CHEMICAL AND FUNCTIONAL PROPERTIES OF PECTIN FROM NAM WA BANANA (*Musa* (ABB GROUP) 'KLUAI NAM WA') PEELS AND ITS POTENTIAL FOOD APPLICATION

NITJAREE MANEERAT 5437709 NUFN/M

M.Sc. (FOOD AND NUTRITION FOR DEVELOPMENT)

THESIS ADVISORY COMMITTEE: NATTAPOL TANGSUPHOOM, Ph.D.,
ANADI NITITHAMYONG, Ph.D.

ABSTRACT

As a result of the fast growth of the banana processing industry, and the rising demands of processed banana products, there is a large quantity of banana peel, especially of Nam Wa banana (*Musa* (ABB group) 'Kluai Nam Wa'), being discarded as waste. Pectin is a polysaccharide that can be extracted from a variety of fruits and vegetables, as well as, from the processing wastes. Pectin is generally used in a food to provide functional properties, along with some physiological effects. This study aims to investigate the usage of Nam Wa banana peels as a source for pectin extraction. Blanched banana peels were extracted using HCl solution (pH 1.5 and 2.3) and DI water (pH 6.0) for 30, 60, 90 and 120 min at 90±5ºC. Chemical and functional properties of Nam Wa banana peels pectin (NBPP) were determined. Extraction at pH 1.5 gave the highest yield of NBPP (7-11% dry weight basis) with galacturonic acid content (GalA) and degree of methylation (DM) of 42-47% and 57-61%, respectively. NBPP extracted at pH 2.3 had lower DM (52-53%) than NBPP extracted at pH 1.5, but there was no significant difference in GalA. NBPP obtained from water extraction contained higher GalA (50-52%) with lower DM (38-39%), and higher viscosity-average molecular weight (M_v) when compared to acid-extracted NBPP at the same extraction time. The prolonged extraction lowered the M_v of NBPP and the viscosity of their solutions. For functional properties, viscosity of 2.5% (w/v) acid-extracted NBPP solution was lower than those from water extraction. Most of NBPP provided high methoxy pectin gelling ability in the presence of acid and sugar, but water-extracted NBPP which is low methoxy pectin did not form gel in the presence of calcium ions. Water- and oil-holding capacities of NBPP were 4.2-5.9 g water/g pectin and 3.3-3.4 g oil/g pectin, respectively. NBPP extracted using HCl solution pH 1.5 and DI water pH 6.0 for 60 min was selected to evaluate their potential use as fat replacers in salad dressing. The addition of NBPP solution (2% w/v) at levels of >25% oil substitution resulted in the decrease in viscosity and the darker color of the reduced-fat salad dressing (RSD). All RSD were stable within 2 weeks of storage period at room temperature. RSD at 30% of oil substitution were "just-about-right" in terms of thickness, although the color was rated between "just-about-right" and "too dark" when their sensory characteristics were evaluated. The results from sensory acceptability test confirmed that both RSD containing acid and water-extracted NBPP at 30% oil substitution level were accepted by 50 panelists. The hedonic scores on color and thickness of RSD were about "like slightly" while the overall acceptability ranked between "like slightly" to "like moderately". Therefore, it can be concluded that Nam Wa banana peels can be used as an alternative source of pectin and the extracted NBPP has a potential use as a fat replacer in food products.

KEY WORDS: PECTIN / BANANA PEEL / CHEMICAL PROPERTIES / FUNCTIONAL PROPERTIES / FAT REPLACER

108 pages

สมบัติทางเคมี สมบัติเชิงหน้าที่ ของเพคตินจากเปลือกกล้วยน้ำว้า (*Musa* (ABB group) 'Kluai Nam Wa') และศักยภาพการนำไปใช้ในผลิตภัณฑ์อาหาร

CHEMICAL AND FUNCTIONAL PROPERTIES OF PECTIN FROM NAM WA BANANA (*Musa* (ABB GROUP) 'KLUAI NAM WA') PEELS AND ITS POTENTIAL FOOD APPLICATION

นิจจารีย์ มณีรัตน์ 5437709 NUFN/M

วท.ม. (อาหารและโภชนาการเพื่อการพัฒนา)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: นัฐพล ตั้งสุภูมิ, Ph.D., อาณดี นิติธรรมยง, Ph.D.

บทคัดย่อ

อุตสาหกรรมการแปรรูปกล้วยที่เติบโตขึ้นอย่างรวดเร็ว ประกอบกับความต้องการผลิตภัณฑ์กล้วยแปรรูปของผู้บริโภคที่เพิ่มขึ้นในปัจจุบัน ทำให้มีเปลือกกล้วยจำนวนมากเหลือทิ้งจากอุตสาหกรรมดังกล่าวโดยเฉพาะเปลือกกล้วยน้ำว้า (Musa (ABB GROUP) 'Kluai Nam Wa') เพคตินเป็นพอลีแซกคาไรด์ชนิดหนึ่งที่สามารถสกัดได้จากผักและผลไม้หลายชนิด รวมถึงของเหลือทิ้งจากการแปรรูปผักและผลไม้ เพคตินมักจะใช้ในอาหารเพื่อสมบัติเชิงหน้าที่ และผลเชิงสุขภาพ งานวิจัยนี้มีวัตถุประสงค์เพื่อใช้เปลือกกล้วยน้ำว้าเป็นแหล่งสำหรับการสกัดเพคติน โดยสกัดเปลือกกล้วยน้ำว้าลวกด้วยสารละลายกรดไฮโดรคลอริก (พีเอช 1.5 และ 2.3) และน้ำปราศจากไอออน (พีเอช 6.0) เป็นเวลา 30, 60, 90 และ 120 นาทีที่อุณหภูมิ 90±5°ซ ศึกษาสมบัติทางเคมี และสมบัติเชิงหน้าที่ของเพคตินจากเปลือกกล้วยน้ำว้า (NBPP) จากผลการทดลองพบว่า การสกัด NBPP ด้วยสารละลายกรดพีเอช 1.5 ให้ผลผลิตสูงสุด (ร้อยละ 7-11 โดยน้ำหนักแห้ง) โดยมีปริมาณกรดกาแลคทูโรนิก (GalA) และปริมาณการแทนที่หมู่เมทิล (DM) ร้อยละ 42-47 และ 57-61 ตามลำดับ NBPP ที่สกัดที่พีเอช 2.3 มี DM ต่ำกว่า (ร้อยละ 52-53) NBPP ที่สกัดโดยใช้สารละลายกรดที่พีเอช 1.5 แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญของ GalA ในขณะที่ NBPP ที่ได้จากการสกัดด้วยน้ำมี GalA สูงกว่า (ร้อยละ 50-52) แต่มีปริมาณ DM ต่ำกว่า (ร้อยละ 38-39) และมีน้ำหนักโมเลกุลเฉลี่ยแบบความหนืด (M_v) สูงกว่า เมื่อเปรียบเทียบกับ NBPP ที่สกัดด้วยสารละลายกรดที่ใช้เวลาในการสกัดเท่ากัน การเพิ่มเวลาในการสกัดส่งผลให้ NBPP ที่สกัดได้มี M_v และความหนืดของสารละลายต่ำลง สำหรับสมบัติเชิงหน้าที่ พบว่า สารละลาย (ร้อยละ 2.5 น้ำหนักโดยปริมาตร) ของ NBPP ที่สกัดด้วยกรด มีความหนืดสูงกว่าสารละลายของ NBPP ที่สกัดด้วยน้ำ NBPP ส่วนใหญ่มีความสามารถในการเกิดเจลแบบเพคตินเมทอกซีสูงในสภาวะที่มีกรด และน้ำตาล แต่ NBPP ที่สกัดด้วยน้ำซึ่งเป็นเพคตินเมทอกซีต่ำไม่สามารถเกิดเจลในสภาวะที่มีแคลเซียมได้ ความสามารถในการอุ้มน้ำและน้ำมันของ NBPP คือ 4.2-5.9 กรัมน้ำต่อกรัมเพคติน และ 3.3-3.4 กรัมน้ำมันต่อกรัมเพคตินตามลำดับ การประเมินศักยภาพการนำ NBPP ที่สกัดด้วยสารละลาย HCl พีเอช 1.5 และน้ำปราศจากไอออน เป็นเวลา 60 นาทีไปใช้เป็นสารทดแทนไขมันในน้ำสลัด พบว่า การเติมสารละลาย NBPP (ร้อยละ 2 น้ำหนักโดยปริมาตร) ที่ระดับการแทนที่น้ำมันมากกว่าร้อยละ 25 ทำให้น้ำสลัดสูตรลดไขมัน (RSD) มีความหนืดลดลง และมีสีเข้มขึ้น RSD ทุกสูตรมีความคงตัวตลอดระยะเวลาการเก็บรักษาที่อุณหภูมิห้องเป็นเวลา 2 สัปดาห์ จากการประเมินความเหมาะสมของลักษณะทางประสาทสัมผัส พบว่า RSD ที่มีการแทนที่น้ำมัน ร้อยละ 30 มีความข้นหนืด "กำลังดี" แม้ว่ามีคะแนนด้านสีอยู่ระหว่าง "กำลังดี" และ "เข้มเกินไป" ผลจากการทดสอบการยอมรับยืนยันว่า RSD ที่มีการแทนที่น้ำมันร้อยละ 30 ด้วย NBPP ที่สกัดด้วยสารละลายกรดและน้ำ ได้รับการยอมรับจากผู้ประเมินจำนวน 50 คน โดยมีคะแนนความชอบด้านสีและความข้นหนืดใกล้เคียง ชอบเล็กน้อย และความชอบรวมอยู่ระหว่าง "ชอบเล็กน้อย" ถึง "ชอบปานกลาง" จึงสรุปได้ว่า เปลือกกล้วยน้ำว้าสามารถนำมาใช้เป็นแหล่งเพคติน โดยเพคตินที่สกัดได้มีศักยภาพในการนำไปใช้เป็นสารทดแทนไขมันในผลิตภัณฑ์อาหาร

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Nitjaree Maneerat

APPENDICES

APPENDIX A

Standard curve for galacturonic acid content determination

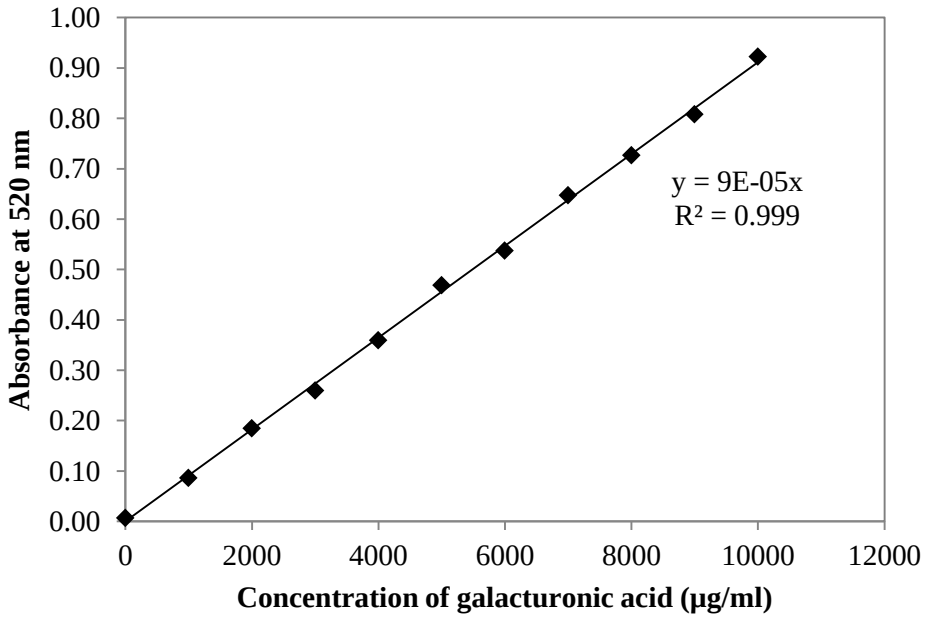

Figure A.1 Standard curve of galacturonic acid (0-10 mg/ml)

APPENDIX B

Recipe for salad dressing

Ingredients

	% w/w
Vegetable oil	50
Sugar	18.5
Egg yolk	10.1
Vinegar	8.5
Water	7.2
Lime juice	3.5
Salt	1.6
Mustard powder	0.6

Procedures

1. Mix sugar, vinegar, lime juice, salt and mustard powder together until completely dissolved
2. Whisk egg yolk using a food mixer (HW-FM02, House Worth, Bangkok, Thailand) at a low speed for 3 min.
3. Add the prepared mixture gradually into the whisked egg yolk with stirring until smooth
4. Add the vegetable oil gradually while stirring
5. Add water and continuously stir for 1 min
6. If any, add pectin solution and gently fold to combine with the emulsion
7. Pasteurize the product by heating at 90ºC for 10 min
8. Hot-fill into glass jars and immediately cool in ice bath for 30 min

APPENDIX C

Questionnaire for sensory evaluation of salad dressing

แบบสอบถามการประเมินผลการทดสอบทางประสาทสัมผัส
ผลิตภัณฑ์น้ำสลัดชนิดครีมเทลดไขมัน

รหัสตัวอย่าง.....

วันที่..... เวลา.....
เพศ (.....) ชาย (.....) หญิง อายุ.....ปี

ตอนที่ 1 กรุณาพิจารณาลักษณะต่างๆของผลิตภัณฑ์ ***ก่อนชิม*** ตัวอย่าง และให้คะแนน โดยขีดเครื่องหมาย ✓ ลงในช่องว่างที่ตรงกับความเห็นของท่านมากที่สุด

สีของผลิตภัณฑ์
.....เข้มเกินไปมาก
.....เข้มเกินไปเล็กน้อย
.....กำลังดี
.....อ่อนเกินไปเล็กน้อย
.....อ่อนเกินไปมาก
ข้อเสนอแนะ……

ความข้น/หนืดของผลิตภัณฑ์
.....ข้นหนืดเกินไปมาก
.....ข้นหนืดเกินไปเล็กน้อย
.....ข้นหนืดกำลังดี
.....เหลวเกินไปเล็กน้อย
.....เหลวเกินไปมาก
ข้อเสนอแนะ……

ตอนที่ 2 กรุณาพิจารณาลักษณะต่างๆของผลิตภัณฑ์ ***หลังชิม*** ตัวอย่าง และให้คะแนน โดยขีดเครื่องหมาย ✓ ลงในช่องว่างที่ตรงกับความเห็นของท่านมากที่สุด (กรุณาบ้วนปากด้วยน้ำสะอาดก่อนทำการทดสอบตัวอย่างถัดไป)

ความรู้สึกในช่องปากของผลิตภัณฑ์
.....เนียนละเอียดเกินไปมาก
.....เนียนละเอียดเกินไปเล็กน้อย
.....เนียนละเอียดกำลังดี
.....ไม่เนียนละเอียดเกินไปเล็กน้อย
.....ไม่เนียนละเอียดเกินไปมาก
ข้อเสนอแนะ……

รสชาติของผลิตภัณฑ์
.....เข้มเกินไปมาก
.....เข้มเกินไปเล็กน้อย
.....กำลังดี
.....อ่อนเกินไปเล็กน้อย
.....อ่อนเกินไปมาก
ข้อเสนอแนะ……

APPENDIX D

Questionnaire for sensory acceptability test of salad dressing

แบบสอบถามการประเมินผลการทดสอบทางประสาทสัมผัส

ผลิตภัณฑ์น้ำสลัดชนิดครีมลดไขมัน

รหัสตัวอย่าง.....

วันที่..... เวลา.....

เพศ (.....) ชาย (.....) หญิง อายุ.....ปี

กรุณาตอบแบบสอบถามให้ครบทั้ง 2 ตอน

ตอนที่ 1 กรุณาพิจารณาลักษณะต่างๆของผลิตภัณฑ์ ***ก่อนชิม*** ตัวอย่าง และให้คะแนนความชอบ โดยขีดเครื่องหมาย ✓ ลงในช่องว่างหน้าข้อความที่ตรงกับความเห็นของท่านมากที่สุด

ลักษณะโดยรวม	**สี**	**ความข้น/หนืด**
.....ชอบมากที่สุด	.....ชอบมากที่สุด	.....ชอบมากที่สุด
.....ชอบมาก	.....ชอบมาก	.....ชอบมาก
.....ชอบปานกลาง	.....ชอบปานกลาง	.....ชอบปานกลาง
.....ชอบเล็กน้อย	.....ชอบเล็กน้อย	.....ชอบเล็กน้อย
.....เฉยๆ	.....เฉยๆ	.....เฉยๆ
.....ไม่ชอบเล็กน้อย	.....ไม่ชอบเล็กน้อย	.....ไม่ชอบเล็กน้อย
.....ไม่ชอบปานกลาง	.....ไม่ชอบปานกลาง	.....ไม่ชอบปานกลาง
.....ไม่ชอบมาก	.....ไม่ชอบมาก	.....ไม่ชอบมาก
.....ไม่ชอบมากที่สุด	.....ไม่ชอบมากที่สุด	.....ไม่ชอบมากที่สุด
ข้อเสนอแนะ……	ข้อเสนอแนะ……	ข้อเสนอแนะ……

ตอนที่ 2 กรุณาพิจารณาลักษณะต่างๆของผลิตภัณฑ์ ***หลังชิม*** ตัวอย่าง และให้คะแนนความชอบ โดยขีดเครื่องหมาย ✓ ลงในช่องว่างหน้าข้อความที่ตรงกับความเห็นของท่านมากที่สุด (กรุณาบ้วนปากด้วยน้ำสะอาดก่อนทำการทดสอบตัวอย่างถัดไป)

ความรู้สึกในช่องปาก	**รสชาติ**	**ความชอบโดยรวม**
.....ชอบมากที่สุด	.....ชอบมากที่สุด	.....ชอบมากที่สุด
.....ชอบมาก	.....ชอบมาก	.....ชอบมาก
.....ชอบปานกลาง	.....ชอบปานกลาง	.....ชอบปานกลาง
.....ชอบเล็กน้อย	.....ชอบเล็กน้อย	.....ชอบเล็กน้อย
.....เฉยๆ	.....เฉยๆ	.....เฉยๆ
.....ไม่ชอบเล็กน้อย	.....ไม่ชอบเล็กน้อย	.....ไม่ชอบเล็กน้อย
.....ไม่ชอบปานกลาง	.....ไม่ชอบปานกลาง	.....ไม่ชอบปานกลาง
.....ไม่ชอบมาก	.....ไม่ชอบมาก	.....ไม่ชอบมาก
.....ไม่ชอบมากที่สุด	.....ไม่ชอบมากที่สุด	.....ไม่ชอบมากที่สุด
ข้อเสนอแนะ……	ข้อเสนอแนะ……	ข้อเสนอแนะ……

