia)	7.00 ± 0.76^{ab}	7.12 ± 0.88^{a}	7.16 ± 0.85^{ab}	6.92 ± 0.75^{a}
P6 (13% garcinia)	7.12 ± 1.12^{ab}	7.20 ± 0.86^{a}	6.64 ± 1.07^{bc}	6.96 ± 0.88^{a}
P7 (15% garcinia)	7.52 ± 0.77^{a}	7.20 ± 0.40^{a}	6.76 ± 1.05^{abc}	6.96 ± 0.67^{a}
P8 (17% garcinia)	7.52 ± 0.65^{a}	7.28 ± 0.67^{a}	7.00 ± 0.91^{ab}	7.16 ± 0.74^{a}
P9 (20% garcinia)	6.96 ± 1.01^{ab}	7.52 ± 1.08^{a}	6.64 ± 1.22^{bc}	7.12 ± 1.16^{a}
P10 (25% garcinia)	5.96 ± 1.36^{c}	6.28 ± 0.97^{b}	6.28 ± 1.30^{c}	5.84 ± 1.14^{b}

Different letters within a column mean significantly difference ($p<0.05$)

The 9 point hedonic scale : 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like or dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely

Table 6. Sensory score of basic and gaciniaKeang-Hleung soup

Formula	Attribute					
	Appearance	**Color**	**Viscosity**	**Flavor**	**Taste**	**Overall**
S1 (Lime 10%)	6.92±1.11^{ab}	7.08±1.11^{ab}	6.96±1.09^{abc}	7.08±0.81^{a}	7.24±0.92^{a}	7.20±0.91^{a}
S2 (3% garcinia)	7.28±0.68^{a}	7.48±0.65^{a}	7.08±0.95^{abc}	6.60±1.04^{a}	6.04±1.30^{d}	6.28±1.02^{d}
S3 (5% garcinia)	7.04±0.73^{ab}	7.04±0.73^{ab}	7.16±0.85^{abc}	6.88±0.88^{a}	5.96±1.09^{d}	6.44±1.00^{cd}
S4 (7% garcinia)	7.40±0.86^{a}	7.16±0.98^{ab}	7.24±0.87^{ab}	6.96±0.88^{a}	6.36±1.38^{cd}	6.84±1.10^{abc}
S5 (10% garcinia)	7.20±0.76^{a}	7.36±0.63^{a}	7.32±0.80^{a}	7.12±0.83^{a}	6.52±1.22^{bcd}	6.80±0.95^{abc}
S6 (13% garcinia)	6.92±0.99^{ab}	7.04±0.78^{ab}	6.96±0.73^{abc}	7.00±1.00^{a}	6.40±0.91^{bc}	6.68±0.74^{abcd}
S7 (15% garcinia)	7.28±0.84^{a}	7.36±0.70^{a}	7.00±0.76^{abc}	7.04±0.88^{a}	7.04±0.84^{ab}	7.08±0.70^{ab}
S8 (17% garcinia)	7.00±0.82^{ab}	7.04±1.01^{ab}	6.44±0.91^{d}	7.08±0.75^{a}	6.64±1.18^{bc}	6.92±1.03^{abc}
S9 (20% garcinia)	7.20±0.91^{a}	7.16±0.74^{ab}	6.76±0.97^{bcd}	6.88±0.72^{a}	7.00±0.81^{ab}	7.00±0.86^{ab}
S10 (25% garcinia)	6.60±0.96^{b}	6.72±1.17^{b}	6.72±0.79^{cd}	6.64±1.07^{a}	6.72±0.84^{abc}	6.64±0.81^{bcd}

Different letters within a column mean significantly difference ($p<0.05$)

The 9 point hedonic scale : 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like or dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely

Conclusions

*L**, *a**, *b** of the paste did not have any relationship with amount of added dried garcinia. However, it was found that the more added dried garcinia the lesser pH value and the higher acidity obtained. When the paste were taken to cook, it was found that pH values and acidity (g/100g) decreased and increased, respectively as amount of added garcinia fruit increased. Though added garcinia fruit leading to high acid content, seemed to not show a strong antimicrobial activity then TVC of all treatments were in the range of 10^2-10^3 cfu/g. Addition of 15% garcinia (P7) is made consumer acceptability of the soup comparable with the soup made from basic paste (P1). Furthermore, addition of 15% garcinia in the paste seemed to be the best formula (score > 6.5) in almost attributes compared with other garcinia Keang-hlueng pastes.

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2. Study of Prebiotic Properties of the Southern Sour Curry Paste, Keang-hleung, with and without Garcinia

Abstract

Thais particulary in the southern of Thailand prefer sour curry soup or Keang-hleung. The paste was made by pounded or blended turmeric rhizome, garlic, shallot and chili together until it became a fine paste. The souring agent that aviable is used, however, dried garcinia fruit, a local fruit of the southern of Thailand is usually used in any sour soup. This study aimed to compare prebiotic properties of the pastes with and without garicia addition. Prebiotic properties showed that both the basic Keang-hleung paste without garcinia (P1) and 15% garcinia Keang-hleung paste (P2) were partially resistance to simulated gastrointestinal conditions hydrolysis. When the partial hydrolyzed paste were taken to ferment with human feces it was found that the numbers of bifidobacteria, lactobacilli and eubacteria were slightly increased whereas the numbers of bacteroids and clostridia were decreased in batch culture by fecal slurry fermentation for 24 h. Garcinia Keang-hleung paste (P2) showed prebiotic index (PI) of 2.75 which was higher than basic Keang-hleung paste without garcinia (P1) with PI of 1.19. However, the concentrations of short-chain fatty acids including lactic acid, acetic acid, propionic acid and butyric acid produced by fecal fermentation of basic Keang-hleung paste without garcinia (P1) was higher than garcinia Keang-hleung paste (P2). Moreover, basic Keang-hleung paste (P1) could produce vitamin B1 (18.38±0.10 µg/ml) and B2 (45.28±2.02 µg/ml) but not folic acid meanwhile garcinia Keang-hleung paste (P2) produced only vitamin B1 (5.99±0.48 µg/ml) at 24 h fermentation.

Key words: Keang-hleung paste, southern sour curry, garcinia, prebiotic,vitamin B1,vitamin B2.

Introduction

Prebiotics are non-digestible oligosaccharides that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health (Gibson and Roberfroid, 1995). The prebiotic concept considers that many potentially affects health- promoting microorgranisms, such as bifidobacteria and lactobacilli, are already resident in the human colon. Prebiotics must be stable in the acid of stomach reach the colon where they are then selectively fermented by positive bacteria and they must not be absorbed in the small intestine (Roberfroid, 2001). In *vitro* models of the gut are often used to screen effects that prebiotic can exert on the colonic microflora. Prebiotics can be screened using batch cultures to ascertain how prebiotics affect the colonic microflora. Glass vessels containing a medium capable of supporting the growth of the colonic microflora are used. The substance to be tested is added just before the addition of faecal slurry (1% w/w total volume), which is used as representation of colonic microflora. The vessels is maintained under anaerobic conditions at 37 °C and sampled periodically. Static batch cultures are generally used with small volumes, these are non-pH controlled, so they best suited to initial screening process. Stirred pH-controlled batch cultures can then be used to obtain more detailed information at a pH representative of distal region of the colon. Ten samples from thirteen fruits and vegetables from southern Thailand were reported as potential sources of natural prebiotics with the highest oligosaccharide content being 9.81% (w/w) (Thammarutwasik *et al.*, 2009). Kumdum and Chomnawang (2005) reported that shallot and garlic consisted of non-digestible carbohydrate (soluble component) which contain inulin 2-6%, oligofructose 2-6% and inulin 9-16%, oligofructose 3-6%, respectively.

Southern sour curry or Keang-hleung soup is traditional popular spicy-sour curry consumed particularly in southern of Thailand. It is also claimed as a healthy food because of low calories due to less fat but high proportion of vegetables containing high fiber content. Moreover, the ingredients used in the paste normally are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds (Ruby *et al*., 1995; Ahsan *et al*. 1999; Cousin *et al*. 2006; Siripongvutikorn *et al*., 2005; Jayaprakasha *et al*. 2006). During cooking the sour soup, lime juice, tamarind, or any label of sour fruits or even leaves is added. However, southern Thais generally used dried garcinia fruit as souring agent in many kind of sour soups. Addition of dried garcinia fruit into the paste may alter some qualities, particulary prebiotic property.

Materials and Methods

Materials

Turmeric rhizomes (*Curcuma longa*), garlic (*Allium sativum*), dried finger chili (*Capsicum annuum*), shallot (*Allium ascalonicum*) and dried garcinia (*Garcinia atroviridis*) were purchased from a local market in Hat Yai, Songkhla, Thailand.

Chemicals

All chemicals reagents and enzyme were obtained from Sigma Chemical Co. (St. Louis, MQ, USA). Hydrochloric acid, sodium acetate, sodium carbonate (Na_2CO_3), absolute ethanol were purchased from Merck (Darmstadt, Germany).

Keang-hleang paste preparation

All spices were sorted, trimmed and washed thoroughly to remove dusts and dirts, then soaked in 150 ppm and 10 ppm of chlorine solution, respectively for 1 minute, drained and then weighed according to basic recipe before addition of 20% salt before devided into 2 groups as basic Keang-hleung paste without garcinia (P1) and garcinia Keang paste with 15% garcina (P2). Based on calculation the compositions of each paste were differences as showed in Table 1. All mentioned ingredients were then ground with blender (Moulinex, TYPE 276, France) to make a fine paste (20-40 mesh).