APPENDIX E

Data from FT-IR analyses of NBPP

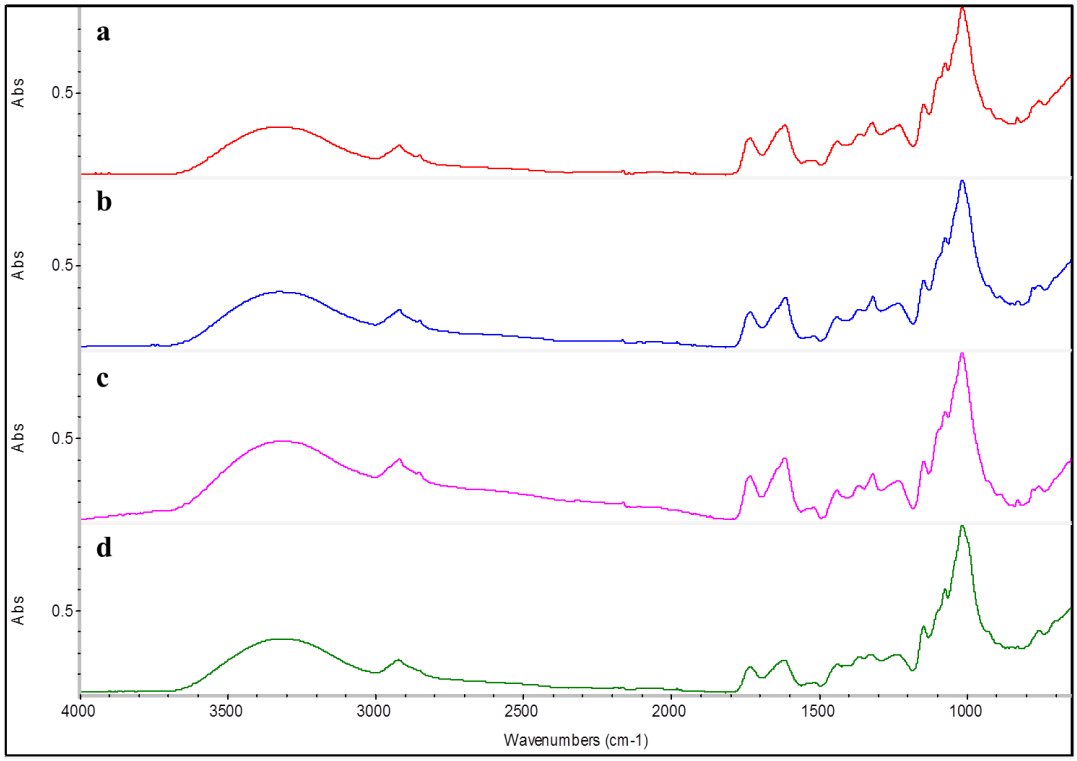

Figure E.1 FT-IR spectra of NBPP extracted with HCl solution pH 1.5 for 30 (a), 60 (b), 90 (c) and 120 (d) min at 90±5°C

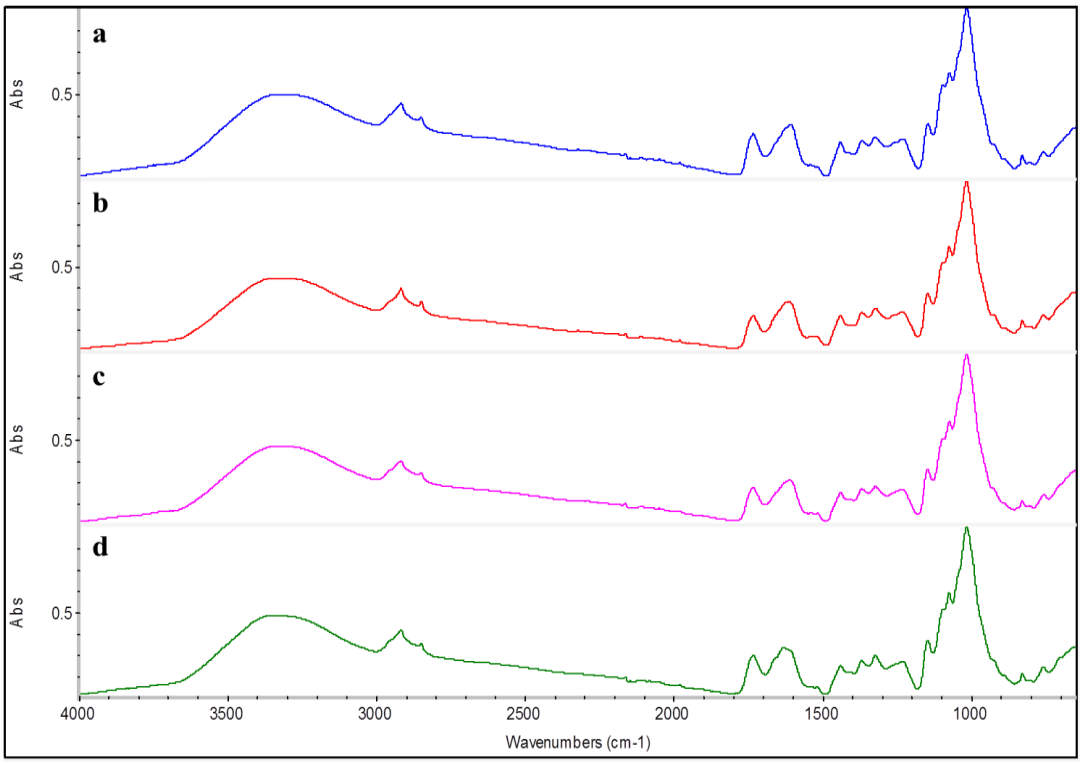

Figure E.2 FT-IR spectra of NBPP extracted with HCl solution pH 2.3 for 30 (a), 60 (b), 90 (c) and 120 (d) min at 90±5°C

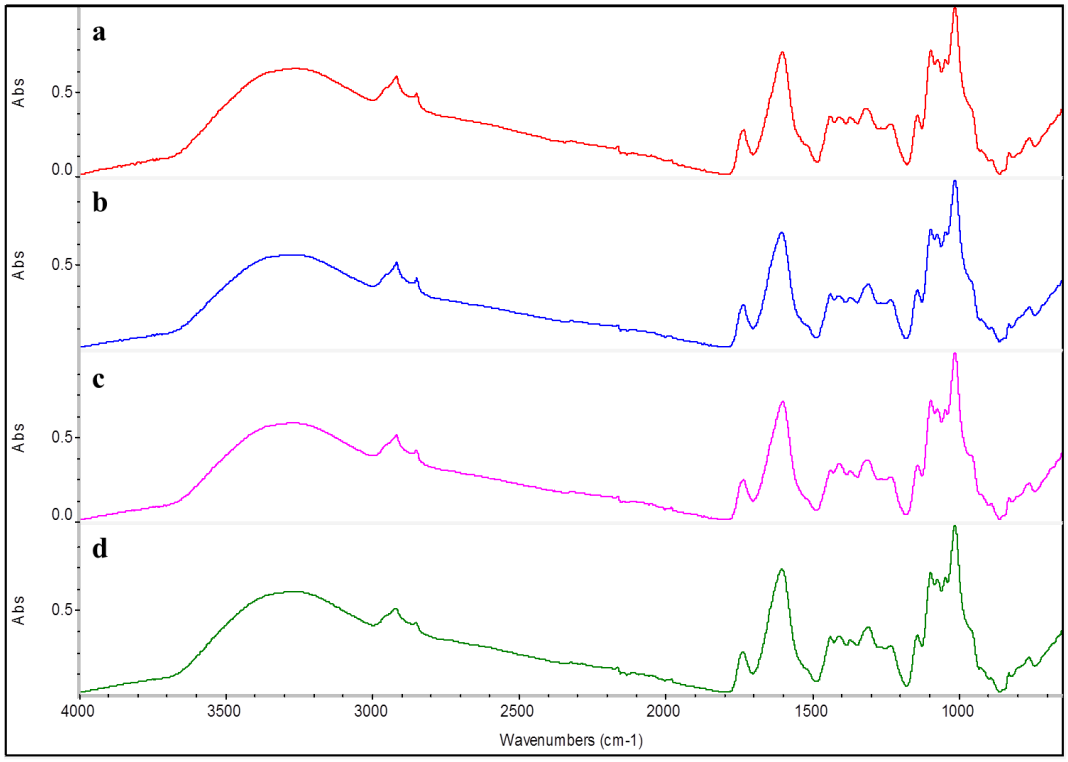

Figure E.3 FT-IR spectra of NBPP extracted with DI water pH 6.0 for 30 (a), 60 (b), 90 (c) and 120 (d) min at 90±5°C

Table E.1 Area of peaks used for degree of methylation determination from FT-IR spectra of NBPP

Extraction condition		**Peak area**[1]	
pH	**Time (min)**	**Peak I (1640-1620 cm^{-1})**	**Peak II (1760-1745 cm^{-1})**
1.5	**30**	20.10±1.10	12.07±0.70
	60	22.01±2.25	15.28±1.46
	90	29.14±2.97	15.75±0.81
	120	16.95±0.30	11.31±0.78
2.3	**30**	30.27±1.95	13.97±0.26
	60	31.74±2.21	10.91±0.67
	90	25.08±2.44	11.07±0.41
	120	16.49±1.43	8.98±1.42
6.0	**30**	53.38±0.80	10.09±0.26
	60	55.58±8.80	9.02±2.24
	90	63.97±4.23	11.59±0.12
	120	62.62±4.51	11.17±0.92

[1]Means ± standard deviation of triplicate samples

APPENDIX F

Data from intrinsic viscosity determination of NBPP

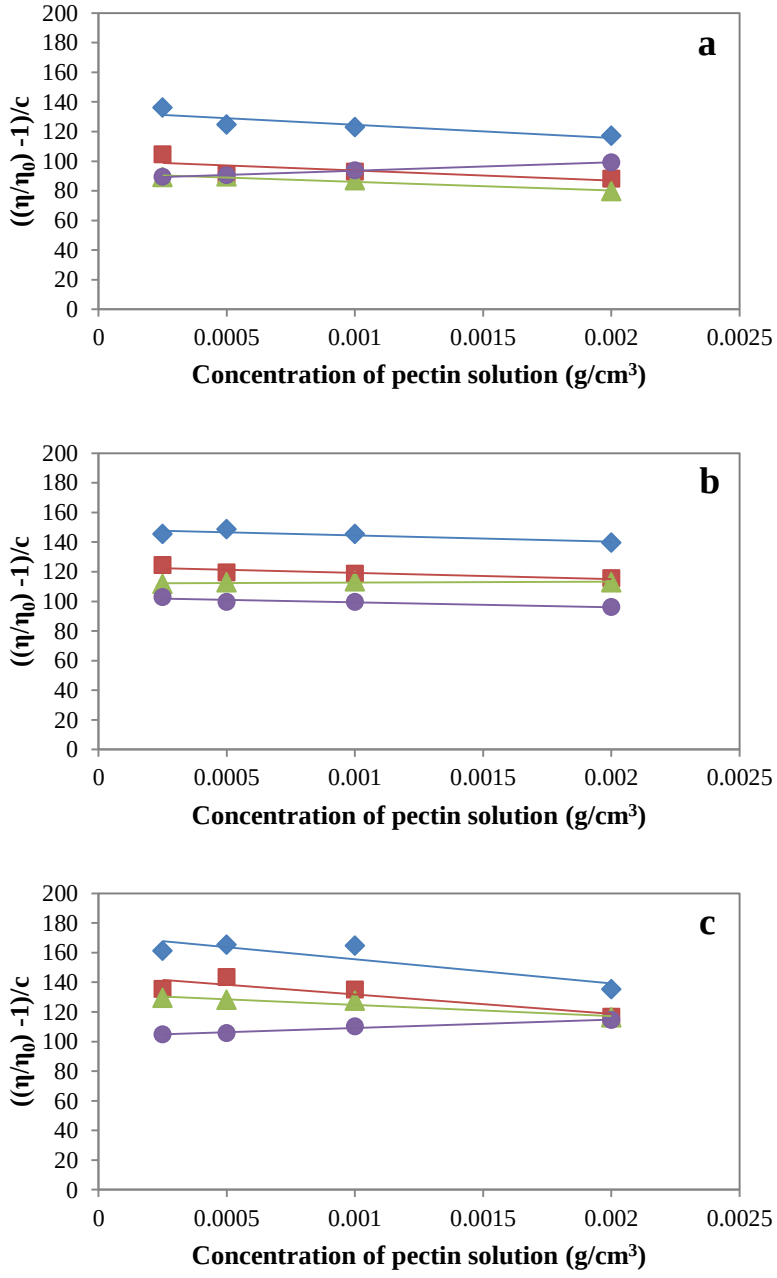

Figure F.1 Huggins plots of NBPP extracted with HCl solutions pH 1.5 (a), and 2.3 (b), and DI water pH 6.0 (c) for (♦) 30, (■) 60, (▲) 90 and (●) 120 min at 90±5°C

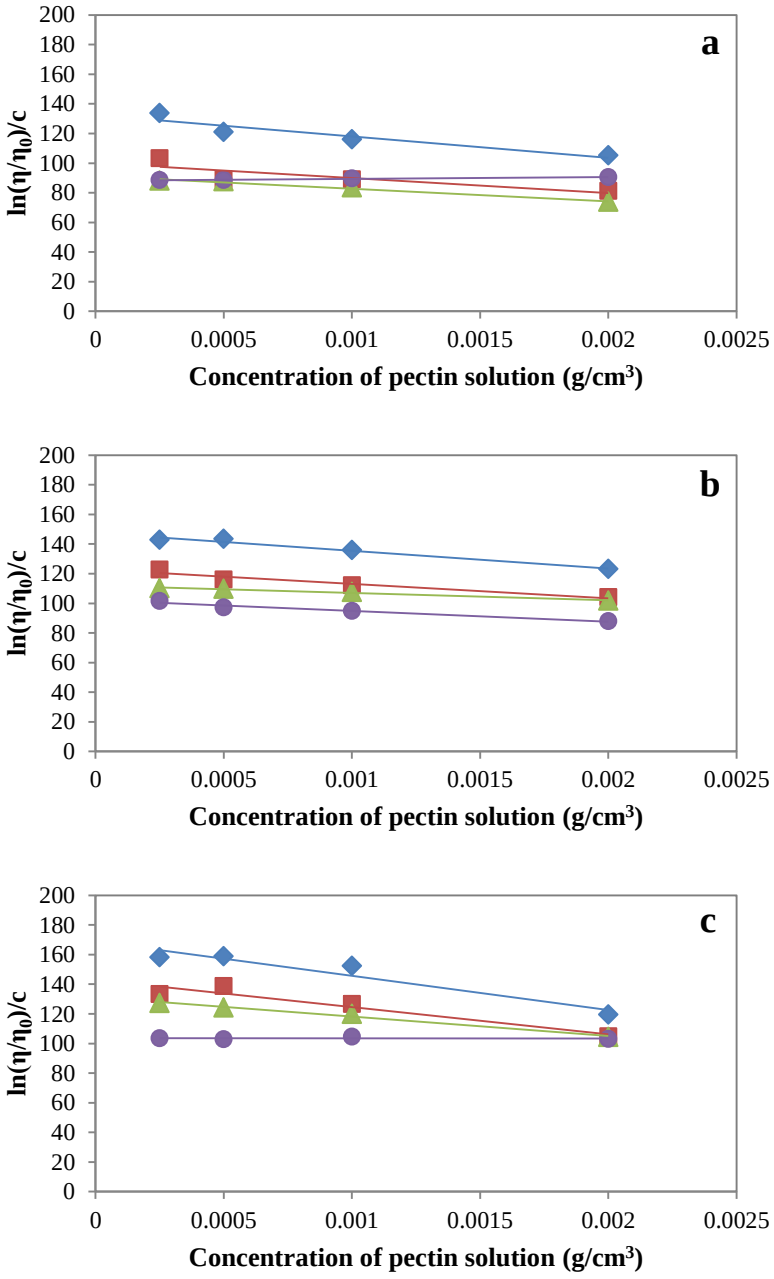

Figure F.2 Kraemer plots of NBPP extracted with HCl solutions pH 1.5 (a), and 2.3 (b), and DI water pH 6.0 (c) for (♦) 30, (■) 60, (▲) 90 and (●) 120 min at 90±5°C

Table F.1 Intrinsic viscosity ([η]) and Huggins constant (k_H) of NBPP from the Huggins plots

Extraction condition		**Huggins plot**		
pH	**Time (min)**	**[η] (ml/g)**	**k_H**	**R^2**
1.5	**30**	133.52	-0.50	0.7517
	60	100.59	-0.68	0.5356
	90	91.90	-0.70	0.9462
	120	87.98	0.73	0.9985
2.3	**30**	148.67	-0.19	0.7415
	60	123.50	-0.28	0.7840
	90	112.19	0.04	0.2849
	120	102.63	-0.31	0.8522
6.0	**30**	171.81	-0.55	0.7686
	60	144.95	-0.63	0.7974
	90	132.33	-0.43	0.9109
	120	103.4	0.54	0.9689

Table F.2 Intrinsic viscosity ([η]) and Kraemer constant (k_K) of NBPP from the Kreamer plots

Extraction condition		**Kreamer plot**		
pH	**Time (min)**	**[η] (ml/g)**	**k_K**	**R^2**
1.5	**30**	132.47	0.82	0.8826
	60	100.04	1.01	0.7185
	90	91.34	1.02	0.9841
	120	88.18	-0.15	0.9630
2.3	**30**	147.50	0.55	0.974
	60	122.82	0.65	0.9437
	90	111.88	0.39	0.9857
	120	102.20	-0.31	0.9636
6.0	**30**	168.84	0.81	0.9236
	60	142.99	0.90	0.9160
	90	131.13	0.76	0.9854
	120	103.54	0.01	0.0042