Table 1. The ingredient compositions in curry paste formula (P1 and P2)

Ingredient	Amount (%, w/w)	
	P1	**P2**
Garlic	10	8
Shallot	30	24.5
Chilli	30	24.5
Turmeric rhizome	10	8
Garcinia	-	15
Salt	20	20

P1 : basic Keang-hleung paste without garcinia

P2 : garcinia Keang-hleung paste with 15% garcinia

Preparation of Keang-hleung paste in simulated gastrointestinal conditions

The study was tested *in vitro* digestion by simulating conditions occurred during the upper gastrointestinal tract of human which consists of three parts, the digestion in the mouth, stomach and small intestine. The enzyme activity was determined by the Sigma quality control test procedures for α-amylase. Enzyme was prepared in solution using cold distilled water (30 ml) and mixed with artificial saliva solution (270 ml) to obtain final concentration of 2 unit/ml α-amylase, pH 6.8. Artificial human saliva contained (g/l); NaCl, 1.60; NH_4NO_3, 0.33; NH_2PO_4, 0.64; KCl, 0.20; $K_3C_6H_5O.7H_2O$, 0.31; $C_5H_3N_4O_3Na$, 0.02; H_2NCONH_2, 1.98; $C_3H_3O_3Na$, 0.15 and 15 ml porcine mucin (Sarker *et al*., 2009). The paste sample (100 g) was mixed with artificial saliva (270 ml) and human saliva α-amylase (30 ml) then incubated in a shaking water bath at 85 rpm and controlled temperature of 37±1 ºC for 2 min. Sample (0.5 ml) was taken every 15 sec intervals to determine reducing sugar and total sugar. Percentage hydrolysis was calculated based on reducing sugar liberated and the total sugar.

Sample was dissolved in reversed osmosis (RO) water to give a 1% (w/v) solution. Artificial human gastric juice was mimicked by using hydrochloric acid buffer containing (g/l): NaCl, 8; KCl, 0.2; $Na_2HPO_4 \cdot 2H_2O$, 8.25; $NaHPO_4$, 14.35; $CaCl_2 \cdot 2H_2O$, 0.1; $MgCl_2 \cdot 6H_2O$, 0.18. The pH of the buffer was adjusted to 1, 2, 3, 4 and 5 using 5 M HCl (Korakli *et al*., 2002). HCl buffer (5 ml) at each pH was added to the sample solution (5 ml) and the reaction mixture was incubated in a shaking water bath at a controlled temperature of 37±1 °C for 4 h. Sample (1 ml) was taken periodically at 0, 0.5, 1, 2, 4 and 6 h. Reducing sugar content in the sample was determined by DNS method (Robertson *et al*., 2001) and total sugar was determined by phenol–sulphuric acid method (Dubois *et al*., 1956). Percentage hydrolysis of sample was calculated based on reducing sugar liberated and total sugar content of the sample (Korakli *et al*., 2002).

After the sample solution (pH 2) was incubated for 30 min then the pH was adjusted to 6.9 to mimic the conditions of digestion in the small intestine. Then human pancreatin α-amylase enzyme 0.75 unit/ml was added and incubated at 37 ºC for 6 h. Sample (0.5 ml) was taken at 1 h intervals to determine reducing sugar and total sugar. Percentage hydrolysis was calculated based on reducing sugar liberated and the total sugar. Sample was cooled by immersion in ice and sample was precipitated by 95 % ethanol (final concentration of 80%). The mixture was left overnight at 4°C. Sample was precipitated and reprecipitated twice to separate the sugar molecules from solution precipitated sample was evaporated with a

rotary evaporator under low pressure and dried using a freeze-drier. The dried powder samples were stored at -20 º C until use.

$$\text{Hydrolysis (\%)} = \frac{\text{reducing sugar released x 100}}{\text{total sugar content-initial reducing sugar content}}$$

Preparation of faecal slurry

Human faecal slurry at concentration of 10% (w/w) was prepared by diluting in PBS for use in a stirred pH-controlled batch culture. Fresh stool sample was weighted in a stomacher bag in pre-weight container then addition of PBS to get desired concentration of faecal slurry. Sample was blended in a stomacher at normal speed for 120 seconds.

Stirred pH-controlled batch culture fermentation

After Keang-hleung paste 100 g of P1 and P2 which moisture content 56 and 58 %, respectively were digested in the stimulated conditions in the mouth, stomach and small intestine then freeze-dried, it was found that 9.8 g and 9.2 g of dried powder P1 and P2 were obtained, respectively. To determine the prebiotic index (PI), a sterile glass fermenter (300 ml capacity) was filled with sterile basal medium 100 ml purged with oxygen-free nitrogen gas to obtain stable condition. Faecal slurries 100 ml were added to each fermenter and maintained the system under a head space of oxygen-free nitrogen gas. Fermentation was carried out at 37 ºC for 24 h, magnetically stirred and culture pH was automatically controlled at 6.8±0.1 by addition of 0.5 N NaOH or HCl during fermentation using a pH-controller. Dried sample powder (8 g) of Keang-hleung paste from Keang-hleung paste in stimulated gastrointestinal as mentioned above was added in to the glass vessel. Samples (5ml) were taken at 0, 6, 12 and 24 h for enumeration of bacteria using FISH technique (Rycroft *et al*. 2001) and short chain fatty acids (SCFA) and vitamins were analyzed by HPLC (Wichienchot, 2006). Prebiotic index (PI) was used as indicator of prebiotic property. The PI of sample was calculated according to an equation given in Fluorescent *in situ* hybridization (FISH) technique.

Fluorescent *in situ* hybridization (FISH) technique

Sample (375 µl) was taken from the batch culture and added to 1.125 ml filtered 4% (w/v) paraformaldehyde solution (pH 7.2), mixed and stored at 4 ºC overnight to fix the bacterial cells. The fixed cells were washed twice in filtered PBS (pH 7) and resuspended in 150 µl filtered PBS. Ethanol (150 µl) was added and the sample was mixed and stored at -20 ºC for at least 1 h or until needed, but no longer than 3 months.

The fixed cell (20 µl) spread on a slide (TEFLON / Poly-L-Lysine) put on a slide warmer at 45 ºC for 10-12 minutes until dried. The slide was dipped in ethanol concentrations of 50, 80 and 96% (v / v). If the slides for lactobacilli drop 20 µl of lysozyme for 15 min and then washed with distilled water before dipping in ethanol. The dip at each concentration from 3 minutes to break the cell walls make DNA probes can bind to specific DNA of bacteria then slide dried on the slide warmer.

Prewarmed hybridization buffer (Appendix A) was mixed with 5 µl of sample, 45 µl of a solution of DNA probes specific for each bacterial species and incubated with hybridization oven (Table 8.). Then curing left for 4 h after the time the slides washed with washing buffer (Rycroft et al. 2001), 50 ml of incubation at the appropriate temperature for each probe by soaking for 15 min. After a time, washed with distilled water 50 ml of chilled for 2 times, then slide the drying immediate addition of antifade (5 µl) into each hole, close by cover slide and counting by fluorescence microscope. A minimum of 15 fields, each containing 10-100 cells was counted for each slide.

Table 2. Probe references and hybridization temperature

Target organisms	**Probe Reference**	**Sequence from 5' to 3'**	**Hybridization temperature (◦C)**
Bacteroides	Bac 303	CCAATGTGGGGGACCTT	48
Bifidobacteria	Bif 164	CATCCGGCATTACCACCC	50
Lactobacilli	Lab 158	GGTATTAGCA(T/C)CTGTTTCCA	50
Clostridia	Chis 150	TTATGCGGTATTAATCT(C/T)CCTTT	50
Eubacteria	Eub 338	GCTGCCTCCCGTAGGAGT	48

Source: Rycroft *et al.* (2001)

Calculation of prebiotic index (PI)

Equation for calculation of prebiotic index is as following :

$$\text{Prebiotic index (PI)} = \alpha + \beta - \gamma - \delta$$

$$\alpha = (Bif_{24}/Bif_0)/\text{Total}$$

$$\beta = (Lac_{24}/Lac_0)/\text{Total}$$

$$\gamma = (Bac_{24}/Bac_0)/\text{Total}$$

$$\delta = (Clos_{24}/Clos_0)/\text{Total}$$

$$\text{Total} = Eub_{24}/Eub_0$$

Eub_0, Eub_{24} is *Eubacterian* numbers or total bacteria numbers at 0 and 24 h

Bif_0, Bif_{24} is *Bifidobacterium* numbers at 0 and 24 h

Lac_0, Lac_{24} is *Lactobacillus* numbers at 0 and 24 h

Bac_0, Bac_{24} is *Bacteroides* numbers at 0 and 24 h

$Clos_0$, $Clos_{24}$ is *Clostridium* numbers at 0 and 24 h

The equation assumed that an increase in populations of bifidobacteria and/or lactobacilli has a positive effect in contrast to an increase in bacteroides and clostridia has negative (Palframan *et al*., 2003).

Short-chain fatty acid (SCFA) analysis

Sample (1.5 ml) was centrifuged (13,000x*g*) for 15 min to remove particulate materials and cells then the supernatant was filtered through a 0.2 µm nylon filter. Sample (20 µl) was injected into an HPLC system attached to a UV detector at 214 nm. The column was a BIO-RAD Aminex HPX-87 H Ion exclusion column (BioRad, Watford, Herts) maintained at 50 °C with a column heater. The eluent, 0.005 M sulphuric acid in HPLC-grade water, was pumped through the column at flow rate of 0.6 ml/min. Data was integrated using the ValueChrom™, software package (Bio-Rad, USA). Using external calibration curves of lactate, acetate, propionate and butyrate to quantify its concentration in the sample (Wichienchot, 2006).

Vitamin B1, B2 and folic analysis

Sample (1.5 ml) was centrifuged (13,000x*g*) for 15 min to remove particulate materials and cells then the supernatant was filtered through a 0.2 µm nylon filter. Sample (20 µl) was injected into an HPLC system attached to a UV detector set at 254 nm. The column was a Inertsil Diol maintained at 40ºC with a column heater. The eluent, acetonitrile: water; trifuroacetic acid (CN_3CN:H_2O: TFA) ratio with 90:10:0.1, was pumped through the column at flow rate of 1 ml/min. Data was integrated using the ValueChrom™, software package (Bio-Rad, USA). Using external calibration curves, vitamin B1, B2 and folic acid were quantified in the sample (Wichienchot, 2006).

Statistic analysis

Bacterial counts at 0 and 24 h of batch culture fermentations were tested for significance using paired *t*-tests, assuming equal variance and considering both sides of the distribution (two tailed distribution). Difference were considered at 99% and 95% significance if $P<0.01$ and $P<0.05$, respectively using SPSS for windows software version 16 (SPSS, Inc., Chicago, IL).

Results and Discussion

The digestability of Keang-hleang paste in simulated gastrointestinal conditions

The digestability of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) with human salivary α-amylase at pH 6.8, 37 °C for 60 sec showed that the degree of hydrolysis increased rapidly within 5 sec and slightly increased until 60 sec then it was gradually decreased. The maximum hydrolysis of P1 and P2 were 3.38% and 3.41%, respectively after 60 sec incubation (Fig. 1). The reducing sugar increased from 144.82 μmole/ml to 282.32 μmole/ml.

Mixed Keang-hleung paste after hydrolysis by human salivary α-amylase was further hydrolyzed by artificial human gastic juice (pH 2). Percentage of hydrolysis increased with increasing incubation time until 120 min then it was almost stable (Fig. 2). Therefore, percentage of hydrolysis of P1 and P2 were 1.46% and 1.58% at 120 min, respectively. The reducing sugar increased from 190.69 μmole/ml to 214.60 μmole/ml.

The maximum hydrolysis of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) after hydrolysis by human pancreatic α-amylase. The result showed that the degree of hydrolysis increased rapidly within 30 min and slight increased until 6 h incubation. The percentage of hydrolysis of P1 and P2 were 6.69% and 6.80%, respectively after 6 h incubation (Fig 3). The reducing sugar increased from 180.01 μmole/ml to 282.06 μmole/ml.

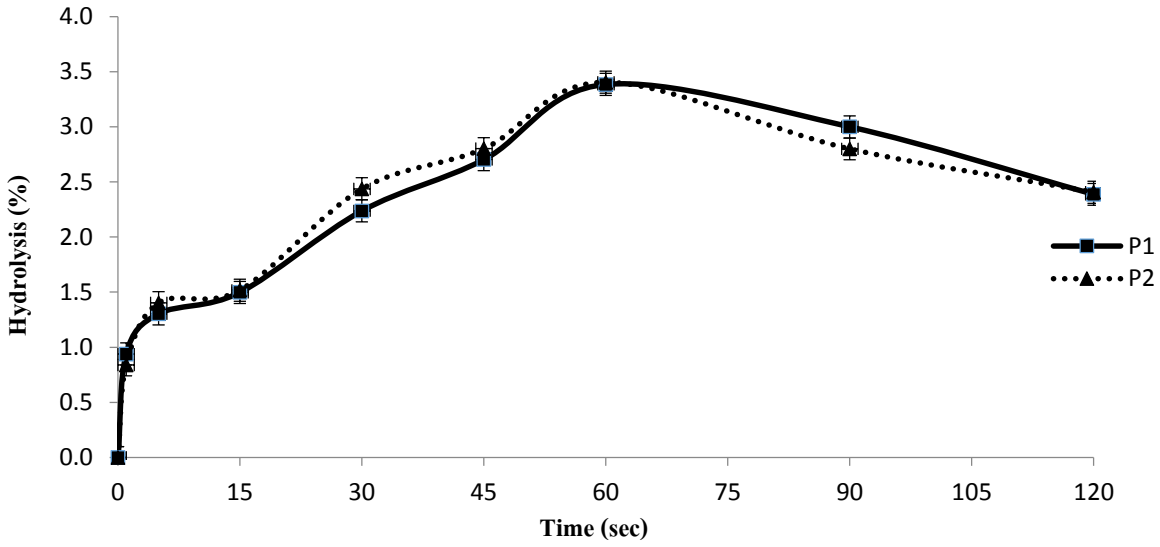

Figure 1. The hydrolysis of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) with human salivary α-amylase at pH 6.8, 37 °C

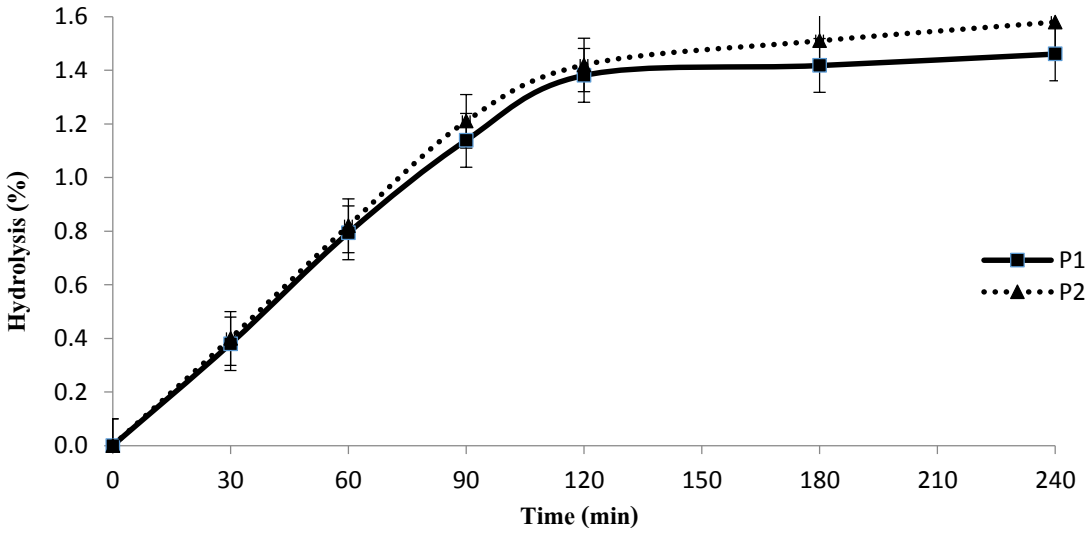

Figure 2. The hydrolysis of basic Keang-hleung paste without garcinia (P1) and garcinia Keang- hleung paste (P2) after hydrolysis by human salivary α-amylase was further hydrolyzed by artificial human gastic juice (pH 2)

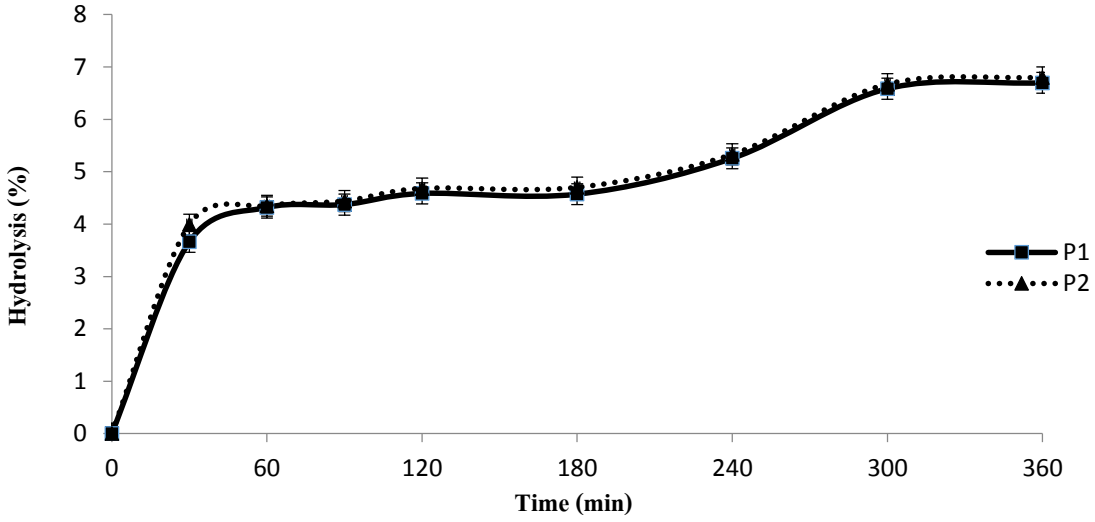

Figure 3. The hydrolysis of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) with human pancreatic α-amylase digestion

Inchuen *et al* (2010) reported that the composition of Thai red curry powder were crude protein, crude fat and crude fiber. However, from this result about 88% of the both Keang-hleung paste (P1 and P2) consumption would reach the colon since some of them was hydrolyzed by saliva α-amylase (3.38 % and 3.41%), by acid in stomach (1.46% and 1.58%) and by human pancreatic α-amylase (6.69% and 6.80%). Therefore, totally partial hydrolyzed stimulates in mouth, stomach and small intestine was about 12%. This result explained that the curry paste (P1 and P2) may have other compositions except carbohydrate were not digested. Typically, the carbohydrates were mainly digested in the small intestine where some of brush-border enzymes, i.e. isomaltase, glucoamylase, maltase, sucrose and lactase hydrolyse α- 1, 4- and α-1,6 linked glucosaccharides present in the small intestine and yielded monosaccharides as end product (Johnson and Schmit, 2005). It has been reported that 88% of inulin and oligofructose reach the colon, using both the ileostomy patient model and the incubaion model (Cummings and Macfarlane, 2002). Therefore, the basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) in this experiment appeared to have partial resistance under stimulated gastrointestinal conditions.

Microbial populations change and prebiotic index calculation

The fermentability of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) by fecal slurry in stirred pH-controlled batch cultures was tested. The changes of microbial populations were determined by fluorescent *in situ* hybridization accordance with the specific DNA probes used for particular 5 genera of bacteria. Microbial populations change of basic Keang-hleung paste without garcinia (P1) (Fig. 4) showed that the number of bifidobacteria increased from 7.96 Log cell/ml to 8.13 Log cell/ml, lactobacilli increased from 7.66 Log cell/ml to 7.76 Log cell/ml and eubacteria increased from 8.64 Log cell/ml to 8.75 Log cell/ml even not significant different while the number of bacteroid decreased from 7.74 Log cell/ml to 7.48 Log cell/ml and clostridia decreased from 9.04 Log cell/ml to 8.63 Log cell/ml even significant different.