APPENDIX G

Data from rheological measurement of salad dressings containing 2% (w/v) solution of NBPP as fat replacer

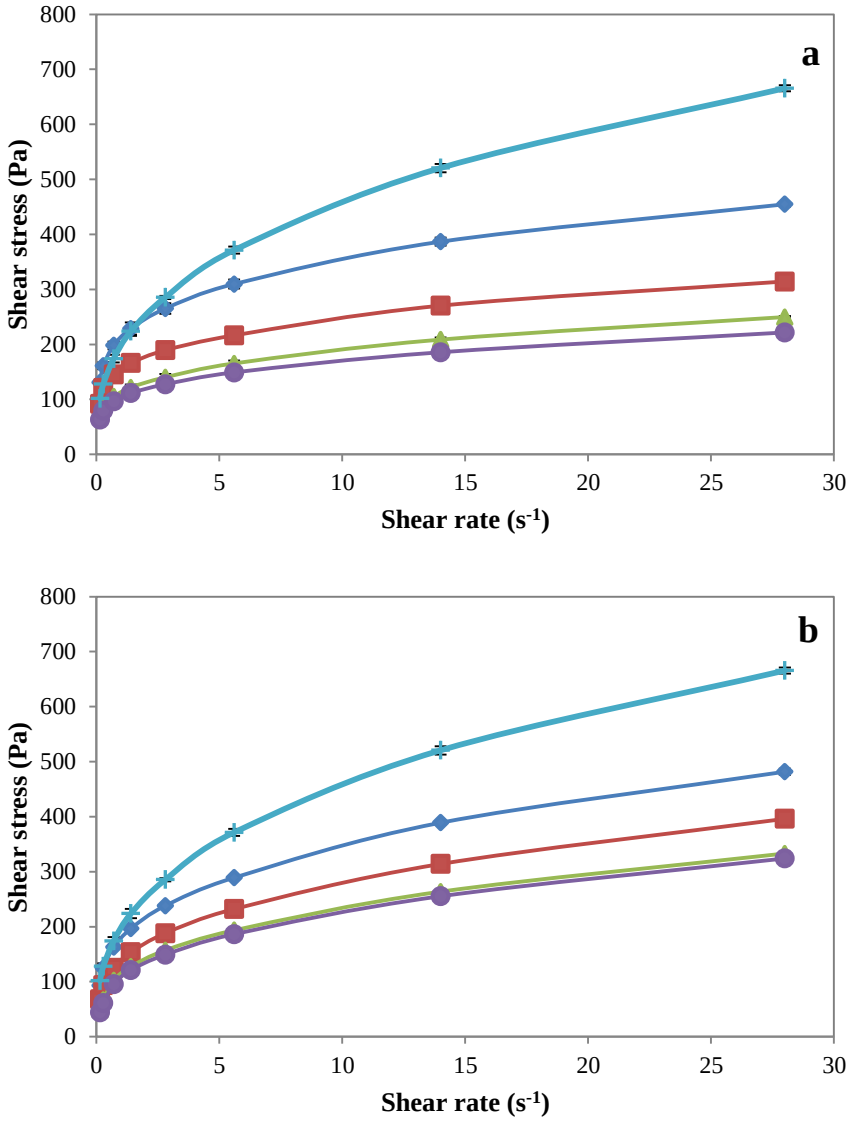

Figure G.1 Power law plots of salad dressings containing NBPP extracted with HCl solution pH 1.5 (a), and DI water pH 6.0 (b) for 60 min at 90±5°C as fat replacer at (+) 0, (♦) 25, (■) 30, (▲) 35 and (●) 40% oil substitution levels

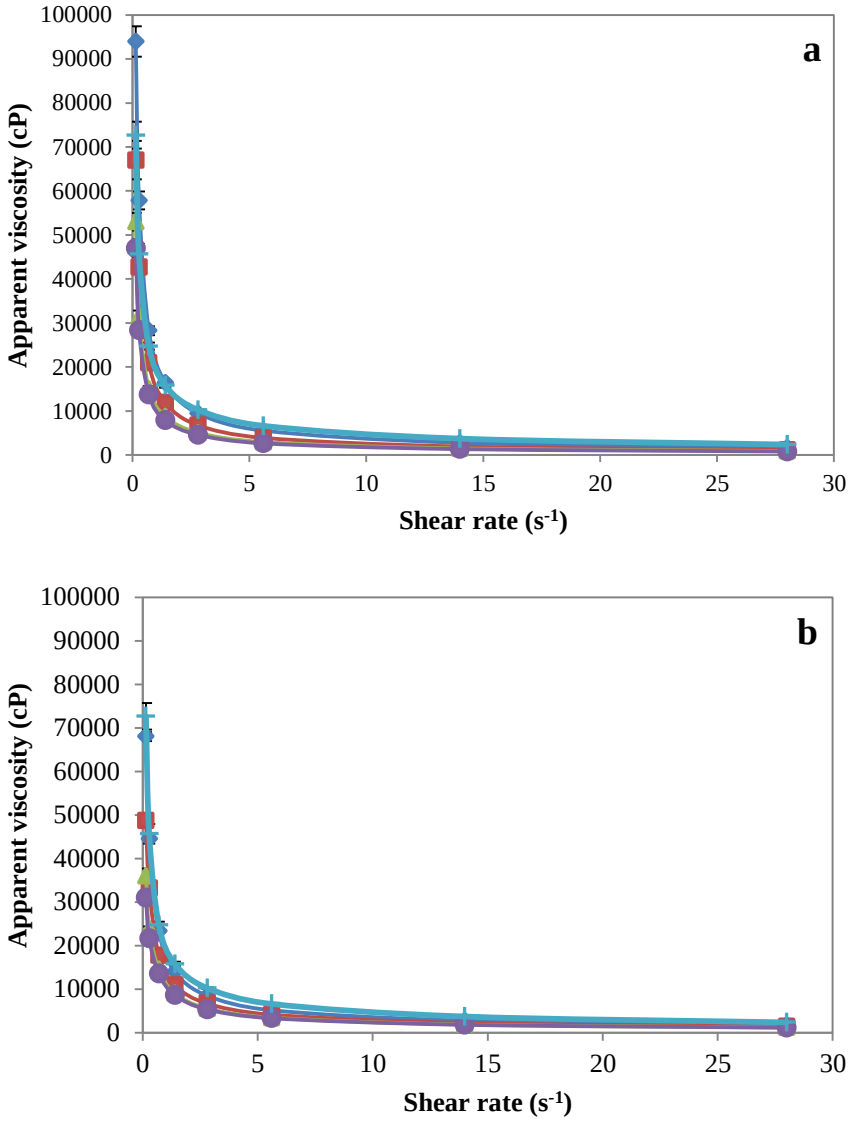

Figure G.2 Flow curve of salad dressings containing NBPP extracted with HCl solution pH 1.5 (a), and DI water pH 6.0 (b) for 60 min at 90±5°C as fat replacer at (+) 0, (♦) 25, (■) 30, (▲) 35 and (●) 40% oil substitution levels

BIOGRAPHY

NAME	Miss Nitjaree Maneerat
DATE OF BIRTH	26th February 1989
PLACE OF BIRTH	Nakhon Pathom, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 2007-2011 Bachelor of Science (Biotechnology) Mahidol University, 2011-2014 Master of Science (Food and Nutrition for Development)
WORK EXPERIENCE	Universal Food (Public) Co., Ltd., 2010: Summer Intern
HOME ADDRESS	46 Moo 1, Thaphraya, Nakhon Chaisi, Nakhon Pathom 73120, Thailand Tel.: +66(0)-8-7155-3917 E-mail: nitjaree.man@gmail.com

CHAPTER V
CONCLUSION

The present study demonstrated that Nam Wa banana (*Musa* (ABB group) 'Kluai Nam Wa') peels can be used as an alternative source for pectin production. Both the conventional, hot acid (pH 1.5 and 2.3) and the more environmental-friendly, water extractions at 90±5ºC could extract pectin from the cell wall of banana peel at appreciable yields (4-11% dry weight basis). The chemical properties of the extracted NBPP depended on the severity of the extraction condition, especially the pH of extraction. The stronger acidity contributed to the higher extraction yield and DM of the obtained NBPP, but reduced GalA and M_v of the pectin. NBPP extracted at acidic conditions contained 33-47% GalA and 52-61% DM. Water-extracted NBPP was larger in M_v than those extracted with acid (21-40 and 17-34 kDa, respectively) and thus provided more viscous solutions. The length of the extraction less affected the properties of pectin. The longer extraction time tended to lower the M_v of the extracted NBPP and the viscosity of their solutions. NBPP obtained from all conditions, except water extraction for 120 min, provided HMP gelling ability; while water-extracted NBPP, which contained 40% DM, did not formed LMP gel in the presence of Ca^{2+}. Water- and oil-holding capacities of NBPP were 4.2-5.9 g water/g pectin and 3.3-3.4 g oil/g pectin, respectively.

NBPP extracted with HCl solution pH 1.5 and with DI water pH 6.0 for 60 min at 90±5°C was selected to evaluate their potential food application according to the yield and functional properties. Food application of the extracted pectin was investigated by incorporated them as fat replacers in salad dressing. The reduced-fat products of which 30% of the fat was substituted with the acid- and water-extracted NBPP solutions were accepted by the panelists, even though the addition of NBPP decreased the measured viscosity and changed the color values of the dressing. All reduced-fat formulas were stable over 2 wk storage under room temperature.

Therefore, it can be concluded that both acid- and water-extracted NBPP have the potentials to use as functional ingredients in food product.

Suggestions for further study

The improved purity and functional properties of the extracted NBPP may be achieved by better controlling the variation of raw materials and modifying the pectin extraction method. Banana peels with the more consistent composition should result in less variation in the chemical properties of the obtained NBPP, although it is difficult to control the varieties and maturity of Nam Wa banana used as raw material. Modified extraction methods using a chelating agent, such as ammonium oxalate and EDTA, as an extractant should yield the pectin with high yield, GalA and molecular weight (12, 140).

According to their WHC and OHC, it is interesting to investigate the emulsifying properties of NBPP. Protein content, degree of acetylation and monosaccharide composition, which are the structural factors responsible for emulsifying properties of pectin (71), should be determined as well as the surface activity and the properties and stability of the pectin-stabilized emulsion. Such information will be useful to evaluate the potential application of NBPP as a natural emulsifier in food products.

Itharat and Sakpakdeejaroen reported that the extract obtained from boiling of ripe banana peel in water contained high amount of total phenolic compound with high antioxidant activity (39). Therefore, determinations of the phenolic compound and antioxidant activity of NBPP and also the developed RSD should be investigated. Despite the fact that the substances with antioxidative activity are likely to be more soluble in alcohol and hence may not much retain in the pectin, which precipitates in alcohol.

It is challenging to investigate more applications of NBPP as fat replacer in food product. Pectins have been reported to be successfully substituted the fat in various products such as cheese, spread, dressing, mayonnaise and baked products (89-92, 134, 135). The *in vitro* lipid digestibility of food containing NBPP solutions as fat replacer should also be determined by using simulative digestion to confirm the

physiological function of pectin in delaying the digestion and absorption of fatty acids in the gastro-intestinal tract (141).

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LIST OF ABBREVIATIONS

cm	centimeter
cP	centipoise
ºC	degree Celsius
DM	degree of methylation
DI water	deionized water
FT-IR	Fourier transform infrared spectroscopy
GalA	galacturonic acid content
g	gram
HMP	high methoxy pectin
h	hour
kcal	kilocalorie
kDa	kilodalton
LMP	low methoxy pectin
µg	microgram
µl	microliter
mg	milligram
ml	milliliter
mm	millimeter
mM	millimolar
min	minute
M	molar
mo	month
NBPP	Nam Wa banana peels pectin
nm	nanometer
OHC	oil-holding capacity
Pa	Pascal
RSD	reduced-fat salad dressing

LIST OF ABBREVIATIONS (cont.)

s	second
M_v	viscosity-average molecular weight
v/v	volume by volume
WHC	water-holding capacity
wk	week
w/v	weight by volume
w/w	weight by weight
y	year

CHEMICAL AND FUNCTIONAL PROPERTIES OF PECTIN FROM NAM WA BANANA (*Musa* (ABB GROUP) 'KLUAI NAM WA') PEELS AND ITS POTENTIAL FOOD APPLICATION

NITJAREE MANEERAT

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2014

Thesis
entitled

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……...
Miss Nitjaree Maneerat
Candidate

……...
Lect. Nattapol Tangsuphoom,
Ph.D. (Food Science)
Major advisor

……...
Asst. Prof. Anadi Nitithamyong,
Ph.D. (Food Science)
Co-advisor

……...
Prof. Banchong Mahaisavariya,
M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……...
Asst. Prof. Sitima Jittinandana,
Ph.D. (Animal and Food Sciences)
Program Director
Master of Science Program in
Food and Nutrition for Development
Institute of Nutrition
Mahidol University

Thesis
entitled

CHEMICAL AND FUNCTIONAL PROPERTIES OF PECTIN FROM NAM WA BANANA (*Musa* (ABB GROUP) 'KLUAI NAM WA') PEELS AND ITS POTENTIAL FOOD APPLICATION

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Food and Nutrition for Development)
on
February 28, 2014

……...
Miss Nitjaree Maneerat
Candidate

……...
Lect. Kanitha Tananuwong,
Ph.D. (Food Science)
Chair

……...
Lect. Nattapol Tangsuphoom,
Ph.D. (Food Science)
Member

……...
Asst. Prof. Anadi Nitithamyong,
Ph.D. (Food Science)
Member

……...
Prof. Banchong Mahaisavariya,
M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……...
Assoc. Prof. Visith Chavasit,
Ph.D. (Food Science)
Director
Institute of Nutrition
Mahidol University

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Nitjaree Maneerat

CHAPTER I
INTRODUCTION

1.1 Background and rationale

Banana is one of the popular crops in Thailand, with the estimated total crop production of 1,600,000 metric tons in 2011 (1). In response to its abundant supplies, a significant amount of banana produced is usually preserved and value-added by further processing into several products, e.g., dried banana and banana chips. The varieties normally used for processing are Cavendish (*Musa* (AAA group, 'Gros Michel') 'Kluai Hom Thong') and Nam Wa (*Musa* (ABB group) 'Kluai Nam Wa') bananas. With the fast growing of banana processing industry and the rising demands of processed banana products, there is a large quantity of banana peels wasted from such industry. In 2006, over 200 metric tons of banana peels is generated each day and tends to continually increase (2). Hence, the uses of banana peels would be beneficial both in reducing the amount and adding the value of industrial waste.

Pectin is a polysaccharide which is rich in galacturonic acid molecules that are joined together by α−1,4−glycosidic bonds. It is generally found within the cell wall of plants (3). Pectin is widely used as a food additive in food industry. It can be used as gelling, thickening and stabilizing agents for many food products, e.g., jam, jelly, juices, bakery products, and dairy products (4, 5). Moreover, it can be used as a fat replacer in various product, e.g., cookies (6) and low-fat frankfurters (7). Due to the fact that pectin cannot be produced locally in Thailand, it is imported up to 682 metric tons per year which costs more than 219 million baht (8). Consequently, other sources of pectin which can be locally produced have been investigated.

Pectin can be extracted from various fruits and vegetables as well as their processing wastes, e.g., apple pomace (9), citrus peels (10), sugar beet (11), mango peel (12), cacao pod husks (13), passion fruit rind (14), pomelo peel (15), lime peel (16), and soy hull (17). Although the extractions of pectin from banana and its peels, have been previously reported in other studies (18-21), the information on properties

and the food application of pectin from peels of banana, especially Nam Wa varieties, are still limited.

Therefore, this study aims to use Nam Wa banana peels, which is the waste from banana processing industry, as an alternative source for the local production of pectin with an emphasis to determine the chemical, and functional properties of Nam Wa banana peels pectin (NBPP) extracted by conventional method using acid solution and that obtained from the more environmental-friendly extraction using water. The potential application in food products of the obtained NBPP was also studied.

1.2 Research objectives

1.2.1 General objectives

To determine the chemical and functional properties of NBPP extracted under different conditions as well as their potential application in food product

1.2.2 Specific objectives

1. To determine the chemical and functional properties of NBPP extracted with acid solutions and water
2. To investigate the potential application of NBPP in food products

1.3 Expected outcome

1.3.1 Information on the chemical and functional properties of NBPP

1.3.2 Potentials to incorporate NBPP into food product as a functional ingredient

CHAPTER II
LITERATURE REVIEW

2.1 Nam Wa banana

Banana is a tropical fruit which is one of the important crops in the world. Total world production of banana is about 102 million metric tons in 2012 (1). Banana is also one of the popular crops in Thailand. In 2012, a significant percentage of the total banana crop in Thailand was 1.65 million metric tons that was held, which was the 13th of world ranking (1). There are various varieties of banana that can be cultivated in Thailand such as Hom Thong or Cavendish banana (*Musa* (AAA group, 'Gros Michel') 'Kluai Hom Thong'), Khai (*Musa* (AA group) 'Kluai Khai'), Nam Wa (*Musa* (ABB group) 'Kluai Nam Wa') and Hak Muk (*Musa* (ABB group) 'Kluai Hak Muk'). Each variety has different shape, size, appearance and physical characteristic (22).

Nam Wa varieties or Kluai Nam Wa or cooking banana is one of the popular banana varieties because it can grow easily in any parts of Thailand and can be harvested all year round. This variety has many sub-varieties which are "Nam Wa Daeng", "Nam Wa Luang", "Nam Wa Khao", "Nam Wa Dam", "Nam Wa Khom" and "Nam Wa Malee Ong" (23). Each sub-variety is differ in their physical properties such as color of core and peel, size, shape and peel thickness (22). **Figure 2.1** represents the typical appearance of Nam Wa banana.

Figure 2.1 Nam Wa banana (24)

The pulp of banana is commonly consumed as fresh and processed form. It contains high amount of carbohydrate and is rich in dietary fiber, vitamin C, potassium and manganese. Moreover, it is also a good source of magnesium (25). The ripe, mature banana is normally consumed as fresh form; whereas, unripe banana is usually cooked prior to consumption. For processed banana, yellow-green ripe or unripe banana is usually used in banana chips production, while yellow ripe banana are used for making other forms including dried, puree, stewed, marmalade, jam, flakes, confectionery and pastry ingredients, sorbets and ice creams (26). In Thailand, over 960 metric tons of processed bananas was exported and create more than 83 million baht of income to the country in 2009. Moreover, processed banana market is growing up increasingly (27) resulting in 200 metric tons of banana peel disposed from processed banana industry each day and tends to continually increase (2).