Garcinia Keang-hleung paste (P2) (Fig. 5) showed that the number of bifidobacteria increased from 8.00 Log cell/ml to 8.30 Log cell/ml, lactobacilli increased from 7.56 Log cell/ml to 7.71 Log cell/ml and eubacteria decreased from 8.71 Log cell/ml to 8.53 Log cell/ml even not significant different while the number of bacteroid decreased from 7.75 Log cell/ml to 7.61 Log cell/ml and clostridia decreased from 8.81 Log cell/ml to 8.12 Log cell/ml even significant different.

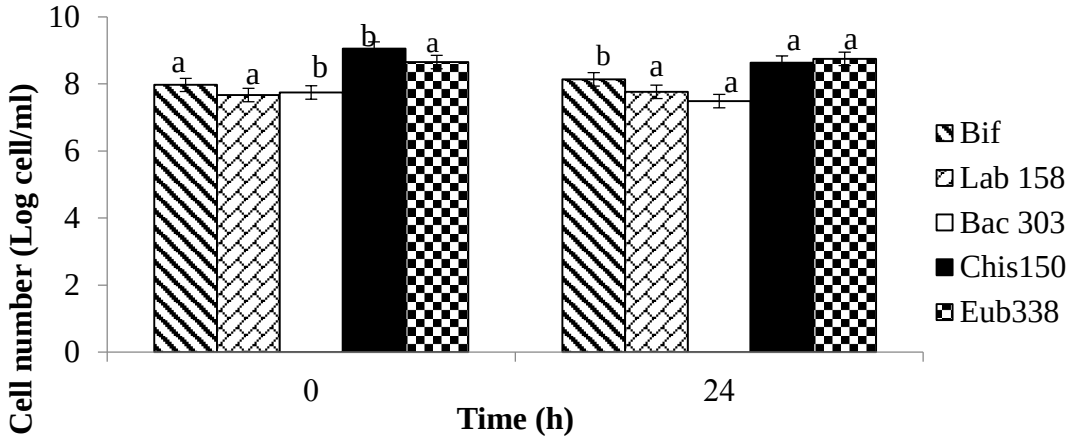

Figure 4. Change in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with basic Keang-hleung paste without garcinia (P1)

Different letters within a same test organism mean significantly difference ($p<0.05$)

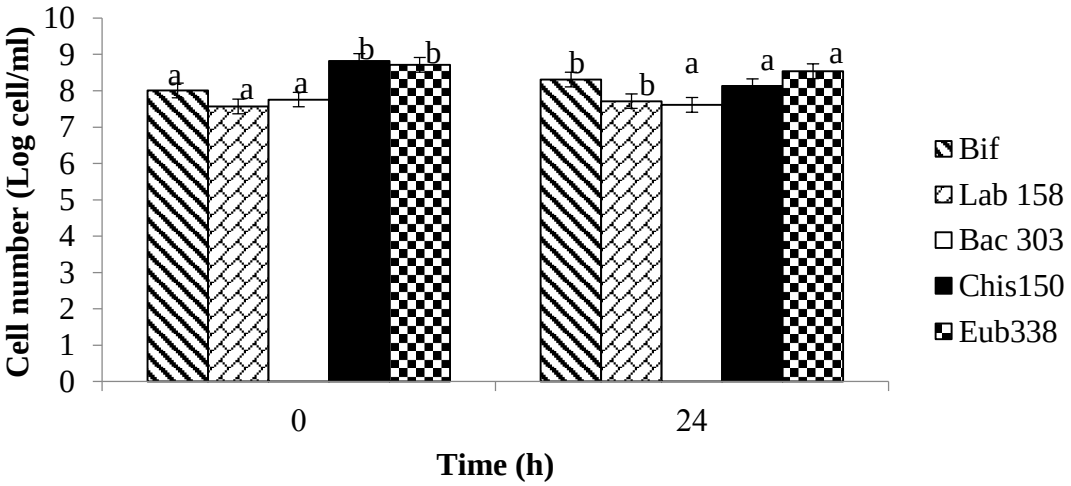

Figure 5. Change in bacterial populations enumerated using fluorescent *in situ* hybridization in stirred pH-controlled batch culture fermentation with Keang-hleung paste with garcinia (P2)

Different letters within a same test organism mean significantly difference ($p<0.05$)

Prebiotic index (PI) of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) in batch culture were calculated according to their bacterial change and it was summarized in Table 3. Garcinia Keang-hleung paste (P2) showed prebiotic index (PI) of 2.75 which was higher than basic Keang-hleung paste without garcinia (P1) with PI of 1.19. The prebiotic index of basic Keang-hleung paste without garcinia (P1) which was lower than garcinia Keang-hleung paste (P2) probably due to garcinia played an important role for inhibition of microbial growth. For example, a decrease of clostridia (Fig. 5) may due to function of hydroxy acid and other weak acids mainly derived from garcinia. The both of pastes stimulate the growth of bifidobacteria and lactobacilli. Kumdum and Chomnawang (2005) reported that shallot and garlic consisted of non-digestible carbohydrate (soluble component) including inulin 2-6%, oligofructose 2-6% and inulin 9-16%, oligofructose 3-6%, respectively. In addition, it was found that total dietary fiber content of garcinia Keang-hleung paste (P2) higher than basic Keang-hleung paste without garcinia (P1). It pointed out that P2 had higher total dietary fiber and prebiotic index compare with P1.

Table 3. Prebiotic index of basic Keang-Hleung paste without garcinia (P1) and garcinia Keang-Hleung paste (P2)

Sample	**Composition (%)**						**Total dietary fiber (%)**	**Prebiotic index**
	Garlic	**Shallot**	**Chilli**	**Turmeric rhizome**	**Garcinia**	**Salt**		
P1	10	30	30	10	-	20	6.38	1.19
P2	8	24.5	24.5	8	15	20	7.97	2.75

Short-chain fatty acids (SCFA) production

Short-chain fatty acids produced by fermentation of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) in batch culture were determined by HPLC. Fermentation of basic Keang-hleung paste produced 120.81 µg/ml lactic acid within 24 h of fermentation (Table 4). High concentration of acetic acid was produced and increased with the increase in fermentation time and the maximum concentration (26.43 µg/ml) at 24 h. Propionic acid was produced at 6, 12 and 24 h of fermentation, giving the maximum concentration of 14.56 µg/ml at 6 h. Butyric acid decreased as fermentation time increased.

Fermentation of garcinia Keang-hleung paste (P2) produced high concentration of lactic acid (82.97 µg/ml) within 12 h then decreased at 24 h of fermentation. Acetic acid was produced and increased as fermentation time increased and the maximum concentration (23.55 µg/ml) was obtained at 24 h. Propionic acid was generated in large quantity at 6 and 12 h of fermentation, giving the maximum concentration of 9.80 µg/ml at 12 h. Butyric acid was slightly decreased as fermentation time increased from 10.65 µg/ml to 9.18 µg/ml at 6 and 12 h of fermentation, respectively.

In comparison between basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) and it was found that concentration of lactic acid produced from P1 higher than from P2 at all fermentation times. It probably due to the number of lactobacilli by fermentation of P1 (7.76 Log cell/ml) seemed to be higher than P2 (7.56 Log cell/ml). As generally known that lactic acid obtained in plant material was mainly produced by lactobacilli (Gibson *et al*., 1998). In addition, concentration of total SCFA by fermentation of P1 was higher than P2. It pointed out that products of fermentation depending on the species and type and availability of substrate that can be converted to various end products (Gibson *et al*., 1998).

Table 10. Short-chain fatt acids (SCFA) production of basic Keang-Hleung paste without (P1) and with garcinia (P2)

Sample	Time (h)	Lactic acid (µg/ml)	Acetic acid (µg/ml)	Butyric acid (µg/ml)	Propionic acid (µg/ml)
basic Keang-Hleung paste (P1)	0	15.64 ±0.01^{d}	5.45±0.27^{d}	29.09±2.65^{a}	1.63±0.02^{c}
	6	109.01±0.20^{b}	20.96±0.03^{b}	9.41±0.50^{cd}	14.56±0.19^{a}
	12	107.20±1.08^{b}	21.09±0.38^{b}	11.16±0.70^{c}	12.29±0.50^{ab}
	24	120.81±6.03^{a}	26.43±1.68^{a}	11.81±0.97^{c}	13.29±1.00^{a}
Garcinia Keng-Hleung paste (P2)	0	11.05±2.29^{d}	3.78±0.34^{d}	16.47±2.45^{b}	1.44±0.74^{c}
	6	79.61±9.87^{c}	17.02±2.39^{c}	10.65±1.72^{c}	8.08±1.69^{b}
	12	82.97±4.67^{c}	20.45±0.91^{b}	10.45±0.57^{c}	9.80±1.71^{b}
	24	72.41±5.34^{c}	23.55±1.74^{ab}	9.18±0.64^{cd}	3.70±0.24^{c}

Different letters within a column mean significantly differences ($p<0.05$)

Vitamin B1, B2 and folic acid production

Vitamin B1, B2 and folic acid produced by fermentation of basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) in batch culture determined by HPLC are shown in Table 5. Basic Keang-hleung paste (P1) produced higher concentration of vitamin B1 with fermentation time increased from 12.82 µg/ml to 18.38 µg/ml at 6 and 24 h, respectively. Vitamin B2 increased from 34.42 µg/ml to 45.28 µg/ml at 6 and 12 h, respectively. Folic acid was not detected throughout the fermentation. Vitamin B1 of garcinia Keang-hleung paste (P2) was sharply decreased from 21.65 µg/ml to 5.17 µg/ml then it was slightly increased to 5.99 µg/ml at 24 h. However, vitamin B2 did not detect at 6, 12 and 24 h and folic acid of this paste (P2) did not detect throughout the fermentation. Gibson *et al* (1998) addressed that various end products of fermentation depending on the species and type and availability substrates in the sample. Nudler and Mironov (2004) reported that vitamin B1 synthesis by Bacilli and vitamin B2 synthesis by *B. subtilis, E. coli and Rhizobium etli.* Therefore, basic Keang-Hleung paste (P1) could be used as a food source for vitamin B1 and B2 production in human gut. In general, these vitamins are benefit to gut health as flavoproteins, essential for the metabolism of amino acid, energy production and activation of folate and pyridoxine to their respective coenzyme form.

Table 5. Vitamin B1, B2 and folic acid production of basic Keang-Hleung paste without (P1) and with garcinia (P2)

Sample	Time (h)	Vit B1 (µg/ml)	Vit B2 (µg/ml)	Folic acid (µg/ml)
Basic Keang-Hleung paste without garcinia (P1)	0	10.93±0.54^{d}	42.75±3.28^{b}	ND
	6	12.82±0.57^{c}	34.42±2.52^{d}	ND
	12	12.71±0.50^{c}	38.59±5.73^{c}	ND
	24	18.38±0.10^{b}	45.28±2.02^{a}	ND
Garcinia Keng-Hleung paste (P2)	0	10.67±0.57^{d}	31±0.09	ND
	6	21.65±1.40^{a}	ND	ND
	12	5.17±0.18^{e}	ND	ND
	24	5.99±0.48^{e}	ND	ND

*ND = Not detected

Different letters within a column mean significantly differences ($p<0.05$)

Conclusion

The basic Keang-hleung paste without garcinia (P1) and garcinia Keang-hleung paste (P2) in this experiment appeared to have resistance under stimulate gastrointestinal conditions approximately 88%. After the remained components were fermented by fecal flora in a pH-controlled batch culture. The numbers of bifidobacteria, lactobacilli and eubacteria slightly increased while the number of bacteroids and clostridia were significantly decreased at 24 h of fermentation. Garcinia Keang-hleung paste (P2) had prebiotic index (PI) higher than the basic Keang-hleung paste without garcinia (P1). However, the concentrations of SCFAs produced by human fecal fermentation of basic Keang-hleung paste without garcinia (P1) was higher than garcinia Keang-hleung paste (P2) in all short-chain fatty acids. Basic Keang-hleung paste (P1) could produce vitamin B1 and B2 but not folic acid. Garcinia Keang-hleung paste (P2) produced vitamin B1 but not vitamin B2 and folic acid. So that addition of garcinia in Keang-hleung paste seemed to increase on prebiotic effect.

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ข้อคิดเห็นและข้อเสนอแนะสำหรับการวิจัยต่อไป

1. การศึกษาผลการเติมส้มแขกสดต่อการเปลี่ยนแปลงคุณสมบัติการต้านออกซิเดชั่นและการต้านจุลินทรีย์ของเครื่องแกงส้มภาคใต้
2. การศึกษาผลการเก็บรักษาต่อสมบัติการต้านการอักเสบของแกงส้มภาคใต้ที่มีและไม่มีส้มแขกแห้ง
3. การศึกษาการหมักด้วยอุจจาระมนุษย์ของเครื่องแกงส้มภาคใต้ที่มีและไม่มีส้มแขกในแบบจำลองลำไส้ใหญ่มนุษย์ (three stage continuous system)

การถ่ายทอดเทคโนโลยี

ได้มีการถ่ายทอดเทคโนโลยีให้กับโรงงานผลิตเครื่องแกงลำดวน อ.เมือง จ. ชุมพร โดยงานวิจัยนี้ได้ใช้สูตรเครื่องแกงส้มภาคใต้ของเครื่องแกงลำดวนมาเป็นสูตรพื้นฐาน เพื่อทางโรงงานจะได้ข้อมูลทางวิทยาศาสตร์เพื่อใช้สนับสนุนและส่งเสริมให้มีการบริโภคแกงในแง่การป้องกันโรคและการมีสุขภาพที่ดี และการเติมส้มแขกลงในเครื่องแกงส้มภาคใต้เพื่อผลิตเครื่องแกงที่มีศักยภาพในการส่งออกสามารถใช้ข้อมูลที่ได้ในการวิจัยเพิ่มยอดขายและสร้างความเชื่อมั่นให้กับการสร้างฐานการผลิตเพื่อการส่งออกไปต่างประเทศเพิ่มขึ้น

บทความวิจัยที่เสนอที่ประชุมวิชาการ (Abstract)

Preeyaporn Promjiam, Sunisa Siripongvutikorn, Worapong Usawakesmanee and santad Wichienchot. "Total phenolic compound content, antioxidant property and quality changes of the southern sour curry paste, Keang-Hleung, as effect of garcinnia, Garcinnia atroviridis,salt during storage", ใน The 2nd Annual International Conference in conjunction with The 8 th IMT-GT UNINET Bioscience Conference . 22-24 Nov.2012. Syiah Kuala University Banda Aceh Indonesia : AIC-UNSYIAH.

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Effect of Added Garcinia Fruit on Total Phenolic Compound Content, Antioxidant Properties and Quality Changes of the Southern Sour Curry Paste, Keang-hleung, during Storage

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ABSTRACT

The favorite soup for Thai particularly in the southern part is Southern sour curry or Keang-hleung soup. The ingredients used in the paste are turmeric rhizome, garlic, shallot and chili. However, for making the sour soup, the souring agent such as lime juice, tamarind pod or garcinia, fruit is added. This study aimed to compare quality changes, total phenolic compound and antioxidant properties of the pastes as affected of added garicia fruit during storage. It was found that the total phenolic compound content of basic paste without the garcinia, garcinia Keang-hleung paste and garcinia Keang-hleung paste without salt decreased as increased storage time. Moreover, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the ferric reducing power (FRAP) activity of the basic paste without the garcinia decreased as increased storage time. However, the DPPH radical scavenging activity and the FRAP activity of the garcinia Keang-hleung paste with and without salt increased during 2 months of storage period and then decreased as increased storage time. Total viable count (TVC) of all paste samples were in the range of 10^2 - 10^3 cfu/g. Yeast and mold counts of basic and garcinia Keang-hlueng paste were less than 30 cfu/g during storage. While, yeast and mold counts of garcinia Keang-hlueng paste without salt were less than 10^2 cfu/g during storage. Lactic acid bacteria counts of garcinia Keang-hlueng paste were less than 30 cfu/g during storage. While, lactic acid bacteria counts of the basic and garcinia Keang-hlueng paste without salt were less than 10^2 cfu/g during storage. However, *Staphylococcus aureus*, *Bacilluus cereus*, *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli* and coliforms were not detected in all treatments throughout the storage period.

Keywords: Keang-hleung Paste; Southern Sour Curry; Garcinia; Antioxidant; Shelf-Life; Thailand

1. Introduction

Free radicals are unstable and highly reactive, and energized molecules have unpaired electron such as superoxide, hydroxyl, peroxyl and alkoxyl. Outside the living cell, these compounds are produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes; however, they are continuously produced in the human body and also controlled by endogenous enzyme (superoxide dismutase, glutathione peroxidase, catalase). An over-production of these species, exposure to external oxidant substance or failure in the defense mechanisms, leads to damaging of valuable bio-molecules (DNA, lipids, proteins) which associated with and increased risk of cardiovascular disease, cancer and other chronic disease [1]. In recent years, human health related to nutrition, fitness and beauty has exaggerated concerns over diet. Therefore, a new diet health paradigm is more interesting.

The meaning of some Thai words such as “keang” means curry which is hot and spicy, while “som” means sour and “hleung” means yellow color as pigment derived from turmeric rhizome. Keang-hleung or Southern sour curry soup is now popular not only in southern part of Thailand but also others. It is also claimed as a healthy

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food because of low calories due to less fat but high propotion of vegetable. Southern sour curry normally contains many kinds of vegetables; therefore it has high fiber which is good for health. Moreover, the ingredients used in the paste are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds [2-5].

For cooking the sour curry soup, souring agent such as lime juice, tamarind juice, and garcinia fruit, or any aviable sour fruit will be used. Hydroxy citric acid, an active compound found in garcinia fruit, a local fruit of the Southern part of Thailand, can help metabolize glucose and carbohydrates and reduce the accumulation of fat [6,7]. Currently, garcinia powder or garcinia extract is used as weight controlling product. Siripongvutikorn *et al*. [8] reported that using garcinia as souring agent in instant Tom-Yum mixed was more acceptable compared with commercial instant Tom-Yum. Therefore, garcinia Keang-hleung is planned to make for convenient product and may also serve some functional property. However, the addition of garcinia in the paste may alter some qualities, and antioxidant property then consumer acceptability of the paste and the soup were also investigated.

2. Material and Methods

2.1. Material

Turmeric rhizomes (*Curcuma longa*), garlic (*Allium sativum*), dried finger chili (*Capsicum annuum*), shallot (*Allium ascalonicum*) and dried garcinia (*Garcinia atroviridis*) were purchased from a local market in Hat Yai, Thailand.

2.2. Chemicals

All chemicals and reagents were of analytical grade. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-s-triazine (TPTZ), gallic acid, ferric chloride hexahydrate ($FeCl_3$ $6H_2O$) were purchased from Fluka, Sigma Chemical Co. (St. Louis, MQ, USA). Hydrochloric acid, sodium acetate, folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), absolute ethanol were purchased from Merck (Darmstadt, Germany).

2.3. Keang-hleang Paste Preparation

All spices were sorted, trimmed and washed thoroughly to remove dust and dirt, then soaked in 150 ppm and 10 ppm of chlorine solution, respectively for 1 minute, then weighed according to basic recipe before added 20% salt then divided in to 3 groups as P1, P2 and P3. Based on calculation the compositions of each paste were differences as showed in **Table 1**. All mentioned ingredients were then ground with blender (Moulinex, TYPE 276, France) to make a fine paste (20 - 40 mesh).