The chemical composition of Nam Wa banana peel is shown in **Table 2.1**. The main component of cooking banana peel is moisture which accounts for about 89% of fresh weight. The peel contains high dietary fiber, ash and fat (28). It is also the good source of lignin, cellulose and hemicellulose (18). The highest mineral content in cooking banana (*Musa* ABB) peel was potassium (50,815 mg/kg dry matter). Other minerals, e.g. phosphorus, calcium, magnesium, sodium, zinc, iron, and copper, are also found in banana peel (29). The banana (*Musa acuminata* Colla AAA) peel extract is also rich in bioactive compounds and exhibits a high antioxidant activity (30). Happi *et al*. reported the effect of stage of maturation to chemical composition of banana peel that soluble sugar content and the degradation of starch

increased while ripening due to the action of endogenous enzymes (29). Nevertheless, the stage of maturation did not affect to the amount of dietary fiber (18).

Banana peel can be used as an animal feed, fertilizer, substrate for biogas production, and also for wine and ethanol production (2, 25, 31-33). A previous study suggested that banana peel can be used as a biosorbent for removal of heavy metal in wastewater treatment (34). There are many studies reported that the peel of banana can be processed to obtain flour which contained high bioactive phenolic compounds while the functional properties of flour were still maintained (35-38). Itharat and Sakpakdeejaroen (39) reported that the extract obtained from boiling of banana peel in water contained high amount of total phenolic compound with high antioxidant activity. They suggested that it may be due to the activity of tannin in banana peels. Moreover, they also reported the possibility to use banana peels extract as anti-inflammatory products because it also showed anti-allergenic and anti-inflammatory effects (39).

Table 2.1 Chemical composition of Nam Wa banana peel

Compositions	**Content (g/100 g dry weight basis)**
Protein	8.60 ± 0.1
Fat	13.10 ± 0.2
Starch	12.78 ± 0.9
Ash	15.25 ± 0.1
Total dietary fiber	50.25 ± 0.2

Adapted from Wachirasiri *et al*. (28)

2.2 Pectin

Pectin is a complex of polysaccharide which containing units of D-galacturonic acid that generally found in the primary cell wall and middle lamella of plants. It plays an important role in giving physical strength of plant cell wall by associating with other polymers such as cellulose, hemicellulose and lignin (40). The structure of plant cell wall is shown as **Figure 2.2**.

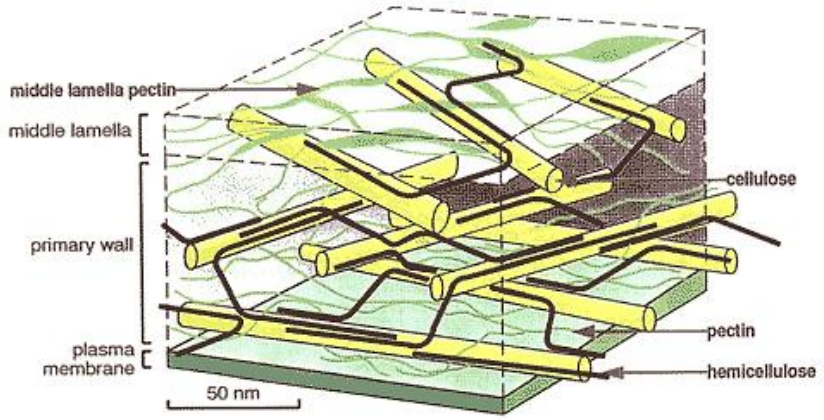

Figure 2.2 Plant cell wall structure (41)

2.2.1 Chemical structure of pectin

Pectin is a natural polymer which consists of polygalacturonic acid chains with α-1, 4 glycosidic linkages (**Figure 2.3**). It mainly contains three polysaccharide structures which are homogalacturonan (HGA), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). In addition to three majors polysaccharide domains of pectin, arabinogalactans, arabinans, and xylogalacturonan are also found in native pectin. The chemical composition and structure of pectin vary among plant source, environmental condition, plant maturity, and extraction mode (5, 40, 42).

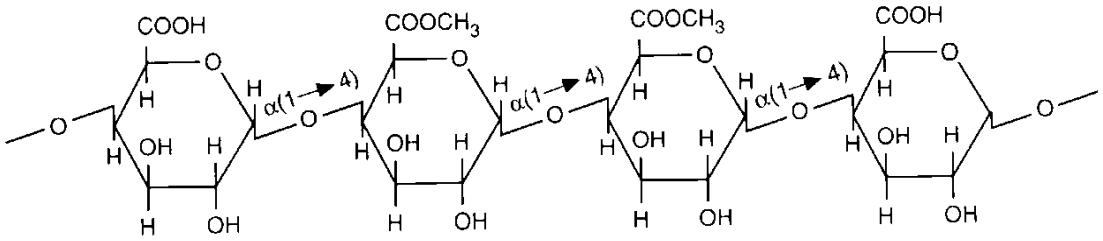

Figure 2.3 Galacturonic acids with α-1, 4 glycosidic linkage (43)

HGA is the linear backbone (smooth region) of pectin. It is composed of (1, 4)-α-linked-D-galacturonic acid and contains galacturonic acid unit around 100-200 units. The other region of pectin is hairy region which comprise of RG-I and RG-II. The RG-I domain is the HGA backbone which interrupted by (1, 2)-α-L-rhamnose residues. The most common sugars of RG-I are being galactose and arabinose. The structure of RG-II domain is more compact because it contains around 9 galacturonic acid units and other sugars , such as D-glucose, D-xylose, D-mannose, L-fucose, and glucuronic acid, are also sometimes found covalently linked to the backbone as side chains (**Figure 2.4**) (5, 40).

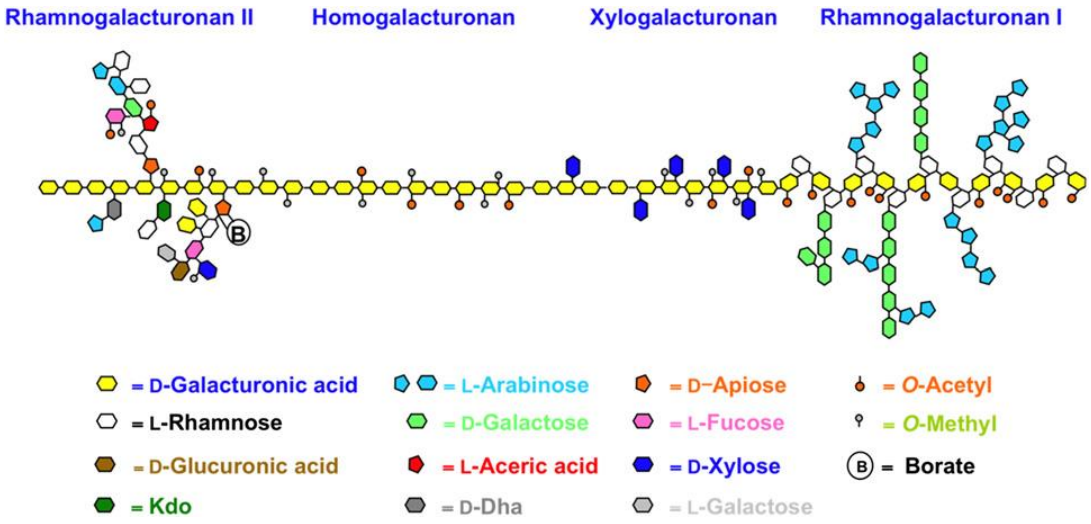

Figure 2.4 Structure of pectin (40)

Likewise, some carboxyl groups (C-6) on galacturonic acid backbone can be partially esterified by methyl group, and galacturonic acid units can be O-acetylated at O-2 and O-3 positions (**Figure 2.5**). The ratio of methyl esterified galacturonic acid groups to total galacturonic acid groups is represented as degree of methylation (DM). While, degree of acetylation is defined as the ratio of acetylated galacturonic acid groups to total galacturonan units (5, 40). The C-6 of methyl ester group of galacturonic acid can be converted to amide group by treating the pectin by ammonia during the production to reach amidated pectin with lower DM (**Figure 2.6**) (5).

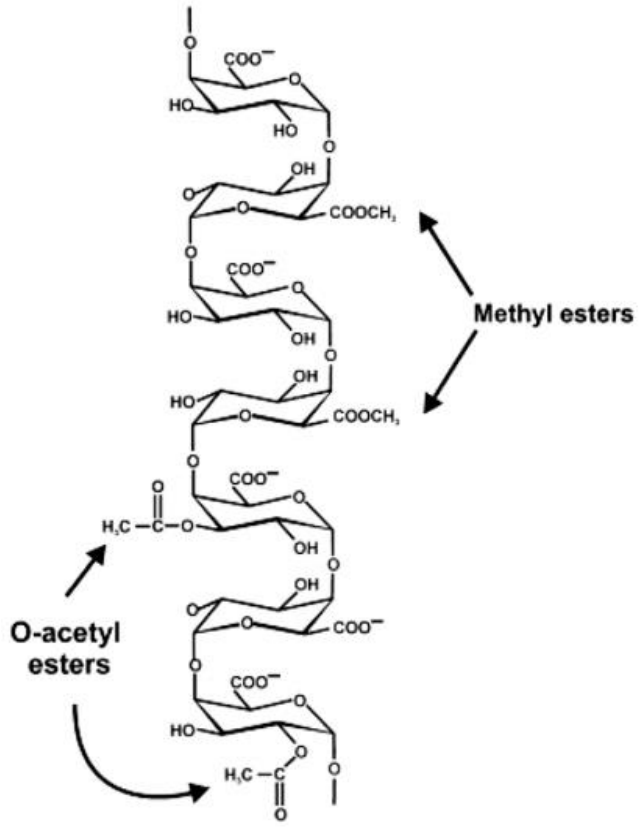

Figure 2.5 Homogalacturonan structure and modification (44)

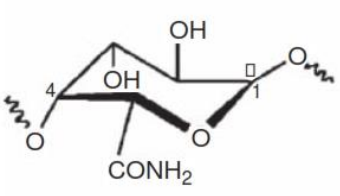

Figure 2.6 Amidated galacturonic acid unit (5)

2.2.2 Classification of pectin

Pectin can be classified into two categories by their DM which refers to percentage of methyl-esterified galacturonic acid units of the pectin chains.

2.2.2.1 High methoxy pectin (HMP) is pectin which was highly methylated. It contains DM of at least 50% (**Figure 2.7**).

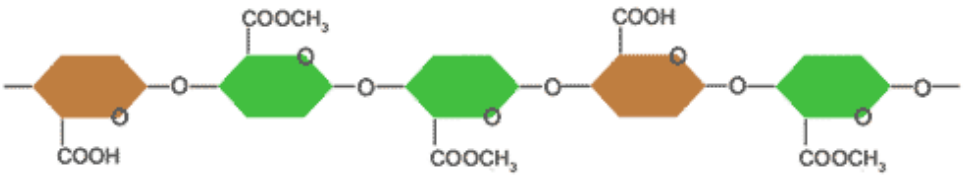

Figure 2.7 Structure of high methoxy pectin (45)

2.2.2.2 Low methoxy pectin (LMP) is pectin which contains less than 50% DM (**Figure 2.8**).

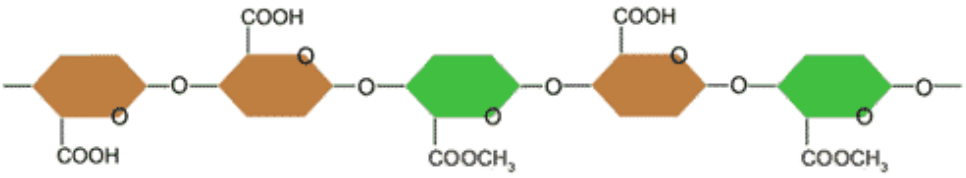

Figure 2.8 Structure of low methoxy pectin (45)

The different amount of methyl groups in polygalacturonic chains of HMP and LMP results in the differences in their properties such as gelling, solubility and rheological properties (42).

2.2.3 Pectin extraction

Pectin can be extracted from various raw materials by different extraction procedures and conditions depending on their sources. The characteristics and quality of pectin are arising from several extracting factors.

2.2.3.1 Source of pectin

Pectin can be extracted from various sources that influent its yield and characteristics. Apple pomace and citrus peel which are by-products from beverage manufacture are widely used for commercial pectin production. The estimated yield of pectin extracted from dried apple pomace ranges from 15-20% (42) with 50-65% GalA and 54.5-79.5% DM (46, 47). For dried citrus peels, their pectin yield is around 30-35% (42) and the GalA and DM is normally around 70%, and 75.5%, respectively (42, 48). Other materials, such as sugar beet pulp (11), sunflower heads (49), have also been used for pectin extraction in the regions where they are available (3). Besides, there are new interesting sources of pectin, including plants, wastes and by-products from agricultural industries, which are being investigated. Those alternative sources being reported are potato fibers, mango peels, cacao pod husks, pineapple skin, passion fruit peels, melon and watermelon rind, lime peels, pomelo peels, soy hull, okra pods and residues from guava, papaya, and coffee processing (12-14, 16, 17, 42, 50-53).

2.2.3.2 Process of pectin extraction

A procedure for commercial pectin extraction is illustrated as **Figure 2.9**.

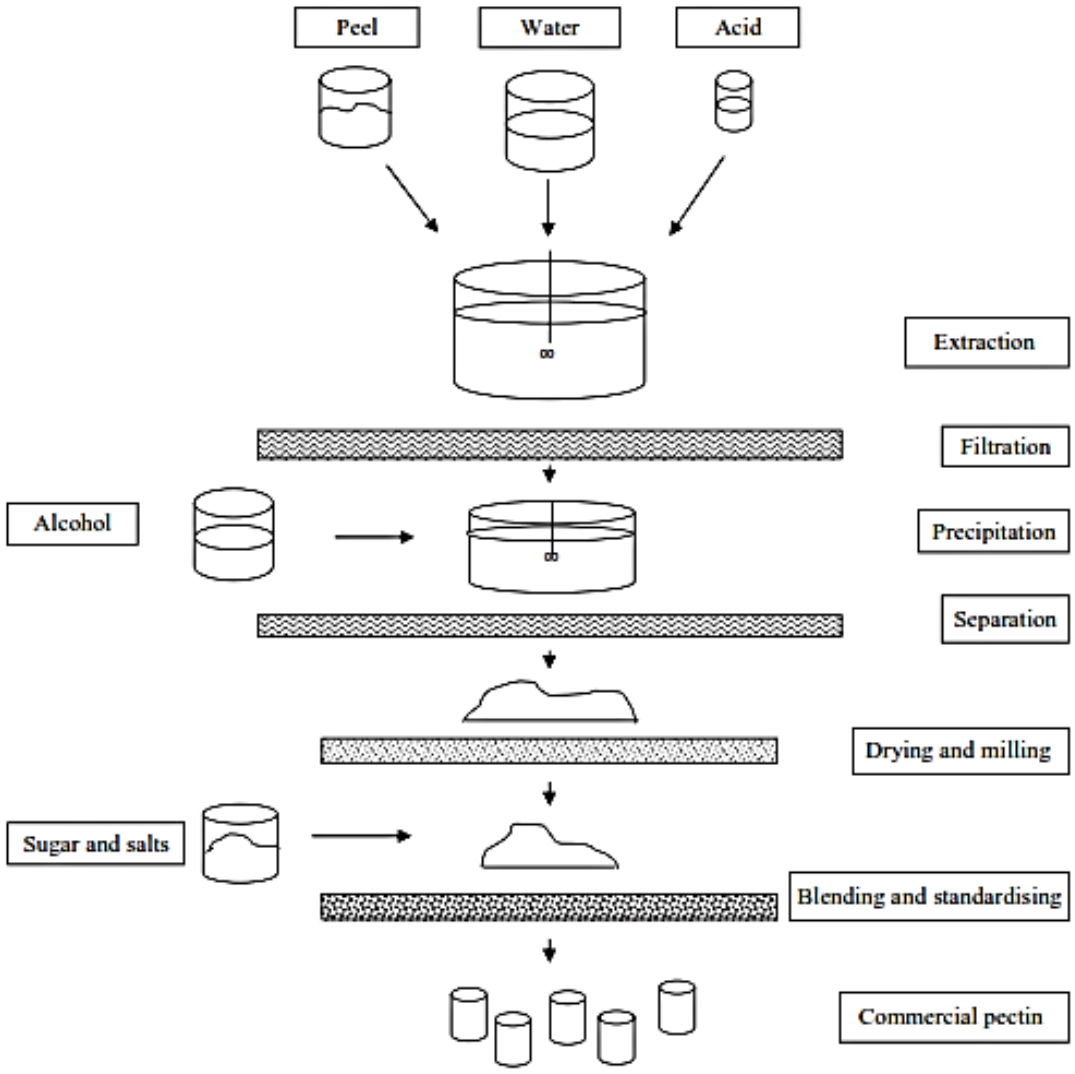

Figure 2.9 Commercial pectin production (5)

Before extraction, the raw materials are pre-treated, either by blanching, washing and drying, to inactivate pectin degrading enzymes and increase the stability of raw material during storage. Generally, commercial pectin is extracted under controlled pH values of 1.5-3 at high temperature (70-90°C) in hot solution of acid such as HCl, HNO_3 or H_2SO_4 to enhance the release of pectin from plant tissues. Under these conditions, depolymerization and methyl deesterification of the pectin are taken place. Low pH, high temperature, and long period of extraction time lead to high yield of pectin. After extraction, pectin is then separated from their residues by filtration or centrifugation. The waste from this step, which is mostly insoluble dietary

fiber, can be used as animal feed. The clear pectin solution is precipitated and washed with alcohol such as ethanol, methanol, and isopropanol. In industrial process, de-esterification is carried out in this step to obtain the pectin with desired DM. Then, the precipitate is pressed to remove soluble impurity, and finally dried and ground to obtain powdered pectin. Besides water-acid extraction, pectin can be extracted with alkali solution but its molecular weight is low because of pectin degradation via β-elimination. (3, 5, 42). However, the use of the chemicals for pectin extraction may cause environmental problems. Therefore, the more environmental-friendly methods have been taken into account for minimizing the use of detrimental chemicals. Those methods include enzymatic extraction with protease (54), hemicellulase (54, 55), carbohydrase (56, 57) and microbial enzymes (58, 59). Several studies have reported on the use of thermo-mechanical technology such as ultrasound (60, 61), autoclaving (62), and extrusion (63), as well as the combined physical-enzymatic extraction (64).