2.4. Extraction Procedure

The paste 100 g (fresh weight) was extracted with 300 ml of distilled water then stirred with a magnetic stirrer 12 hr before being subjected to filter through cheesecloth and centrifuge at 2000 g for 25 min. Thereafter, the supernatant was dried with freezed dryer and kept at −20˚C until used.

2.5. Physical Qualities

2.5.1. pH Values

Ten g of the paste was homogenized in 40 ml distilled water for 1 min with the homogenizer (Wiggen Hauser D500, Germany) and measured for pH at room temperature with a Satorius Docu-pH Meter (Germany) [9].

2.5.2. Color Values

Color values of the extracts were measured using a color meter (Hunter Lab Universal Software).The color values were express as CIE Lab* coordinates where L^* represents the luminosity (0 = black; 100 = white), a^* the redness ($a^* > 0$) or ($a^* < 0$) and b^* the blueness ($b^* > 0$) or yellowness ($b^* < 0$).

2.6. Chemical Qualities

Titratable Acidity

The 5 ml of sample was diluted with 45 ml distilled water and titrated with 0.1 N sodium hydroxide to reach pH 8.1. The results were expressed as percentage of citric acid (g citric acid/100 g) as equation below.

$$\%\ \text{Total acidity}(\text{citric acid}) = (\text{vol. of NaOH} \times \text{Normality of NaOH} \times 0.064 \times 100)/\text{wt. of sample}[10].$$

2.7. Analyses

2.7.1. Determination of Total Phenolic Compound Content

Total phenolic content of the extracted sample was determined using the Folin-Ciocalteu assay reported by Kahkonen *et al*. [11] with some modification. Briefly, the extracted sample (20 µl) was introduced into 96 well plates, followed by 100 µl of Folin-Ciocalteu's reagent and 80 µl of sodium carbonate (7.5% w/v). The plates were shaken vigorously and left at ambient temperature (29˚C ± 2˚C) for 30 min in the dark. Then the absorbance

Table 1. The ingredient compositions in any paste formula.

Component	Formulation (%)		
	P1	P2	P3
Garlic	10	8	10.5
Shallot	30	24.5	32
Chilli	30	24.5	32
Turmeric Rhizome	10	8	10.5
Garcinia	-	15	15
Salt	20	20	-

P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

was measured at 765 nm using the microplate reader (PowerWare X, Biotek, USA). Gallic acid was used as antioxidant standard, and reported as g gallic/100g sample of dry weight.

2.7.2. Determination of Antioxidant Activity

1) DPPH Scavenging Activity

DPPH scavenging activity was described by Wu *et al.* [12] with some modification. Briefly, a 100 µl of each sample was mixed with 100 µl of 0.3 mM DPPH dissolved in 75% ethanol. The mixture was shaken vigorously and left at ambient temperature for 30 min in the dark. The DPPH scavenging activity was determined by measuring the absorbance at 517 nm using the microplate reader (PowerWare X, Biotek, USA).

2) FRAP Antioxidant Activity

The FRAP assay was done according to Benzie and Strain [13] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37˚C before used. The extracted sample (15 µl) was allowed to react with 285 µl of the FRAP solution for 30 min in the dark condition. Reading of the colored products [ferrous tripyridyltriazine complex] was then taken at 593 nm using the microplate reader (PowerWare X, Biotek, USA).

2.8. Microbiological Analyses

Total viable count (mesophilic bacteria), Coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens*, *Salmonella* and Lactic acid bacteria as well as yeast and mold were followed BAM, [14]. Briefly, Twenty-five grams of the paste was blended with 225 ml of 0.1% peptone water. Serial dilution was made at 10^{-1} to 10^{-6} by using the 0.1% peptone water. Appropriate dilution was determined as mentioned in BAM [14].

2.9. Statistical Analysis

Data were subjected to analysis of variance, and mean comparison were made using Duncan's new multiple range test. Statistical analyses were carried out using the SPSS statistical software version 6 (SPSS, Inc., Chicago, IL)

3. Results and Discussion

3.1. Quality Changes of Keang-Hleung Paste During Storage at Ambient Temperature (29˚C ± 2˚C) and 4˚C ± 2˚C

Physical and Chemical Properties

The color value interm of *L**, *a** and *b** of the basic and garcinia Keang-hleung with and without 20% salt then stored at ambient temperature and 4˚C were presented in **Tables 2** and **3**, respectively. A decreased of color values particularly *a** value, redness, during storage may due to

Table 2. Color values of basic and garcinia Keang-hleung paste during storage at ambient temperature.

Treatment	Storage (month)	Color		
		*L**	*a**	*b**
Basic Keang-hleung Paste	0	29.82 ± 0.01^{bc}	36.44 ± 0.09^{a}	45.76 ± 0.10^{c}
	1	29.27 ± 0.01^{bc}	34.96 ± 0.03^{b}	44.32 ± 0.07^{cd}
	2	29.22 ± 0.04^{bc}	32.82 ± 0.02^{bc}	44.41 ± 0.33^{cd}
	3	28.01 ± 0.12^{c}	32.88 ± 0.26^{bc}	44.98 ± 0.33^{cd}
	4	28.16 ± 0.03^{c}	30.87 ± 0.06^{c}	44.55 ± 0.07^{cd}
	5	28.39 ± 0.20^{c}	30.38 ± 0.17^{c}	43.16 ± 0.06^{cd}
	6	25.86 ± 0.06^{e}	30.59 ± 0.07^{c}	43.04 ± 0.14^{cd}
Garcinia Keang-hleung Paste with Salt 20%	0	28.72 ± 0.13^{c}	34.18 ± 0.20^{b}	47.66 ± 0.05^{b}
	1	28.14 ± 0.01^{c}	32.21 ± 0.02^{bc}	47.30 ± 0.22^{b}
	2	28.97 ± 0.11^{c}	34.11 ± 0.05^{b}	45.14 ± 0.21^{c}
	3	28.44 ± 0.07^{c}	30.87 ± 0.12^{c}	45.64 ± 0.25^{c}
	4	27.63 ± 0.03^{d}	31.82 ± 0.04^{c}	45.59 ± 0.04^{c}
	5	26.56 ± 0.06^{d}	31.13 ± 0.11^{c}	43.12 ± 0.44^{cd}
	6	26.53 ± 0.51^{d}	30.09 ± 0.19^{c}	43.33 ± 0.26^{cd}
Garcinia Keang-hleung Paste without Salt 20%	0	31.14 ± 0.02^{a}	34.22 ± 0.06^{bc}	48.99 ± 0.14^{a}
	1	31.23 ± 0.03^{a}	33.82 ± 0.06^{bc}	48.68 ± 0.05^{a}
	2	30.81 ± 0.02^{ab}	33.00 ± 0.06^{bc}	48.75 ± 0.26^{a}
	3	29.35 ± 0.03^{bc}	32.87 ± 0.02^{c}	48.50 ± 0.28^{a}
	4	28.75 ± 0.06^{c}	30.46 ± 0.07^{c}	48.71 ± 0.20^{a}
	5	28.33 ± 0.08^{c}	30.31 ± 0.16^{c}	48.66 ± 0.07^{a}
	6	28.29 ± 0.14^{c}	30.69 ± 0.15^{c}	46.69 ± 0.15^{bc}

Each value is expressed as a mean ± SD (n = 3); a - e mean that with different letters within a column are significant different ($p < 0.05$).

Table 3. Color values of basic and garcinia Keang-hleung paste during storage at 4˚C ± 2˚C.

Treatment	Storage (month)	Color		
		*L**	*a**	*b**
Basic Keang-hleung Paste	0	29.82 ± 0.01^{a}	36.44 ± 0.09^{a}	45.76 ± 0.10^{b}
	1	29.53 ± 0.01^{a}	34.11 ± 0.01^{b}	44.89 ± 0.16^{bc}
	2	29.55 ± 0.05^{a}	34.64 ± 0.07^{b}	44.50 ± 0.38^{bc}
	3	28.84 ± 0.07^{ab}	34.04 ± 0.16^{b}	44.88 ± 0.14^{bc}
	4	28.83 ± 0.04^{ab}	34.53 ± 0.06^{b}	44.10 ± 0.08^{bc}
	5	28.66 ± 0.23^{ab}	31.20 ± 0.10^{d}	44.66 ± 0.06^{bc}
	6	25.90 ± 0.05^{d}	30.56 ± 0.09^{d}	43.60 ± 0.10^{c}
Garcinia Keang-hleung Paste with Salt 20%	0	28.72 ± 0.13^{ab}	34.18 ± 0.20^{b}	47.66 ± 0.05^{b}
	1	27.86 ± 0.05^{bc}	29.05 ± 0.11^{e}	45.99 ± 0.38^{bc}
	2	27.52 ± 0.03^{bc}	29.51 ± 0.10^{e}	45.07 ± 0.25^{bc}
	3	26.88 ± 0.04^{c}	29.46 ± 0.04^{e}	46.54 ± 0.24^{b}
	4	27.80 ± 0.02^{bc}	30.15 ± 0.05^{de}	46.31 ± 0.19^{b}
	5	27.18 ± 0.02^{bc}	26.22 ± 0.06^{f}	46.99 ± 0.14^{b}
	6	27.90 ± 0.03^{bc}	26.13 ± 0.13^{f}	46.17 ± 0.07^{b}
Garcinia Keang-hleung Paste without Salt 20%	0	31.14 ± 0.02^{a}	34.22 ± 0.06^{b}	48.99 ± 0.14^{a}
	1	30.92 ± 0.05^{a}	32.03 ± 0.19^{c}	48.82 ± 0.18^{a}
	2	30.80 ± 0.01^{a}	30.15 ± 0.08^{de}	48.96 ± 0.26^{a}
	3	29.42 ± 0.01^{a}	30.38 ± 0.26^{de}	48.46 ± 0.20^{a}
	4	28.47 ± 0.03^{ab}	30.73 ± 0.07^{de}	48.15 ± 0.11^{a}
	5	28.35 ± 0.02^{ab}	27.03 ± 0.03^{f}	47.40 ± 0.08^{ab}
	6	28.22 ± 0.02^{ab}	27.59 ± 0.07^{f}	47.50 ± 0.40^{a}

Each value is expressed as a mean ± SD (n = 3); a - f mean that with different letters within a column are significant different ($p < 0.05$).

degradation of carotenoids oxidation process mainly *β*-carotene during storage period [15,16]. Similar result was found in study of Ketsa and Pangkoolm [17,18] who reported that the fading of durian pulp color was most probably due to degradation of *β*-carotene because the curcuminoids were readily decomposed when exposed to bright light [19]. Moreover, Coneellon *et al*. [20] addressed that oxidized products such as mono-, di-phenol and quinones of phenolic compounds were unstable and rapidly react with amino acid or protein particularly at 30˚C, generating brown pigments by polymerization. However, kepting the paste in lower temperature, 4˚C, retained more color values particularly *b** value. It pointed out that enzymatic oxidation of natural phenolic compounds was partly inhibition. The difference of *a** value between the basic paste and garcinia paste with and without salt was noticed. The basic paste had more *a** value compared with the garcinia paste may due to *β*-carotene bleaching affect [21] as function of hydroxyl citric acid derived from garcinia leading to redness reducing and yellowness increasing.