The method of pectin extraction could also affect the properties of pectin. The combined of physical and enzymatic treatments of apple pomace produced pectin with 632 mg/g GalA and 4.6% yield. Even though the pectin yield of physical/enzymatic treatments was lower than that of chemical extraction, the DM of pectin from such method (69%) is higher than chemically-extracted pectin (58%) (9). Moreover, the microwave- and ultrasound-assisted methods were also investigated for extraction of pectin from grapefruit. When compared to the conventional method, the use of microwave power gave the higher yield (27.8%), GalA (74.9%), DM (79.4%). While, the yield, GalA and DM of pectin extracted by using the ultrasound-assisted extractions, which are 17.9%, 68.2% and 75.1%, respectively, are lower than microwave and conventional method (61).

2.2.3.3 Extraction condition

The extraction condition significantly affects the yield, characteristics and properties of pectin.

A. pH

Many studies reported that different extracting pH led to the pectin with different properties. Pectin can be degraded rapidly by de-esterification and de-polymerization when it is exposed to unsuitable pH. Acid extraction at low pH values yields pectin with high DM. Under acidic condition, acid hydrolysis occurs rapidly than β-elimination, resulting in the lowering in molecular weight of pectin (65). On the other hand, pectin from alkali extraction contains low DM because of the saponification of ester groups. For the extraction under neutral and alkali conditions, pectin chains are degraded as a result of de-polymerization via β-elimination at C-4 position of methylated galacturonic acid units (5, 66, 67). The mechanism of β-elimination of pectin molecules is illustrated as **Figure 2.10**. For pectin from banana peels, the higher acidity of extraction has been reported to increase the yield but decrease GalA and molecular weight of the obtained pectin (19).

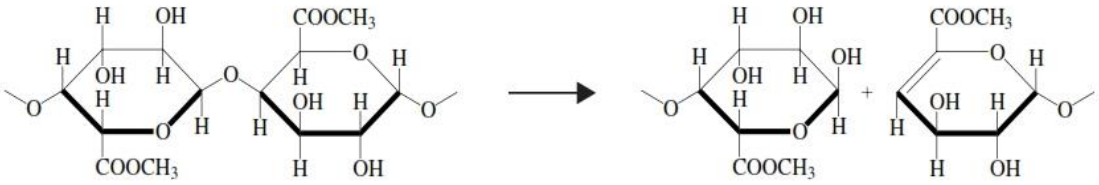

Figure 2.10 β-elimination of pectin molecules (68)

B. Extracting time

The yield of pectin extracted with long period of time is higher than that of pectin obtained from short time extraction, despite that the methoxyl content and equivalent weight of pectin are decreased due to partial degradation (14). For banana peels pectin extraction, it has been reported that the yield of pectin increased with the increasing extraction time while GalA was not influenced by extraction time. On the other hand, there were more methyl residues in pectin extracted for short period of time, suggesting the decrease in the content of de-esterified galacturonic acid when the extracting time was increased (19).

C. Extracting temperature

The characteristics of pectin are also arising from extracting temperature. High extracting temperature increases the pectin yield, while decreases the methoxyl content and equivalent weight of pectin (14). The elevated extracting temperature can accelerate the degradation rate of pectin chain and progressively increases the cleavage of glycosidic bonds of galacturonan backbone (68). Happi *et al.* reported that GalA of banana peels pectin was not influenced by extraction temperature. In contrast, the lower extracting temperature lead to the higher DM (19).

Consequently, the characteristic and properties of pectin depend on source of pectin and the extracting factors. The selecting and controlling the extracting factors during pectin production can be led to the desired characteristics of the extracted pectin.

2.2.4 Functional properties of pectin

The functional properties of pectin in food products are arising from its physicochemical properties. Major functional properties of pectin are gelling and rheological properties, while it also has a potential use as an emulsifier.

2.2.4.1 Gelling property

Pectin is widely used as a gelling agent in food industry because of its ability to form gel. Pectin gel strength generally increases when its molecular weight is higher. The most important factor for gelation of pectin is its DM. HMP and LMP have different conditions and mechanisms of gelling (42).

A. Gelling mechanism of high methoxy pectin

HMP requires low pH and water activity to form thermo-irreversible gel. The high amount (55-85%) of sugar or other soluble solids and acid condition (pH<3.8) is general condition for HMP gelation. Because high amount of sugar or soluble solid leads to low water activity condition, it promotes pectin-pectin interaction more highly than pectin-solvent interaction at the junction zone (**Figure 2.11**). Low pH also reduces the dissociation of carboxyl groups, so the electrostatic repulsion between pectin chains is decreased. The gelling mechanism of HMP depends on the hydrogen bonding between non-dissociated carboxyl and secondary alcohol groups, and hydrophobic interactions between methyl ester groups (5). Moreover, HMP is divided into several types based on its DM, setting time, and temperature. There are ultra-rapid set (74-77% DM), rapid set (71-74% DM), medium rapid set (66-69% DM), slow set (61-65% DM), and extra slow set (58-60% DM) pectin (5).

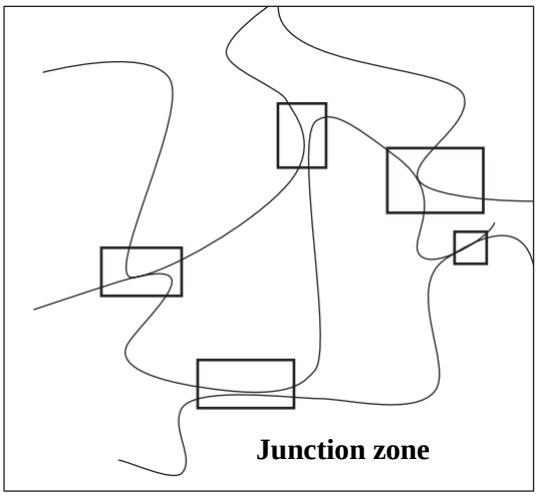

Figure 2.11 The gelling mechanism of high methoxy pectin (5)

B. Gelling mechanism of low methoxy pectin

The gelling mechanism of LMP is different from HMP because it requires divalent ions, usually calcium, and low pH of 3.5-4 to form gels. This mechanism is illustrated by the "egg-box" model which shown in **Figure 2.12**. The main pectin chain has two-fold symmetry thus the divalent cations and pectin molecule together can form the series of electronegative gaps by different affinities. The dimers of polygalacturonic chains are formed by ionic interactions between the cations and the free carboxylic groups on the pectin backbone. Due to the interaction between cations and de-esterified galacturonic acid units, the lower DM form gel (5).

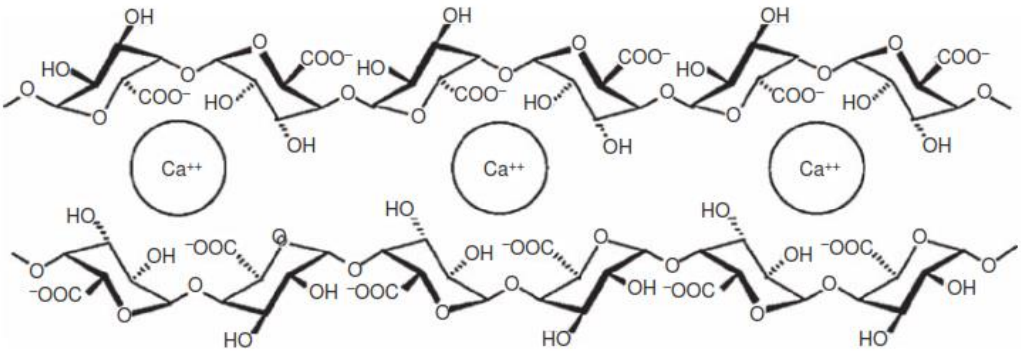

Figure 2.12 The gelling mechanism of low methoxy pectin: "egg-box" model **(5)**

2.2.4.2 Rheological property

Pectin provides a viscous solution similarly to other water-soluble polymers. However, pectin solutions are relatively low in viscosities compared to other hydrocolloids. The viscosity of pectin depends on its concentration, molecular size, conformation, solvent, pH, temperature and the presence of salts.

In general, the viscosity increases with the increasing pectin concentration, DM, and molecular weight. Pectin solutions exhibit Newtonian behavior at lower concentrations (<0.5%), in contrast, shear-thinning and non-Newtonian behavior is found in the solutions of higher concentrations of pectin. When the shear is reduced or stopped, pectin solution retrieves its viscosity (5).

Pectin with high molecular weights and rigid molecules exhibits higher viscosity than the compact and low molecular weight pectin. The intrinsic viscosity can be used to determine molecular weight of polymers including pectin. The viscosity of dilute solutions of a polymer depends on the dimension of the polymer chain (69).

Due to the fact that pectin is polyelectrolyte, pH and ionic strength could affect the viscosity of pectin solution due to charge shielding of the polymer chain. In a good solvent, such as NaCl solution, the pH and degree of ionization of the pectin decreases because the carboxylic groups on pectin chain are charged by the cation and thus suppresses the repulsive force between the pectin chains. So, the pectin solution exhibits lower viscosity (5, 42). In contrast, viscosity of pectin solution increases with the addition of divalent cations, such as calcium due to the association of pectin chains and calcium ions to form the structure known as “egg-box” model.

The co-solute, such as sugar, also affects the viscosity of pectin solution. Kar and Arslan reported that different types of sugar added to pectin solution significantly affect the solution’s viscosity. Dextrose and maltose increase the viscosity of pectin solutions since they can decrease the dielectric constant of the solvent, the dehydration action, and the formation of hydrogen bonding. Whereas, the effect of ionic impurity in dextrin preparation led to the decrease in viscosity of pectin solution (70).

2.2.4.3 Emulsifying property

Even though the pectin is not generally used as an emulsifying agent, there are some studies reported about the emulsifying properties of pectin. The acetyl groups enhance the hydrophobicity of pectin and hence promote its surface activity. So, the highly-acetylated pectin can act as an interfacial agent in oil-in-water emulsions (5, 71). Moreover, the depolymerized citrus pectin with a low molecular weight (about 60-70 kDa) and a high DM (70%) showed appreciable emulsifying properties at pH 4.7. Akhtar *et al.* explained that the obtained emulsifying properties resulting from hydrophobic/hydrophilic balance of depolymerized pectin that was modified to a proper ratio and the molecular structure that was changed. Additionally, the appropriate molecular size of the depolymerized pectin might enhance the rapid adsorption of the protein to the oil-water interface during emulsification and prevent the droplet flocculation caused by pectin bridging (72). Leroux *et al*. suggested that the acetyl groups may reduce calcium sensitivity which can cause a flocculation that contributes to the instability of emulsion. The combination of hydrophobic acetyl groups and protein also plays a key contribution to the emulsifying properties of pectin (73).

2.2.5 Physiological function of pectin

Pectin exhibits a wide range of physiological and nutritional effects to human health. Due to the fact that pectin is a soluble dietary fiber which is not digested by enzymes in human body, it is not absorbed in gastrointestinal tract. It can prevent diarrhea and constipation, and improve the intestinal health. The study of Parker *et al.* reported the gut health benefit of kiwifruit pectin that it enhanced the adhesion of *Lactobacillus rhamnosus* and decreased the adhesion of pathogenic bacterium more than inulin which is a prebiotic fructo-oligosaccharide (74). There are some evidences suggested that pectin may have an antimicrobial effect on *Echerichia coli* under a certain *in vitro* condition (75). According to its high water binding capacity, pectin can increase satiety after being consumed, leading to the reduction of energy intake (76). Pectin affects carbohydrate and lipid metabolisms, and also the absorption of other nutrients. Several studies reported that a meal fortified with pectin can delay the gastric emptying but have no impact on the post-prandial glucose

response in healthy adults (77-80). Jenkins *et al.* demonstrated that the addition of pectin in carbohydrate-containing meals can reduce postprandial blood sugar and the level of serum insulin after consumption in diabetic patients (81). It is partly due to the decrease in diffusion rate of available carbohydrates to the absorptive mucosal surface (76). Pectin can lower and maintain normal blood cholesterol concentrations in human. Many studies suggested that the consumption of pectin at least 6 g/d reduced total cholesterol, and higher amount of consumption at 10-50 g/day reduced low-density cholesterol and very low-density cholesterol, as well as the low-density to high-density cholesterol ratio (68, 78). There are some studies showed that pectin can reduce risks of cancer. Zhang *et al.* reported that sweet potato pectin exhibited antiproliferation effects on human colon cancer cells and human breast cancer cells (82). Citrus pectin also has been reported to inhibit the proliferation of two-colon carcinoma and an erythroleukemia cell lines (83). Another study reported the inductive effect of pectic rhamnogalacturonan from okra pods on apoptosis in melanoma cells (84). Pectin was also proven to have an effective ability to remove mercury and lead from gastrointestinal tract and respiratory organs. The urinary excretion of lead is probably due to the binding of lead cations to monomeric galacturonic acid which was produced by enzymatic degradation (85).

2.2.6 Food application of pectin

Pectin is widely used as a food additive in food industry. It can use as gelling, thickening and stabilizing agents. DM is the most important property of pectin for chooses type of pectin in food application. The traditional application of pectin in food industry is as a gelling agent in jam and jellies which the gelling ability of HMP and LMP. HMP is suitable for high-sugar jam and jelly because it needs high amount of sugar and low pH to form irreversible gel, while LMP is ought to use for low-sugar product because it forms heat-reversible gel at low level of sugar in the presence of calcium ions. LMP is also used for glazing in jams and jellies as a result of its heat-reversible gel property. Pectin is used as a thickener because of its thixotropic behavior and it can improve mouthfeel in food system. For beverage production, HMP can inhibit aggregation of casein on heating in acidified milk products, drinkable yoghurt, blends of milk, and it also used in fruit beverages to restore the mouthfeel of

the product due to it has viscosity and mouthfeel properties. In yoghurt production, small amounts of LMP increase firmness, mouthfeel and creaminess through excellent water-binding ability, calcium reactivity and interaction with milk proteins. Moreover, the stabilizing property of pectin is useful in multiphase systems for stabilization of emulsion, suspension, and foam. Due to the thickening and stabilizing properties of HMP and LMP, they can be partly of fully replaced fat content in miscellaneous foods, i.e. salad dressings, fruit and vegetable sauces, spreads, mayonnaise, and ice cream, and other food products such as process meats, desserts and bakery products. Pectin provides viscosity in food, and prevents phase separation by acts as an interfacial agent in oil/water and air/water system due to the acetyl groups in pectin chain which enhance the emulsifying property of pectin. Furthermore, pectin is interested in bakery production. As the excellent water-binding property of pectin, it is used to increase bread volume and improve moisture retention during storage to ensure a soft and spongy of end product. (5, 42, 73, 86-88) In low-fat products, pectin is used as a fat-substitute where it binds water and consequently improves the emulsion stability. The study of Cheng *et al.* shows the possibility to use the combination of fish gelatin and medium set pectin in low-fat spread (89). The use of pectin as fat replacers in low-fat cheese production can retain the texture, rheology and sensory characteristic of the product (90, 91). The study of Angpanitcharoen reported that the combination of pectin and modified starch can be used as carbohydrate-based fat replacers in low-fat and cholesterol-free spoonable salad dressing which maintained the acceptability from the panelists (92).

2.3 Pectin from banana peel

Due to the chemical composition of banana peel of which the dietary fiber content is high, the extraction of dietary fiber, especially pectin, from the peel has been investigated in a number of studies. One of them reported that the yield of pectin from banana (*Musa* AAA) peels varies from 10-21% dry matter. It was divided into water-soluble pectin (2.2-4.1%), chelating-soluble pectin (2.8-3.7%) and acid-soluble pectin (4.8-13.9%) depending on the extraction condition. GalA, DM, degree of

acetylation and molecular weight of pectin from banana peels is about 40-69%, 41-70%, 0.7-4.1% and 252-573 kDa, respectively (18). This study is also indicated that the greatest yield of pectin was found from banana peels at the 5^{th} stage of ripening (18). The study of Duan *et al.* reported that the decrease in pectin yield during ripening was due to the solubilization and depolymerization of pectin polysaccharides in plant cell wall (93). Main significant factor affecting the yield and composition of pectin from banana peel is pH of the extraction. The lower pH increases the pectin yield, in contrast, it decreases the GalA and molecular weight of pectin (19).

2.4 Fat replacer

Fat plays an important role in sensory characteristic of food products. It contributes flavor, palatability, appearance, texture, mouthfeel, creaminess, thickness, and lubricity of the product (94, 95). In reduced- or low- fat products, fat can be reduced or replaced by traditional techniques such as directly removing fat from the formula, substituting water or air for fat, and changing processing method from frying to baking or frying under vacuum. Moreover, the fat can be replaced or substituted by lipid-, protein- or carbohydrate-based ingredients known as fat replacers (96). There are two terms used for describing the ingredients which can replace fat in food products: (96).

- **Fat substitute** is a macromolecule which intends to theoretically replace fat content in food product on weight-to-weight basis. It usually has a similar chemical and physical structure to fat, stable at cooking and frying temperature, and resistant to hydrolysis by degradation enzymes (94, 96).

- **Fat mimetic** is a compound that imitates organoleptic or physical properties of fat. It requires a substantial amount of water to provide the functionalities and it cannot replace fat on weight-to-weight basis. Protein- and carbohydrate-based fat replacers or their combinations are commonly used as fat mimetics. Fat mimetics

are susceptible to digestion by digestive enzymes and hence provide caloric value ranged from 0-4 kcal/g (94, 96).

Fat replacers can be classified by the types of macronutrient into three main categories, i.e. protein-, carbohydrate-, and lipid-based fat replacer. Each fat replacer provides a variety of functional and sensory properties. The applications and general functions of the selected fat replacer in some food products are listed in **Table 2.2**.

Table 2.2 The applications and general functions of fat replacers (95)

Specific application	**Fat replacer**	**General functions**
Baked products	Protein-based	Texturize
	Carbohydrate-based	Retain moisture, retard staling
	Lipid-based	Emulsify, provide cohesiveness, tenderize, carry flavor, replace shortening, prevent staling, prevent starch retrogadation, condition dough
Frying	Lipid-based	Texturize, provide flavor and crispiness, conduct heat
Salad dressing	Protein-based	Texturize, provide mouthfeel
	Carbohydrate-based	Increase viscosity, provide mouthfeel, texturize
	Lipid-based	Emulsify, provide mouthfeel, hold flavorants
Frozen desserts	Protein-based	Texturize, stabilize
	Carbohydrate-based	Increase viscosity, texturize, thicken
	Lipid-based	Emulsify, texturize
Margarine, spreads, butter, shortening	Protein-based	Texturize
	Carbohydrate-based	Provide mouthfeel
	Lipid-based	Provide spreadability, emulsify, provide flavor and plasticity
Dairy products	Protein-based	Stabilize, emulsify
	Carbohydrate-based	Increase viscosity, thicken, aid gelling, stabilize
	Lipid-based	Provide flavor, body, mouthfeel, and texture; stabilize, increase overrun
Soups, sauces, gravies	Protein-based	Texturize
	Carbohydrate-based	Thicken, provide mouthfeel, texturize
	Lipid-based	Provide mouthfeel and lubricity

2.4.1 Protein-based fat replacers

Protein-based fat replacers are derived from a variety of protein sources including egg, milk, whey, soy, gelatin, and wheat. Microparticulated protein is one of protein-based fat mimetics which forms microscopic coagulated round deformable particles that can mimic the sensory function of fat. Some fat mimetics are modified to achieve other functionalities, such as water binding and emulsifying properties. It is generally used in dairy products, salad dressing, mayonnaise, sauce, frozen dessert, cheese, butter and margarines, but it is not suitable for frying products (96).

2.4.2 Carbohydrate-based fat replacers

Carbohydrate-based fat replacers are widely used to partially or totally replace fat content in many food products. There are a few caloric values provided from non-digestive carbohydrates, while digestible carbohydrates such as modified starch and dextrins provide 4 kcal/g. Most of carbohydrate-based fat replacers are used as thickening, stabilizing, gelling and bulking agents. Due to their high water binding capacities, gums, starches, pectin, cellulose and other carbohydrate can maintain the functions of fat and also provide the viscosity, texture, mouthfeel, and opacity of the reduced- and low-fat products. Corn syrups, syrup solids, high-fructose corn syrups and various types of polyols can be used to control water activity of many reduced-fat products. According to the properties of carbohydrates, it is not suitable for frying but can be used as fat barrier in fried and baked food (95, 96).

2.4.3 Lipid-based fat replacers

Lipid-based fat replacers are synthetic substances produced by reengineering, redesigning, chemically altering or synthesizing from the conventional fats and oils to retain the physical and functional properties of fat. The physical and chemical properties of these compounds are similar to triacylglycerols. These chemically-altered lipids provide few or no calories because they are not hydrolyzed and absorbed in gastrointestinal tract. Due to the thermal stability of lipid-based fat substitutes, they are widely used in frying or high-temperature cooking and also in desserts and dairy products (97).

2.5 Salad dressing

Salad is a popular menu that can promote vegetable and fruit consumption. Salad dressing is an oil-in-water emulsion used to enhance the palatability, desirability and flavor of salad (98).

2.5.1 Types of salad dressing

There are three main categories of salad dressing, including mayonnaise, spoonable and pourable salad dressings, which are classified by their fat contents regardless of the ingredients. The US regulations identified that mayonnaise should contain at least 65% vegetable oil by weight. The spoonable salad dressing generally contains 35-50% oil, while pourable salad dressing may contain less fat contents (99). The spoonable and pourable salad dressing are also different in their rheological characteristics. The high fat content of spoonable salad dressing leads to a strong plastic-like characteristic that a spoon must be used to remove the product from the container; whereas the pourable product can flow when being poured from the bottle (98). However, starch and gums are sometime added as a thickening agent to maintain the texture, body and stability of the products. The Thai Industrial Standard requires that mayonnaise should contain at least 65% fat with no sugar added, while salad dressing should contain fat in the range of 30 to 65% by weight (100).

Since salad dressing normally contains high amount of fat, attempts have been made to formulate the products with lower fat content. However, the removal of their fat contents can cause the changes in texture, lubricity, stability, color, flavor and stability of the dressings. Therefore, fat substitutes or fat mimetics are added in the reduced-fat or fat-free salad dressing to maintain the texture, organoleptic attributes and appearances of the final products. Various types of fat replacer are used such as gums, maltodextrins, dextrins, starch, modified starch and pectin. Each fat replacer provides different functional properties in salad dressing. Lipid-based fat replacers also be used in reduced-fat salad dressing, the non-digestive fat-like molecules or some designed triacylglycerols can provide the quality attributes of the conventional fat. According to the Notification No. 182/2541 of the Ministry of Public Health of Thailand, a fat substitution level of at least 25% of the total fat or energy from the regular formula needs to be achieved for being labeled as reduced- or lower-fat, while

low-fat product needs to contain total fat equal or less than 3 g, or provide energy equal or less than 40 kcal per serving (~30 g). For light product, it needs to reduce the total fat content at least 50% from the regular formula that provide energy from fat at least 50% of total energy, or reduce energy at least 1/3 of regular formula that provide energy from fat less than 50% of total energy. Free- or non-fat product needs to contain total fat less than 0.5 g or provide energy less than 5 kcal per serving. (101). **Table 2.3** shows the amount of fat content in various type of salad dressing.

Table 2.3 Typical fat contents of mayonnaise and salad dressings (98, 99)

Product	**Approximate fat content (% w/w)**
Regular mayonnaise	75-84
Reduced-fat mayonnaise	50
Light mayonnaise	25
Low-fat mayonnaise	20
Fat-free mayonnaise	0
Spoonable salad dressings	30-60
Reduced-fat dressings	22-45
Light dressings	15-30
Low-fat dressings	5-10
Fat-free dressings	0
Italian dressings	50-60
Italian dressings (low calorie)	30
French dressings	36-40
Thousand Island dressings	30-45
Russian dressings	30-40

2.5.2 Salad dressing ingredients

The interactions between the ingredients of salad dressing affect the physical and chemical properties and the quality of the products. The main ingredients of salad dressing are vegetable oil, emulsifying agent, acidifying ingredient, flavoring ingredients and thickening agent.

2.5.2.1 Oil

Oil is the most important ingredient in salad dressing. It plays an important role in food emulsion to provide body, texture, appearance, lubricity and flavor. Since salad dressing is an oil-in-water emulsion, the oil present in the dressing as minute droplets thoroughly dispersed in the aqueous phase. The oil also affects the shelf life of the product because the oil containing more unsaturated fatty acids is more prone to oxidation which is the cause of rancidity. The edible triglyceride oils derived from plant sources, i.e., soybean, canola, sunflower seed, cotton seed and olive, are commonly used in salad dressing formulation (102).

2.5.2.2 Emulsifying agent

An emulsifying agent or emulsifier is a surface active material that can reduce the surface tension between oil and aqueous phase due to its amphiphilicity (containing both hydrophilic and hydrophobic parts). An emulsifier can align itself at the interface between oil and water in food emulsion and hence prevent the coalescence of oil droplets (103). Egg yolk, lecithin, dairy and vegetable proteins, gum arabic, guar gum, polysorbates, propylene glycol alginate, pectin and mixture thereof can be used as emulsifiers in salad dressing (99, 102). Egg yolk is a natural emulsifying agent which is generally used in salad dressing formulation. The major components of egg yolk are lipoproteins, phospholipids and cholesterols. The lipoproteins form the interfacial film surrounding oil droplets. Lecithin which is phospholipid also contributes the emulsifying ability of egg yolk in salad dressing (99).

2.5.2.3 Acidifying ingredient

The addition of acidifying ingredient in salad dressing is mainly for its function as flavoring agent and preservative. The lower pH resulted from acidifying agent can preserve the product from microbial spoilage. Salad dressing is an acid food with typical pH value of 4.1 or lower (100). The edible acids which can be used in the formulation are including vinegar, lemon juice, lime juice, and other acidulants such as acetic, lactic, citric, tartaric and malic acid (102). The Thai Industrial Standard specified that the added acidulants in salad dressing should lower than 5 g/ kg product (100).

2.5.2.4 Flavoring ingredients

The most common flavoring ingredients in salad dressing are mustard powder, sugar and salt. The addition of mustard powder mainly provides flavor and small amount of color. It also contributes to the emulsion stability by its particle mechanism that can stabilize the emulsion by the adsorption of particles at the droplet interface (99). Sugar and salt are added to enhance the flavor of salad dressing. Besides of their flavoring functions, their presence also reduce the water activity of the product to the level that can inhibit the microbial growth (102).

2.5.2.5 Thickening agent

Thickener is a chemical component or mixture of components that can enhance the emulsion stability by thickening a food system. They also act as bulking agent to provide the viscosity and forming networks in the continuous phase to prevent oil separation of the emulsion. There are a variety of thickeners used in salad dressing formulation, and each type can perform different functional properties. Gums are often added to salad dressing to stabilize the emulsion, improve flow behavior, texture and appearance, control pourability, improve cling, and suspend the solid particles. Starch and modified starch can be used to provide the desired structure of the final product, and can also act as bulking agents or fat mimetics to improve the body and mouthfeel of salad dressing. Pectin can be used to form the network between oil and water phases that could stabilize the emulsion and prevent phase separation.

The combination of hydrocolloids can improve the rheological behavior and textural characteristics of salad dressing (102).

2.5.3 Physicochemical properties of salad dressing

2.5.3.1 Appearance

Typically, salad dressings are homogeneous semisolid emulsions with light-yellow color (100). The relatively high droplet concentration leads to extensive light scattering that affect the opaque appearance of dressing. Therefore, the reduction of fat content in salad dressing results in the decrease in lightness of the product. This is due to the fact that non-fat particles such as protein, polysaccharide and their combinations which are added into the reduced-fat dressing may not scatter the light. However, the color of can be altered by the ingredients used in the formulation since they adsorb radiation in the visible region of the electromagnetic spectrum and contribute their color to the color of the product (98)

2.5.3.2 Rheological properties

The major effective factor on perceived quality of salad dressing is rheological properties which relate to the texture of the product. The terms of “pourability,” flowability,” “thickness” and “creaminess” are extremely relying on rheological characteristics of the dressings. Rheological properties also affect the processing steps in the production of dressing such as mixing, stirring, homogenization, and pumping through pipes. Spoonable salad dressing usually exhibit strong shear-thinning characteristics of which the apparent viscosity decreases with the increasing shear rate and may also time. Yield stress, apparent viscosity and shear-thinning behavior are crucial to determine the mouthfeel and texture of the products. The higher consistency index implied the more viscous characteristic which is corresponding to a strong network structure of the products. The higher yield stress implies the stronger interactions in the emulsion and higher ability of salad dressing to retain in the container before pouring. The closely packed oil droplets also result in the dressing that flows slowly from the container. The yield stress or high viscosity of the emulsion at low shear stresses can also slow down or hinder the coalescence and

creaming of oil droplets in the dressing. Salad dressing also exhibits viscoelastic behavior that can be categorized in terms of a complex modulus. Storage and loss modulus refer to elastic and viscous properties, respectively. For spoonable salad dressing and mayonnaise, the storage modulus is higher while loss modulus is lower, which refers to a gel-like structure of a flocculated and entangled network. In lower fat dressings, thickeners or fat mimetic need to be added to the aqueous phase to maintain their rheological characteristics. The uses of texture modifier can generate the viscosity, yield stress and shear-thinning behavior to the products by promoting droplets aggregation and forming an interfacial membrane around the oil droplets to increase the effective volume fraction of droplets. (98, 99, 102)

2.5.3.3 Flavor

A combination of volatile odor molecules, nonvolatile taste molecules, and mouthfeel affects the overall flavor of salad dressing. The additions of acidifying agents and flavoring agents mainly affect the taste and aroma of the product. Lipid oxidation from chemical degradation reactions during storage causes the changes in flavor profile that lead to undesirable flavor components and off-flavor. The changes in flavor profile also occurred in reduced-fat salad dressing due to the removal of fat droplets and the binding of flavors and the added texture modifier (99).

2.5.3.4 Stability

The stability of salad dressing is a relative terms according to the ability of the emulsion to preserve all sensory characteristics such as texture, taste and appearance throughout the shelf life. The quality deterioration such as lipid oxidation or hydrolysis affects the stability of the final product because it leads to chemical and biochemical changes, undesirable off-flavors and potentially harmful reaction products (98). Tocopherol, ascorbic acid, butylated hydroxyanisole (BHA) and calcium disodium ethylenediaminetetraacetate (calcium disodium EDTA) can be used as anti-rancidity agents in salad dressing. They should not be added to the product at any level exceed 240, 500, 140 and 75 mg/kg, respectively (100).

Instability or creaming of the lower fat emulsions, i.e., pourable or reduced-fat or low-fat or fat-free salad dressing, can be prevented by the

addition of gum or starch, which is thickening or gelling agents, to decelerate droplet movement. Although the addition of texture modifiers may promote droplet flocculation by a depletion mechanism, the emulsion is still stable due to the high viscosity of the aqueous phase. The presence of emulsifiers or surface active materials can form highly viscoelastic interfacial membrane that may protect the oil droplets from coalescence (98).

For microbial stability, the addition of acidifying agents such lead to the lower pH that can prevent microbial spoilage. The preservatives and antimicrobials which can retard bacterial growth are used in many dressings to preserve the microbiological stability of the products. Weak lipophillic organic acids, i.e., benzoic acid, sodium benzoate, potassium benzoate, and calcium benzoate, are commonly used in food emulsion with various permitted level depending on the legislation in different countries (98, 102). The permitted level of antimicrobial preservatives in salad dressing is not stated in the specification of Thai Industrial Standard (100), but the Notification No. 281/2547 of the Ministry of Public Health of Thailand announced that the addition of food additives in food products should be used according to the Codex General Standard for Food Additives (GSFA) (104). The GSFA specified that the emulsified sauce and dips such as mayonnaise, salad dressing and onion dip can use benzoates and sorbates as antimicrobial agents at the maximum level of 1000 mg/kg (105).

CHAPTER III
MATERIALS AND METHODS

This study consists of two major parts as illustrated in **Figure 3.1**. The first part was emphasized on the characterization of NBPP extracted using acid solution and water under different conditions. Certain acid- and water-extracted NBPP were selected to further investigate their potential application in food product. In the second part, the selected NBPP were used for fat substation in salad dressing and the quality of the product was evaluated.

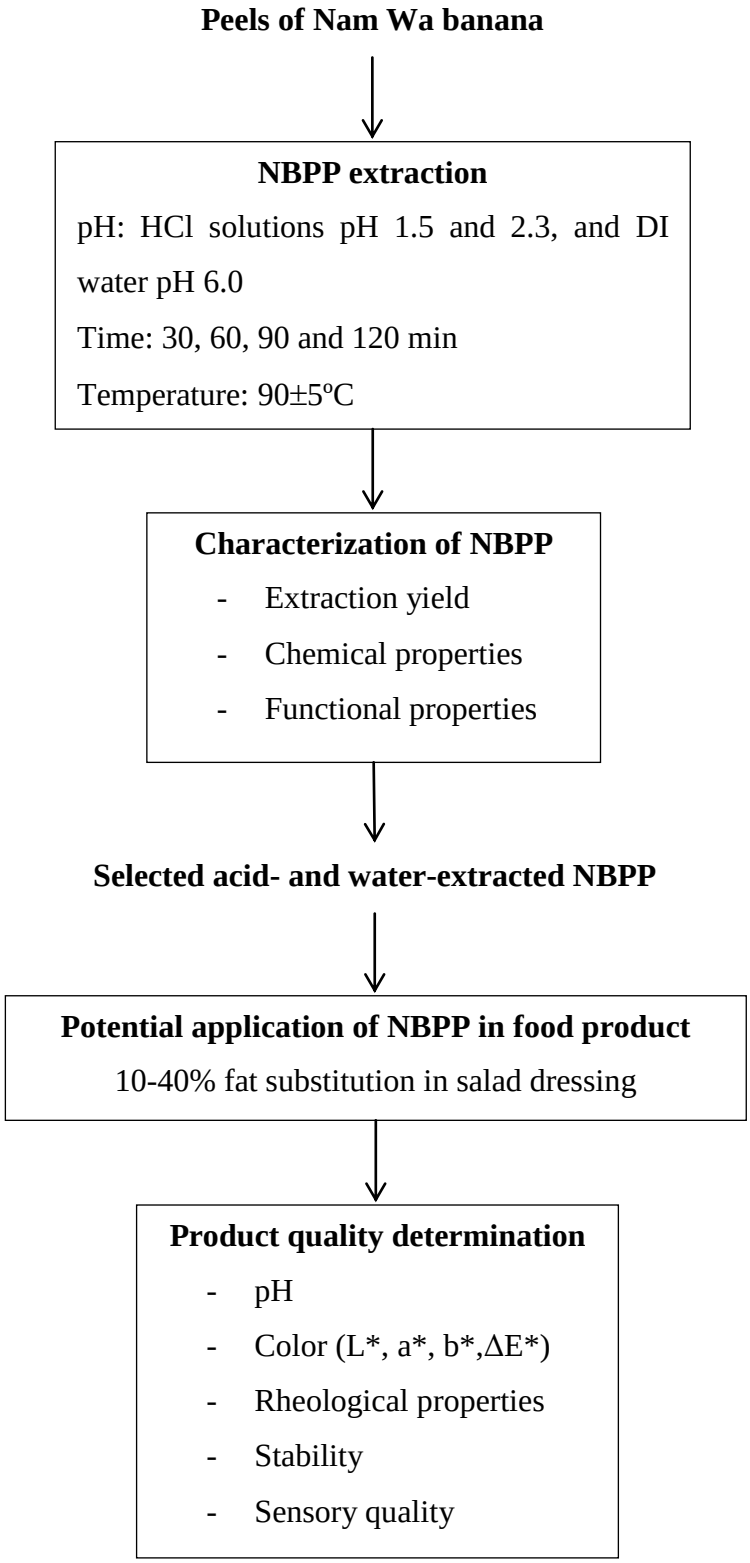

Figure 3.1 Experimental framework

3.1 Materials

Peels of Nam Wa banana (*Musa* (ABB group) 'Kluai Nam Wa') at the 5th stage of ripening were collected from the small-scale processed banana producers in Nakhon Pathom province during September 2012 and January 2013. Within 3 h after collection, the peels were washed twice in tap water prior to blanching in distilled water at 100ºC for 5 min. The peels were chopped into pieces of 1x1 cm^2, packed in sealed low-density polyethylene (LDPE) bags and stored in the freezer at -20°C until being used for pectin extraction. Chemical composition of the peels, analyzed according to AOAC Official Methods (106), is shown in **Table 3.1**, is presented as **Figure 3.2**.