The pH values and acidity (g/100g) during storage time of the Keang-hleung paste were presented in **Tables 4** and **5**, respectively. pH values and acidity of basic Keang-hleung paste kept at ambient temperature and 4˚C ± 2˚C tended to decrease and increase, respectively during storage. This may due to growth of lactic acid bacteria producing lactic acid or acetic acid [22,23]. As expected, the lower of pH and higher of acidity (g/100 g) were found in the paste added with garcinia may due to organic acid mainly hydroxy citric acid containing in garcinia fruit.

The A_w of Keang-hleung paste during storage at ambient temperature and 4˚C ± 2˚C were presented in **Tables 4** and **5**, respectively. The result showed that A_w did not

Table 4. pH values, acidity (g/100g) change and A_w of Keang-hleung paste during storage at ambient temperature.

Treatment	Storage (months)	pH	Acidity (g/100g)	A_w
Basic Keang-hleung Paste	0	5.08 ± 0.01^{a}	0.39 ± 0.01^{c}	0.79 ± 0.001^{b}
	1	5.04 ± 0.01^{a}	0.44 ± 0.01^{c}	0.80 ± 0.001^{b}
	2	4.99 ± 0.01^{a}	0.44 ± 0.00^{c}	0.79 ± 0.001^{b}
	3	4.98 ± 0.02^{a}	0.44 ± 0.02^{c}	0.80 ± 0.001^{b}
	4	4.98 ± 0.01^{a}	0.46 ± 0.03^{c}	0.79 ± 0.001^{b}
	5	4.91 ± 0.01^{a}	0.48 ± 0.03^{c}	0.80 ± 0.001^{b}
	6	4.85 ± 0.03^{a}	0.48 ± 0.00^{c}	0.79 ± 0.001^{b}
Garcinia Keang-hleung Paste with Salt 20%	0	3.38 ± 0.02^{c}	1.41 ± 0.03^{b}	0.81 ± 0.001^{b}
	1	3.30 ± 0.00^{c}	1.40 ± 0.02^{b}	0.81 ± 0.001^{b}
	2	3.29 ± 0.01^{c}	1.43 ± 0.01^{b}	0.83 ± 0.001^{b}
	3	3.28 ± 0.01^{c}	1.50 ± 0.02^{b}	0.83 ± 0.001^{b}
	4	3.26 ± 0.01^{c}	1.56 ± 0.04^{b}	0.82 ± 0.001^{b}
	5	3.26 ± 0.01^{c}	1.56 ± 0.03^{b}	0.82 ± 0.001^{b}
	6	3.18 ± 0.00^{c}	1.43 ± 0.01^{b}	0.83 ± 0.001^{b}
Garcinia Keang-hleung Paste without Salt 20%	0	3.62 ± 0.01^{b}	1.59 ± 0.04^{b}	0.98 ± 0.001^{a}
	1	3.63 ± 0.01^{b}	1.78 ± 0.03^{ab}	0.97 ± 0.001^{a}
	2	3.55 ± 0.01^{b}	1.85 ± 0.01^{a}	0.98 ± 0.001^{a}
	3	3.55 ± 0.01^{b}	1.82 ± 0.03^{a}	0.97 ± 0.001^{a}
	4	3.54 ± 0.01^{b}	1.81 ± 0.03^{a}	0.97 ± 0.001^{a}
	5	3.51 ± 0.01^{b}	1.95 ± 0.05^{a}	0.97 ± 0.001^{a}
	6	3.45 ± 0.01^{b}	1.99 ± 0.05^{a}	0.98 ± 0.001^{a}

Each value is expressed as a mean ± SD (n = 3); a - c mean that with different letters within a column are significant different ($p < 0.05$).

Table 5. pH values, acidity (g/100 g) and A_w of Keang-hleung paste during storage at 4˚C ± 2ºC.

Treatment	Storage (month)	pH	Acidity (g/100 g)	A_w
Basic Keang-hleung Paste	0	5.08 ± 0.01[a]	0.39 ± 0.01[c]	0.79 ± 0.001[b]
	1	5.03 ± 0.00[a]	0.44 ± 0.01[c]	0.79 ± 0.001[b]
	2	4.94 ± 0.01[a]	0.44 ± 0.01[c]	0.79 ± 0.001[b]
	3	4.93 ± 0.02[a]	0.43 ± 0.00[c]	0.80 ± 0.001[b]
	4	4.95 ± 0.01[a]	0.43 ± 0.01[c]	0.79 ± 0.001[b]
	5	4.85 ± 0.01[a]	0.45 ± 0.00[c]	0.79 ± 0.001[b]
	6	4.84 ± 0.03[a]	0.46 ± 0.01[c]	0.79 ± 0.001[b]
Garcinia Keang-hleung Paste with Salt 20%	0	3.38 ± 0.02[c]	1.41 ± 0.03[b]	0.81 ± 0.001[b]
	1	3.32 ± 0.00[c]	1.40 ± 0.02[b]	0.82 ± 0.001[b]
	2	3.28 ± 0.01[c]	1.43 ± 0.03[b]	0.81 ± 0.001[b]
	3	3.25 ± 0.01[c]	1.50 ± 0.01[b]	0.82 ± 0.001[b]
	4	3.24 ± 0.02[c]	1.56 ± 0.04[b]	0.82 ± 0.001[b]
	5	3.19 ± 0.01[c]	1.56 ± 0.03[b]	0.82 ± 0.001[b]
	6	3.20 ± 0.01[c]	1.43 ± 0.02[b]	0.81 ± 0.001[b]
Garcinia Keang-hleung Paste without Salt 20%	0	3.62 ± 0.01[b]	1.59 ± 0.04[b]	0.98 ± 0.001[a]
	1	3.79 ± 0.01[b]	1.78 ± 0.05[ab]	0.98 ± 0.001[a]
	2	3.71 ± 0.02[b]	1.85 ± 0.04[a]	0.98 ± 0.001[a]
	3	3.66 ± 0.01[b]	1.82 ± 0.07[a]	0.98 ± 0.001[a]
	4	3.53 ± 0.04[b]	1.81 ± 0.04[a]	0.98 ± 0.001[a]
	5	3.52 ± 0.01[b]	1.95 ± 0.05[a]	0.98 ± 0.001[a]
	6	3.50 ± 0.01[b]	1.99 ± 0.06[a]	0.98 ± 0.001[a]

Each value is expressed as a mean ± SD (n = 3) a - c means that with different letters within a column are significant different ($p < 0.05$).

change when during storage increased due to property of packaging (LLDPE/Nylon) which protected moisture permeability and oxygen. The lowest A_w was found in the basic paste while added garcinia in the paste particularly without salt yielded more A_w. This evident pointed out that washing step reabsorbed water as free water resulting to higher A_w. It is the reason why draining step needs to be property applied otherwise shorten shelf-life would be occur afterward. In addition, it confirmed that certain salt content could improve shelf-life by reducing A_w. As well known food products having A_w less than 0.85 are quite safe for pathogenic or spoilage bacteria and have more shelf-life compared with another product having A_w higher than 0.85 [24].

The results also showed that storage temperature did not have any effect on pH, % acidity and A_w. The major factors controlling these mentioned values depended on the added ingredients particularly garcinia and salt.

3.2. Total Phenolic Content and Antioxidant of Keang-hleung Paste with and without Garcinia

Total phenolic contents of the pastes during storage at 29 ± 2 and 4˚C ± 2˚C were presented in **Figures 1** and **2**, respectively. Total phenolic content in the garcinia paste without added salt was highest compared with other pastes may due to highest of spices used. However, at the end of storage (6 mo), total phenolic content of each paste was quite similar meant that faster degradation of phenolic compounds in the garcinia paste without added salt occurred. It pointed out that salt concentration was major role to inhibit enzymatic reaction derived from spice materials. Surprising, total phenolic content in the garcinia paste with added salt kept at ambient temperature was lowest after storage for 2 mo (**Figure 1**) meant that both salt and garcinia may destroy or bleach some phenolic compounds. However, the basic paste kept at 4˚C ± 2˚C (**Figure 2**) seemed to be lowest compared with others even at end of storage (6 mo) was not different.