All chemicals, unless stated otherwise, were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Figure 3.2 Blanched Nam Wa banana peels

Table 3.1 Proximate composition and dietary fiber content of blanched banana peels

Composition	**Content (g/100 g wet weight basis)[1]**
Moisture	89.5
Protein (N x 6.25)	1.0
Crude fat	1.4
Ash	1.0
Carbohydrates (by subtraction)	7.1
Total dietary fiber	7.4
Soluble dietary fiber	1.2
Insoluble dietary fiber	6.2

[1]Mean from duplicate analysis

3.2 NBPP extraction

Extractions of NBPP were performed according to the procedure described by Kulkarni *et al.* (14) with some modifications. The frozen peels were thawed prior to mixing with appropriate extracting solution, i.e., 0.05 M HCl or deionized (DI) water, at a solid-to-liquid ratio of 1:2 (w/v). The pH of the mixtures was then adjusted to 1.5, 2.3 or 6.0 with 1 M HCl or 1 M NaOH. The extraction was performed at 90±5°C for 30, 60, 90 or 120 min with agitation. After extraction, the extracted banana peels was filtered through double-layer nylon cloth. The filtrate was mixed with ethanol (95% v/v, Government Pharmaceutical Organization, Bangkok, Thailand) at the filtrate-to-ethanol volume ratio of 1:2 and left at room temperature (25±5°C) for 12 h to precipitate the pectin. The precipitate was harvested and washed twice with ethanol at the volume ratio of 1:1, and dried at 50°C in a hot air oven until dry (<10% moisture). The dried precipitate was ground, packed in sealed LDPE bags and kept in the desiccator at room temperature for further experiments.

3.3 Characterization of pectin from Nam Wa banana peels (NBPP)

3.3.1 Extraction yield

Yield of NBPP was determined by calculation on a dry-weight basis as follows:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried pectin (g)} \times 100}{\text{Dry weight of dried peel taken for extraction (g)}}$$

3.3.2 Chemical properties

3.3.2.1 Galacturonic acid content

The colorimetric assay using *m*-hydroxydiphenyl according to the procedure described by Blumenkrantz and Asboe-Hansen (107) was used to determine the galacturonic acid content (GalA) of NBPP. One milliliter of NBPP solution (0.005% w/v in DI water) was cooled in an ice bath. Then, each sample was mixed with 6 ml of 0.0125 M sodium tetraborate in sulfuric acid and heated in water bath at 95ºC for 5 min. After the test tube was immediately cooled in an ice bath, 100 µl of 0.15% (w/v) *m*-hydroxydiphenyl in 0.5% (w/v) NaOH was added into each tube and mixed thoroughly. The mixture was measured for absorbance at 520 nm by a UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). GalA of NBPP was determined against the standard curve prepared with galacturonic acid monohydrate (1-10 mg galacturonic acid/ml, R^2=0.9987), given as **Appendix A**.

3.3.2.2 Degree of methylation

Degree of methylation (DM) was determined by Fourier transform infrared (FT-IR) spectroscopy. Pectin powder was desiccated in a desiccator before being analyzed as is. Sample was placed on an attenuated total reflectance sampling accessory (Smart iTR, Thermo Fisher Scientific, Waltham, MA, USA) of an FT-IR spectrometer (Nicolet 6700, Thermo Fisher Scientific) equipped with a single bounce diamond crystal. FT-IR spectra of pectin were obtained by co-adding 64 scans at the resolution of 4 cm^{-1} in mid-infrared region (4000-400 cm^{-1}). The obtained spectra of each sample were subtracted with a blank spectrum collected from

atmosphere. The areas of peak at 1760-1745 and 1640-1620 cm^{-1}, which represent esterified carbonyl group (C=O) and free carboxylic group (COO^{-}), respectively were integrated using a computer software (OMNIC version 8.1, Thermo Electron, Madison, WI, U.S.A.). The following linear correlation was used to calculate DM of the samples:

$$\text{DM (\%)} = 87.609 \left(\frac{\text{Area}_1}{\text{Area}_1 + \text{Area}_2} \right) + 25.768$$

where $Area_1$ and $Area_2$ are areas of the peaks appeared between 1760-1745 cm^{-1}, and between 1640-1620 cm^{-1}, respectively (108).

3.3.2.3 Viscosity-average molecular weight

Dilute solutions of NBPP (0.025-0.2% w/v in 0.1 M NaCl) were filled in a Cannon-Fenske routine viscometer (capillary No. 50, internal diameter 0.44 mm; Schott-Geräte, Hofheim, Germany). The temperature was controlled at 30°C by placing the viscometer in a temperature-controlled water bath. Viscosity of pectin solution was calculated from the flow time recorded using a stopwatch and its density. The intrinsic viscosity of pectin was determined graphically by extrapolation of Huggins and Kraemer plots to zero concentration (109, 110). The following equations are Huggins and Kraemer equations, respectively:

$$\frac{(\eta/\eta_0) - 1}{c} = [\eta] + k_H[\eta]^2 c$$

$$\frac{\ln(\eta/\eta_0)}{c} = [\eta] + k_K[\eta]^2 c$$

where η is the viscosity of pectin solution (g/cm·s), η_0 is viscosity of the solvent, i.e., 0.0899×10^{-2} g/cm·s for 0.1 M NaCl (111), $[\eta]$ is intrinsic viscosity (cm^3/g), c is concentration of pectin solution (g/cm^3), and k_H, k_K are Huggins and Kreamer constants, respectively, which obtained from the corresponding plots.

Viscosity-average molecular weight (M_v) of NBPP was determined from their averaged intrinsic viscosities from the Huggins and Kraemer plots according to Mark-Houwink-Sakurada equation:

$$[\eta] = K(M_v)^{\alpha}$$

where K and α are temperature-depending parameter constants, which are 0.0436 and 0.78, respectively for pectin dissolved in 0.1 M NaCl pH 7.0 (42).

3.3.3 Functional properties

3.3.3.1 Thickening ability

Ability of NBPP in providing thickening effect was determined from rheological properties of their solutions. Apparent viscosity at the shear rate of 122 s^{-1} of NBPP solution (2.5% w/v in DI water) was measured at room temperature using a Brookfield viscometer (RVTDV-II, Brookfield Engineering Laboratories, Middleboro, MA, U.S.A.) fitted with a UL Adapter, which consists of a cylindrical spindle rotating inside a sample tube. Sixteen milliliters of sample was added to the sample tube and allowed to equilibrate for 5 min prior to analysis.

3.3.3.2 Gelling ability

The gelling ability of NBPP was determined by observing its ability to form HMP and LMP gels according to the methods of Fishman *et al*. (112) and Zykwinska *et al*. (113). For the preparation of HMP gel, 50 mg of NBPP was dissolved in 2 ml of 50 mM citrate buffer (pH 3), The mixture was stirred overnight in a 10 ml glass beaker covered with parafilm. After overnight stirring, 3 g of sugar (Mitr Phol™, Mitr Phol Sugar, Bangkok, Thailand) was added to obtain the mixture of 1 and 60% (w/w) NBPP and sucrose, respectively. The mixture was heated at 90ºC for 10 min and left undisturbed at room temperature for 20 h to allow gel setting before being observed. Gel formation was justified by turning the beaker upside down and observing whether the mixture can flow.

LMP gel was prepared by dissolving 100 mg of NBPP with 4.8 ml of DI water in a 10 ml glass beaker and stirring overnight with parafilm covered at room temperature. The mixture was adjusted to pH 6 with 0.5 M NaOH and heated at 70ºC for 15 min with agitation. After that, a preheated solution of 2 ml of 2 mM $CaCl_2$ (pH 6) was dropped into 2 ml of the sample under continuous stirring. The mixture

was left undisturbed at room temperature for 20 h before being observed by the similar method as HMP gel.

3.3.3.3 Water- and oil-holding capacities

Water-holding capacity (WHC) of NBPP was evaluated by the method of Aziz *et al.* (114). Two hundred fifty milligrams of NBPP was dissolved in 25 ml of DI water, stirred for 30 min and left at room temperature for 30 min. The mixture was then centrifuged at 2300xg for 30 min and the supernatants were disposed. The residue was weighed and WHC was calculated by the following equation:

$$\text{WHC (g water/ g pectin)} = \frac{\text{weight of residue (g)} - \text{ weight of pectin (g)}}{\text{weight of pectin (g)}}$$

Oil-holding capacity (OHC) was evaluated using the same method as WHC except that soybean oil (A-ngoon™, Thai Vegetable Oil, Bangkok, Thailand) was used instead of DI water. The following equation was used to calculate OHC of NBPP:

$$\text{OHC (g oil/ g pectin)} = \frac{\text{weight of residue (g)} - \text{weight of pectin (g)}}{\text{weight of pectin (g)}}$$

Based on extraction yield and functional properties, certain acid- and water-extracted NBPP samples were selected to evaluate their potential food application in the further study.

3.4 Potential application of NBPP in food product

3.4.1 Preparation of salad dressing

The selected acid- and water-extracted NBPP from the previous part were determined for their potential use as fat-replacer in salad dressing, of which the recipe is given as **Appendix B**. Based on results from preliminary trials, 2% (w/v) NBPP solutions in DI water were used to substitute up to 40% of vegetable oil in the control recipe.

3.4.2 Determination of product quality

Quality of the salad dressings with different fat substitution levels was determined as follow and compared with the control recipe.

3.4.2.1 pH

The pH value was measured using a pH meter (EcoMet P25, Istek, Seoul, Korea) at room temperature

3.4.2.2 Color

The color values (L*, a*, b*) were measured using spectrophotometer (ColorFlex EZ, Hunter Associates Laboratory, Reston, VA, U.S.A.). The L*, a* and b* values represent the lightness, redness and yellowness, respectively. Color difference (ΔE*) of each RSD and the control formula was calculated according to the following equation:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where L^*_0, a^*_0 and b^*_0 are color values of the control formula.

3.4.2.3 Rheological properties

Rheological properties were determined at room temperature using a Brookfield viscometer (RVTDV-II) fitted with Small Sample Adapter, which consists of a cylindrical cone spindle rotating inside a sample chamber. Eleven milliliters of samples was loaded into the sample chamber tube and allowed to equilibrate for 5 min prior to analysis. The apparent viscosity and shear stress were recorded every 2 min while the shear rate was increased from 0.14 to 28 s^{-1} over 16 min. Yield stress was determined by extrapolating the plot between shear rate and shear stress to the intercept on the stress axis (115).

Flow behavior and consistency indices were determined by fitting the obtained flow curves using a power law equation:

$$\sigma = K\gamma^n$$

where σ is shear stress (Pa), γ is shear rate (s^{-1}), K is consistency index ($Pa \cdot s^n$) and n is flow behavior index (69).

3.4.2.4 Stability

Stability of salad dressing was evaluated by observing the separation of cream layer or oil from the samples during 2 wk storage at room temperature in sealed glass jars.

3.4.2.5 Sensory characteristics

Sensory attributes of salad dressings with different levels of fat substitution by NBPP were evaluated by 20 untrained panelists. The panel composed of 15% male and 85% female aged 22-52 y, who are staffs and graduate students of the Institute of Nutrition, Mahidol University. Samples were prepared one day before the sensory test day and stored at 4ºC in refrigerator. The samples were taken out of the refrigerator and set aside until reached room temperature prior to serving. The test was conducted under daylight fluorescent lamp in air-conditioned, individual testing booths. Ten grams of each sample was served, in a random order, in a 30 ml clear polypropylene plastic cup labeled with three-digit random code number on a square white melamine tray together with iceberg lettuce. The photograph taken of a presented sample tray is given in **Figure 3.3**.

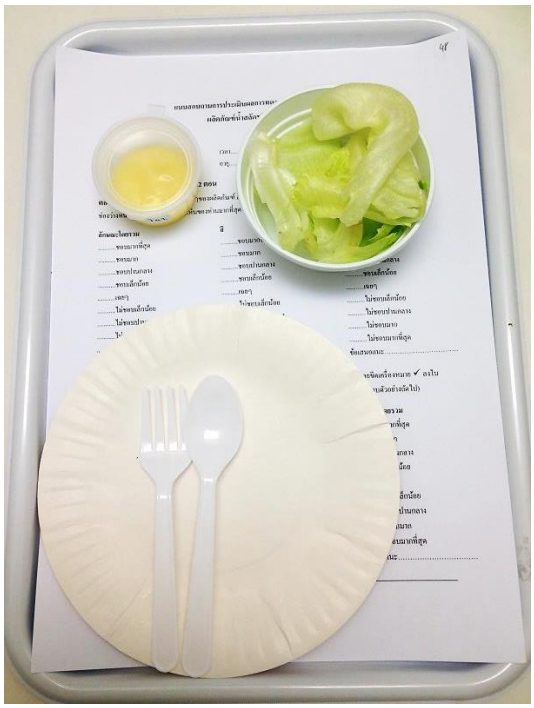

Figure 3.3 Sample presentation for sensory evaluation of salad dressings

Panelists were asked to evaluate the appropriateness of color, viscosity, smoothness and taste of each sample using a five-point just-about-right scale. The sensory score ranged from 1 to 5, where 1 means “much too weak”, 3 means “just-about-right”, and 5 means “much too strong”. The questionnaire is presented in **Appendix C**. Panelists rinsed their mouths with drinking water between tasting each sample.

Acceptability of the samples with appropriate level of fat substitution by NBPP, which were selected according to the quality and sensory score, were evaluated by untrained 50 panelists. The panel composed of 24% male and 76% female aged 22-58 y who are staffs and graduate students of the Institute of Nutrition, Mahidol University. The test was conducted separately in a similar manner as described earlier except that a nine-point hedonic scale was used to assess the preference on general appearance, color, thickness, smoothness, taste and overall acceptability of the samples. The scale ranged from 1 to 9, where 1 means “dislike extremely”, 5 means “neither like nor dislike”, and 9 means “like extremely”. The questionnaire is presented in **Appendix D**.

3.5 Statistical analysis

Experiments on extraction and characterization of NBPP were conducted in a 3x4 Factorial in Completely Randomized Design. Experiments related to the potential application of NBPP in salad dressing were performed in Completely Randomized Design, except the sensory evaluation which was conducted in Randomized Complete Block Design. All experiments, except the sensory evaluation, were conducted in three replications of separate sets of experiment. Data were analyzed using a computer statistics program (SPSS 17.0, SPSS, Chicago, IL, U.S.A.) and are presented as mean and standard deviation. Data obtained from 5-point just-about-right test were presented as the distribution of sensory scores. One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test was used to assess the mean difference among treatments. Factorial ANOVA was used to determine the effects of extracting factors and their interactions on NBPP properties. All statistical analyses were determined at 5% level of probability ($p \leq 0.05$).

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CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of extraction condition on the properties of NBPP

Extraction condition is an important factor that affects the composition and properties of pectin. In this study, pectin was extracted from blanched banana peel using different extracting pH, i.e., 1.5 and 2.3 with HCl solutions which follow the conventional method for pectin extraction using acid, and 6.0 with DI water which is considered to be more environmental-friendly, and time, i.e., 30, 60, 90 and 120 min, at 90±5°C. The extraction yield and chemical- and functional properties of the obtained NBPP were determined and the results were used for selecting of appropriate NBPP for further study.

4.1.1 Extraction yield

Yield of extraction determines the appropriateness and effectiveness of condition used for pectin extraction. **Table 4.1** shows yield of NBPP extraction using different extraction condition. The yield percentage of NBPP varied from 4.5 to 11 on a dry weight basis, of which the highest and the lowest yields were obtained from acid extractions at pH 1.5 for 90 min and at pH 2.3 for 30 min, respectively. The highest yield obtained in this study is similar to that of pectin extraction from banana skin reported previously, i.e., 11.9% on a dry weight basis (50). Acid extraction at pH 1.5 gave higher NBPP yields than at pH 2.3 and water extraction at pH 6.0 at any extraction time. There was no significant difference in extraction yield obtained from the extractions at pH 6.0 and 2.3. The extraction yield of NBPP increased when the peels were extracted under stronger acidic condition at lower pH. Yapo *et al.* (11), Levigne *et al*. (116) and Emaga *et al.* (19) also observed the same trends in the extraction of pectins from sugar beet pulp and banana peel (*Musa* AAA) under different pH. It has been reported that pH of the extraction is the major influencing parameter for pectin isolation at constant extracting time and temperature (12, 116).

This is due to the fact that the stronger acidic condition could enhance cell wall disruption and hence increase pectin release (117).

For the effect of extraction time, yield of acid extraction at pH 1.5 significantly increased when extraction time increased from 30 to 60 min and remained unchanged at longer extraction time. Similar trend was also observed in the yield acid extraction at pH 2.3 and water extraction at pH 6.0 but to a lesser extent. Although there are several studies reported that the longer extraction time resulted in the higher extraction yield of pectins from sugar beet pulp, banana peel and leaves of Khruea Ma Noi (*Cyclea barbata* Miers) (11, 19, 118), a study of Garna *et al.* reported that the extraction time did not influent the extraction yield of pectin from apple pomace (119). Moreover, Kulkarni and Vijayanand reported that the yield of pectin extracted from passion fruit peel increased with the longer extraction time from 30 to 60 min, but further increasing extraction time to 90 min did not significantly increase the pectin yield (14).