3.3. Antioxidant Activities

3.3.1. DPPH• Free Radical Scavenging

DPPH• is a free radical compound that has been widely

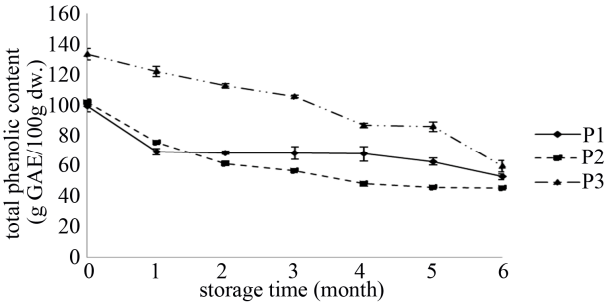

Figure 1. Total phenolic contents of the Keang-hleung paste during storage at 29˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

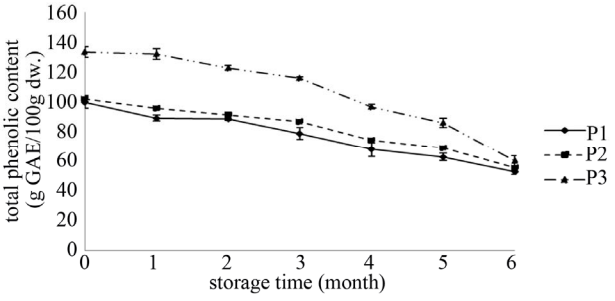

Figure 2. Total phenolic contents of the Keang-hleung paste during storage at 4˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

used to determine the free radical scavenging capacity of various samples [25,26] because of its stability (in radical form), simplicity and fast assay [27]. The DPPH radical scavenging activities of the pastes during storage at 29 ± 2 and 4˚C ± 2˚C were presented in **Figures 3** and **4**, respectively. The results showed that the $DPPH^{\bullet}$ radical scavenging activity of the basic paste kept at ambient temperature and 4˚C decreased as storage time increased. Surprising again, initial DPPH radical scavenging activity was highest in the basic paste even low in total phenolic content. This result confirmed that antioxidant activity may not well relate to total phenolic content [28]. DPPH radical scavenging activity of the garcinia paste without added salt kept in both storage temperatures increased in the first 2 mo before decreased and was lowest at end of storage. An increase of the activity may cause by polyphenol oxidase [20] at the first period of time yielding some active compounds however, without salt and high temperature may allow microbial growth as show in **Table 6**. It meant that degradated compounds derived from the paste were low in H^{+} donor ability. A decrease of DPPH radical scavenging activity well relates to a decrease of total phenolic compounds as mentioned before.

3.3.2. Ferric Reducing/Antioxidant Power (FRAP)

The FRAP activity of the basic paste kept in both temperatures decreased at 1 mo before kept constant for 3 mo then sharply decreased until got the lowest value compared with other pastes. On the other hand , the FRAP activity of garcinia paste with and without added salt kept in the both storage temperature increased at first 2 mo before kept constant for 3 mo then decreased but still higher than the basic paste (**Figures 5** and **6**). From the results of DPPH radical scavenging activity and FRAP activity, it pointed out that these parameters of the paste did not have the same change trends at the first 4 mo. This may be a good explanation of using many

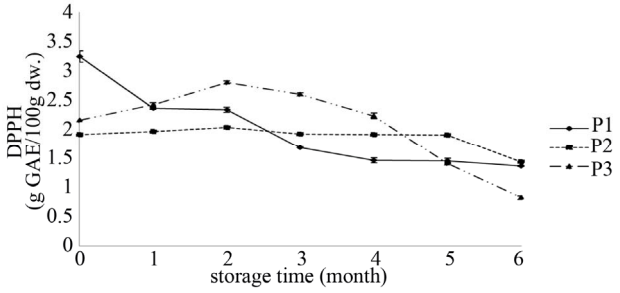

Figure 3. The DPPH scavenging activity of Keang-hleung paste during storage at 29˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

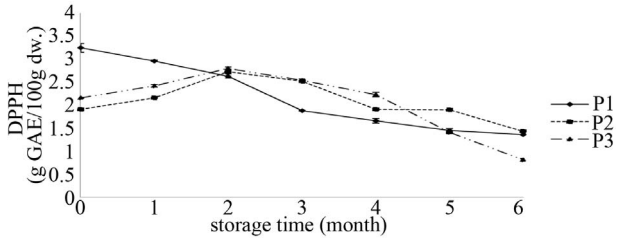

Figure 4. The DPPH scavenging activity of Keang-hleung paste during storage at 4˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

Table 6. Microbiological quality in basic and garcinia Keang-hleung paste with and without salt during storage at ambient temperature and 4˚C ± 2˚C.

Treatment	Bacteria Count (cfu/g)			
	Storage (months)	TVC	Lactic Acid Bacteria	Yeast and Mold
Basic Keang-hlueng Paste AT	0	1.89×10^3	3.10×10^2	<30
	2	4.70×10^3	9.70×10^2	<30
	4	4.30×10^3	6.20×10^2	<30
	6	8.00×10^3	3.00×10^2	<30
Basic Keang-hlueng Paste 4˚C	0	1.89×10^3	3.10×10^2	<30
	2	8.70×10^2	3.20×10^2	<30
	4	1.28×10^3	4.80×10^2	<30
	6	7.60×10^3	<30	<30
Garcinia Keang-hlueng Paste AT	0	6.60×10^2	<30	<30
	2	4.70×10^2	<30	<30
	4	3.00×10^3	<30	<30
	6	2.13×10^3	<30	<30
Garcinia Keang-hlueng Paste 4˚C	0	6.60×10^2	<30	<30
	2	5.30×10^2	<30	<30
	4	6.90×10^2	<30	<30
	6	1.71×10^3	<30	<30
Garcinia Keang-hlueng Paste without Salt AT	0	1.95×10^3	<30	<30
	2	4.70×10^2	3.50×10^2	5.20×10^2
	4	7.40×10^2	3.00×10^2	<30
	6	1.22×10^4	<30	<30
Garcinia Keang-hlueng Paste without Salt 4˚C	0	1.95×10^3	<30	<30
	2	5.30×10^2	3.20×10^2	4.80×10^2
	4	3.50×10^2	3.70×10^2	<30
	6	2.53×10^3	<30	<30

AT = Ambient Temperature.

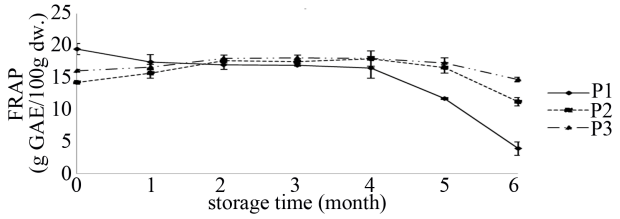

Figure 5. The FRAP activity of Keang-hleung paste during storage at 29˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

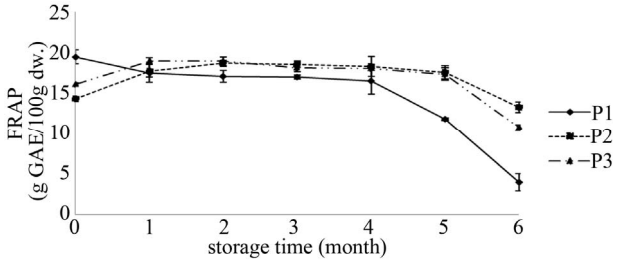

Figure 6. The FRAP activity of Keang-hleung paste during storage at 4˚C ± 2˚C. P1: Basic Keang-hleung paste. P2: Garcinia Keang-hleung paste with 20% salt. P3: Garcinia Keang-hleung paste without 20% salt.

assays to evaluate antioxidant activity [29]. However, some researchers mentioned that in vitro antioxidant activity such as DPPH, FRAP, ABTS and metal chelation activity may not responsible for antioxidant activity in vivo or even food system therefore using cellular antioxidant activity assay was more useful and close to body system [28,30].

3.4. Microbiological Quality in Basic and Garcinia Keang-hleung Paste during Storage at Ambient Temperature (29˚C ± 2˚C) and 4˚C ± 2˚C

The basic and garcinia Keang-hleung paste with and without salt stored at ambient temperature and 4˚C monitored for TVC, yeasts and moulds, *staphylococcus aureus*, *Bacilluus cereus*, Lactic acid bacteria, *Clostridium perfringens*, *Salmonella*, *Escherichia coli* and coliforms were showed in **Table 6**. At the initial stage, TVC of all treatments were in the range of 10^2 - 10^4 cfu/g and increased as the storage time increased (**Table 6**). TVC of any paste kept at both temperatures was not more than 1.22×10^4 cfu/g within 6 mo. It pointed out that both salt and garcinia played an important role for microbial growth. However, using certain salt concentration as 20%, garcinia and chilled storage as hurdle parameters seemed to pronounce more inhibitory effect. Yeast and mold counts of the basic and garcinia Keang-hlueng paste with addition salt were under 30 cfu/g during storage. Without salt, yeast and mold counts of the garcinia Keang-hlueng paste increased and reached 10^2 cfu/g before declined to lower than 30 cfu/g at the end of storage. An increase of yeast and mold at the first 2 mo may due to proper germination period of the fungal spore, thereafter a decrease of yeast and mold may due to function of hydroxy acid and other weak acids mainly derived from garcinia. Lactic acid bacteria were lowest in the garcinia paste with added salt. However, it was found that the basic paste had lactic acid bacteria throughout the storage except sample kept at 4˚C for 6 mo. This result indicated that the salt concentration, garcinia content and storage temperature played their antimicrobial role. It also pointed out that using only salt or garcinia may not enough to control some organism and may stimulate the lactic acid bacteria growth for a period of time. However, there were no *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens*, *Salmonella*, *Escherichia coli* and coliforms detected in all treatments throughout the storage period.

4. Conclusion

In general, color values of the basic and garcinia Keang-hleung paste kept at ambient temperature and 4˚C ± 2˚C decreased during storage increased. The pH values and acidity of all pastes tended to decrease and increase, respectively when storage time increased. A decrease of total phenolic content of during storage at ambient and 4˚C ± 2˚C of the paste was found particularly in the garcinia paste without added salt. A decrease of total phenollic content of the basic paste was concomitant with a decrease of DPPH radical scavenging and FRAP activity. However, there was not a good relationship between DPPH radical scavenging activity and FRAP activity in the garcinia paste with and without added salt. Salt and garcinia in the paste help to prolong to the shelf-life of the paste in term of microbiological quality.

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