Table 4.1 Extraction yield of NBPP from different extraction conditions

Extraction time (min)	**Extraction yield (% dry weight basis)[1]**		
	pH 1.5	**pH 2.3**	**pH 6.0**
30	7.33 ± 0.97^{b}	4.46 ± 0.67^{e}	4.82 ± 0.16^{de}
60	11.05 ± 0.66^{a}	5.77 ± 0.44^{cd}	5.11 ± 0.08^{de}
90	11.13 ± 0.73^{a}	6.63 ± 1.03^{bc}	5.38 ± 0.15^{de}
120	10.61 ± 0.96^{a}	6.79 ± 0.59^{bc}	5.73 ± 0.66^{cd}

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts are significantly different ($p \leq 0.05$)

It should be noted that the yields of extraction at pH 1.5 for 60-120 min were similar to the soluble dietary fiber content of blanched Nam Wa banana peel used in this study (**Table 3.1**), suggesting that nearly all of the soluble dietary fiber in banana peels is pectin and that such acidic condition could extract most of the pectin from the peel.

4.1.2 Chemical properties

Chemical properties of the obtained pectin depend on the condition used for pectin extraction. NBPP extracted using various conditions were analyzed for GalA, DM, intrinsic viscosity and M_v.

4.1.2.1 Galacturonic acid content

GalA is the property which implies the purity of pectin because the main structure of pectin chain is polygalacturonic acid backbone connecting to other substances such as neutral sugars (5, 40). GalA of the extracted NBPP varied from 33-52 g/100 g dry pectin (**Table 4.2)**. Although water-extracted NBPP contained higher GalA than those extracted with acid at pH 1.5 and 2.3, there was no significant difference in GalA of NBPP from all extraction conditions, except among those extracted for 90 min. The major reason behind this was the large standard deviations in GalA of NBPP, suggesting the variations in the component of banana peels from different batches. The length of extraction also did not significantly affect GalA of the extracted NBPP. However, it is seemingly that the purity of NBPP obtained from water extraction was higher than those from acid extraction.

Table 4.2 Galacturonic acid content of NBPP from different extraction conditions

Extraction time (min)	Galacturonic acid content (g/100 g dry pectin)[1]		
	pH 1.5	**pH 2.3**	**pH 6.0**
30	46.17 ± 5.96^{a}	39.43 ± 4.62^{a}	51.77 ± 13.74^{a}
60	44.53 ± 10.01^{a}	37.03 ± 12.55^{a}	50.03 ± 11.92^{a}
90	42.00 ± 6.81^{a}	33.73 ± 5.93^{a}	50.73 ± 9.25^{a}
120	46.50 ± 12.87^{a}	33.43 ± 7.05^{a}	52.07 ± 14.81^{a}

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts are significantly different ($p \leq 0.05$)

According to the specification of the Food and Agriculture Organization of the United Nations and the European Union Commission (120), all NBPP in this study should be classified as pectin-enriched materials since their GalA were less than 65%. Pectins from banana peel have been reported to contain various GalA in different studies depending on the severity of the extractant and extracting condition used. In a study of Emaga *et al.*, GalA of banana peel (*Musa* AAA) pectin extracted under resembling condition was 40-46% (19). In another work using natural weak acid as extractant, the higher GalA of banana peels pectin (68.4%) has been observed. However, GalA of acid-soluble pectin (42%) was reported to be lower than that of water-soluble pectin (66.4%) extracted from banana peel of the same stage of maturation using the same extraction condition (18). The higher pH of extraction has been reported to give the higher GalA of banana peel (*Musa* AAA) pectin. However, in this study water-extracted NBPP contained the highest GalA; while pectin with the lowest GalA was obtained from the less acidic condition at pH 2.3 than pH 1.5 (**Table 4.2**). This could possibly because pectin was degraded under strong acid condition into smaller molecules that also precipitated in alcohol (19) Moreover, non-pectic substances, especially insoluble dietary fiber which was present in a high amount in

blanched Nam Wa banana peel (**Table 3.1**), probably as cellulose and hemicellulose, could also be hydrolyzed during NBPP extraction using acid. Those degraded molecules could co-precipitate with pectin in ethanol and resulted in the higher NBPP yield from acid extraction (**Table 4.1**). The results on GalA suggested that water-extracted NBPP is purer than NBPP extracted from acid extraction. The longer extraction time did not significantly affect the free sugar content of NBPP samples.

In addition to galacturonic acid, pectin also consists of neutral sugar as parts of polygalacturonic acid backbone and side chains. The sugars usually present in pectin include arabinose, galactose, rhamnose and xylose. Other sugar such as glucose, xylose, mannose, fucose, and glucuronic acid, are sometimes found in side chains (42). It has been reported that banana (*Musa* AAA) peel pectin extracted with water at 60ºC for 2 h contained 11% glucose, 2.7% mannose, 2.5% galactose, 2.2% arabinose, 0.8% xylose and 0.5% rhamnose (18). However, the total sugar content and monosaccharide composition of NBPP were not analyzed in this study.

4.1.2.2 Degree of methylation

DM is a chemical property of pectin that is used to categorized pectin into 2 major groups. It also implies the chemical structure as well as the functional properties of pectin (42). In this study, DM of the obtained NBPP was determined from their FT-IR spectra. The typical FT-IR spectra of NBPP extracted from water and acid extraction at 90±5ºC for 60 min are shown in **Figure 4.1**. The characteristic peaks of polysaccharides from cell wall of plants which correspond to their major chemical group were detected. The peak in a broad band of absorption between 3600-2900 cm^{-1} relates to O-H stretching. The peak of O-CH_3 stretching from methyl ester of the galacturonic acid molecules is represented at 2930 cm^{-1}. The peaks at 1760-1730 and 1640-1600 cm^{-1} derived from esterified carbonyl group (C=O) and carboxylate ion stretching band (COO^-), respectively. The observed peaks between 1400-950 cm^{-1} region are related to the typical profile of polygalacturonic acid (108, 121, 122). Finally, the characteristic peaks at 1052 cm^{-1} corresponding to C−O−C stretching of the galactouronic acid (123). The intensity ratio of the peaks at 1760-1730 and 1640-1600 cm^{-1} was used to calculate the DM of NBPP. The FTIR spectra and peak areas of each NBPP sample is presented in **Appendix E**.

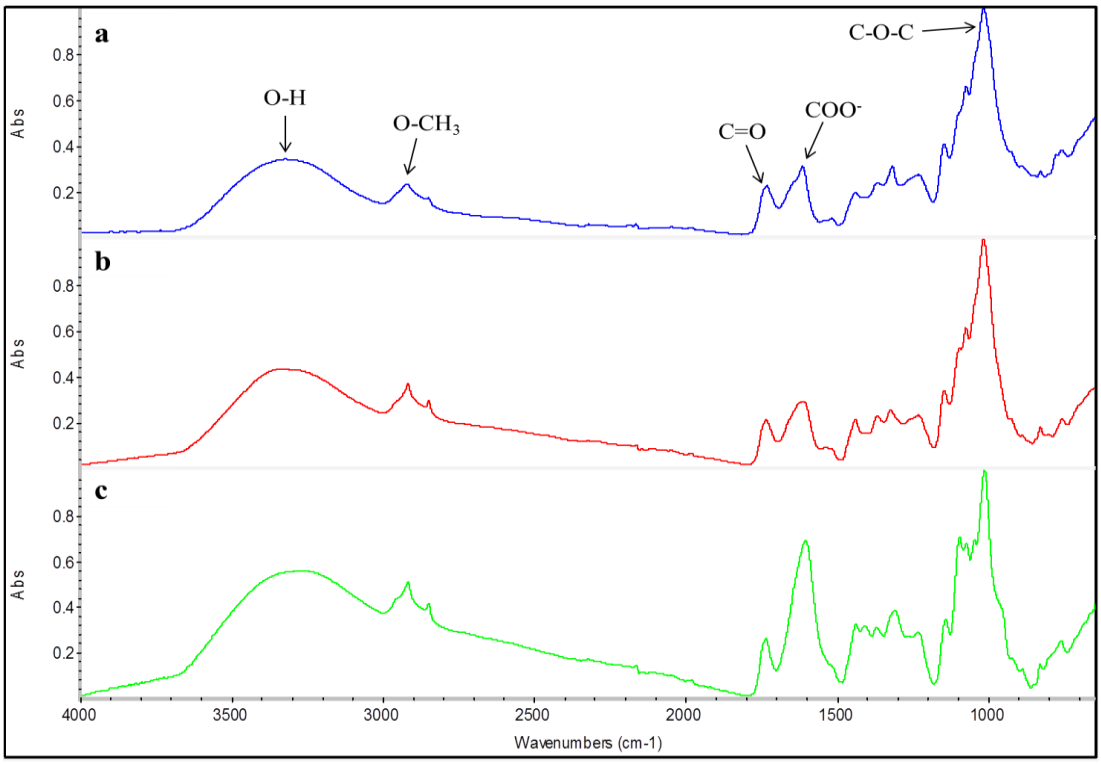


Figure 4.1 Typical FT-IR spectra of NBPP extracted with HCl solutions pH 1.5 (a), and 2.3 (b), and DI water pH 6.0 (c) for 60 min at 90±5°C

DM of NBPP samples ranged from 38-61% (**Table 4.3**). The pH of extraction significantly affected DM of NBPP. NBPP extracted at the higher pH contained lower DM. Water-extracted NBPP, of which the DM were below 40%, are classified as LMP; while acid-extracted NBPP were more methylated and are considered to be HMP. The length of extraction did not significantly affect DM of NBPP extracted using water but slightly affected DM of acid-extracted NBPP, especially at pH 2.3. DM of NBPP from acid extraction at pH 1.5 remained constant at any extraction time between 30 to 90 min but significantly increased when the extraction time increased from 90 to 120 min. Therefore, pH of extracting solution more strongly affected the DM of NBPP than the extraction period. The similar DM (49-80%) was also reported for banana (*Musa* AAA) peel pectin extracted using acid aqueous solution (19). DM of the extracted pectin from mesocarp of citrus fruit had been reported to increase when extracting pH was decreased (124).

Table 4.3 Degree of methylation of NBPP from different extraction conditions

Extraction time (min)	Degree of methylation (%)[1]		
	pH 1.5	pH 2.3	pH 6.0
30	57.70 ± 0.87^{b}	54.18 ± 1.34^{c}	38.96 ± 2.09^{e}
60	58.69 ± 2.61^{b}	51.89 ± 3.08^{d}	38.55 ± 1.92^{e}
90	57.10 ± 1.19^{b}	54.00 ± 1.23^{c}	37.99 ± 1.99^{e}
120	61.38 ± 2.29^{a}	52.68 ± 3.30^{cd}	37.69 ± 1.82^{e}

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts are significantly different ($p \leq 0.05$)

4.1.2.3 Viscosity-average molecular weight

M_v of the extracted NBPP was determined according to Mark-Houwink-Sakurada equation. The intrinsic viscosity of NBPP samples were determined by using capillary viscometer followed by graphically extrapolating the Huggins and Kraemer plots to zero concentration. The Huggins and Kraemer plots of each NBPP and values obtained from each plot are listed in **Appendix F**. The intrinsic viscosities from the Huggins and Kraemer plots of NBPP were averaged and used for the calculation of M_v. The pH and the duration time of the extraction significantly affected M_v of NBPP (**Table 4.4**). Water-extracted NBPP gave the highest M_v followed by those obtained from acid extraction at pH 2.3 and 1.5, respectively. Water extraction of which the pH was higher gave NBPP with higher M_v than NBPP extracted with acid solutions. Acid-extracted NBPP was depolymerized into shorter polygalacturonic acid chains due to the acid hydrolysis under acidic condition, hence the M_v was lower (65). The longer extraction time resulted in the lower M_v of NBPP at any extraction pH since the hydrolysis proceeded longer. M_v of NBPP from all extractions in this study were lower than that of pectin extracted from banana (*Musa* AAA cv. Poyo, Cavendish subgroup) cell wall material by using hot citric acid (pH

1.8) at 75°C for 60 min, which was reported to be 87.3 kDa (125). The higher weight-average molar mass (87-248 kDa) was also reported for banana (*Musa* AAA) peel pectin extracted using hot acid aqueous solution under various conditions in another study (19). This could be due to the variations in banana variety and composition and characteristic of banana peel as well as the different analytical techniques used in each study.

Table 4.4 Viscosity-average molecular weight of extracted NBPP

Extraction time (min)	Viscosity-average molecular weight (kDa)[1]		
	pH 1.5	pH 2.3	pH 6.0
30	29.32 ± 1.32^{c}	33.66 ± 1.91^{b}	40.27 ± 2.48^{a}
60	20.42 ± 0.44^{fg}	26.57 ± 1.35^{d}	32.46 ± 1.56^{b}
90	18.18 ± 0.69^{gh}	23.54 ± 1.30^{e}	28.97 ± 1.99^{cd}
120	17.29 ± 1.30^{h}	20.97 ± 0.74^{ef}	21.25 ± 1.00^{ef}

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts are significantly different ($p \leq 0.05$)

The results from factorial ANOVA for the effects of extracting factors and their interaction on the properties of pectin are shown in **Table 4.5**. The extraction yield and all chemical properties of NBPP were significantly affected by pH of the extraction ($p \leq 0.05$). The length of pectin extraction significantly affected only on pectin yield and M_v of NBPP ($p \leq 0.05$). Effect of interaction between pH and duration time was found on the yield, DM and M_v of the obtained NBPP ($p \leq 0.05$).

Table 4.5 Analysis of Variance for the effects of pH of extraction and extraction time on the extraction yield and chemical properties of NBPP

Factors	Value	Yield	GalA	DM	M_v
pH	F	179.552	53.114	904.204	126.525
	p	0.000	0.000	0.000	0.000
Time	F	21.643	0.515	1.167	163.331
	p	0.000	0.676	0.327	0.000
pH*time	F	4.512	0.247	4.581	5.486
	p	0.003	0.956	0.000	0.001

4.1.3 Functional properties

The important functional properties of pectin in food, i.e., thickening and gelling abilities as well as water and oil holding capacities, of NBPP from different extraction conditions were determined.

4.1.3.1 Thickening ability

Thickening ability is one of the functional properties of pectin that indicates the use of pectin in food product. In this study, thickening ability of NBPP was determined by measuring the apparent viscosity of 2.5% (w/v) NBPP solutions at a shear rate of 122 s^{-1}. The results show that the apparent viscosity of NBPP varied from 8-15 cP (**Table 4.6**). The pH of the extraction did not affect the viscosity of the solution of the obtained NBPP. Nevertheless, longer extraction time significantly decreased the thickening ability of the obtained NBPP, resulting in the lower viscosity of its solution. Such effect was more pronounced in the solutions of acid-extracted NBPP. The results from factorial ANOVA analysis showed that pH, extraction time, and interaction between pH and time significantly affected the apparent viscosity of NBPP solution ($p \leq 0.05$, data not shown). The lower viscosity of

solution the NBPP extracted at pH 1.5 suggested its less ability to provide thickening effect, which corresponded well with its lower M_v (**Table 4.4**). Similar to any other hydrocolloids, ability of pectin in providing the thickening effect depends largely on its molecular weight. Pectin with higher molecular weight and more rigid structure provides more viscous solution (5). The longer time and more acidic condition could enhance pectin hydrolysis and hence resulted in the more degraded, shorter pectin chain. Moreover, prolonged extraction at high temperature (90°C) used in this study could also decrease the viscosity of NBPP solution due to the accelerating effect of heat on depolymerization reaction (65).

Apart from the molecular weight of NBPP, pH of the NBPP solution could also affect their thickening ability. Solutions of NBPP were different in their pH values, depending on the pH of extraction, because the extracts were not neutralized prior to precipitating in alcohol. Solutions of pectin have different viscosity at different pH because pectin is a polyelectrolyte. At lower pH, the ionization of carboxylic groups is suppressed, resulting in the decrease in the hydration of carboxylic acid on pectin chain. It has been demonstrated that the intrinsic viscosity of commercial citrus and apple pectin solution decreases with the decreasing pH from 7 to 3. The reason behind that is the condensation of polyelectrolyte chain which is the result of the diminution of intramolecular electrostatic repulsions (42, 126). It should be noted that the pH of 2% (w/v) solution of NBPP obtained from the acid extraction at pH 1.5 was about 3.0, which is lower than the pK_a of galacturonic acid (pK_a=3.5). Therefore, those NBPP were not ionized in the solutions, which might also be the reason for the lower apparent viscosity and thickening ability of acid-extracted NBPP.

Table 4.6 Apparent viscosity of 2.5% (w/v) solutions of NBPP from different extraction conditions

Extraction time (min)	Apparent viscosity at 122 s^{-1} (cP)[1]		
	pH 1.5	pH 2.3	pH 6.0
30	14.40 ± 1.84^{a}	14.7 ± 2.12^{a}	14.60 ± 3.06^{a}
60	8.36 ± 1.90^{bc}	10.46 ± 3.17^{abc}	11.55 ± 2.25^{ab}
90	7.29 ± 1.86^{bc}	10.33 ± 2.66^{abc}	11.53 ± 1.74^{ab}
120	6.71 ± 1.66^{d}	8.67 ± 2.28^{bc}	9.09 ± 1.65^{bc}

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts are significantly different ($p \leq 0.05$)

4.1.3.2 Gelling ability

Under appropriate condition, pectin chains in the solution may form network among themselves to provide the rigid gel structure The characteristics of gel formation depend largely on the chemical structure of the polygalacturonic acid chain, especially DM (42). The abilities of NBPP to form gels, both under HMP and LMP conditions, were determined. All acid-extracted NBPP samples formed HMP gel in the presence of sugar under acidic condition (pH~3) (**Table 4.7**), which corresponded to their high DM (**Table 4.3**). The HMP gel is formed due to the mechanism of pectin-pectin interactions, which is promoted by high content of soluble solids, e.g., sugar and acid, that creates low water activity condition, rather than pectin-solvent interactions at the junction zone (127). In addition, the low pH leads to low ionization of carboxyl groups which also minimizes the electrostatic repulsive forces between pectin chains. HMP gel is stabilized by hydrogen bonding between non-dissociated carboxyl and secondary alcohol groups at the junction zones, and the hydrophobic interaction of the ester groups (128, 129).

It should be noted that although water-extracted NBPP in this study were LMP (**Table 4.3**), all of them formed gel under HMP gelling condition, except that extracted for 120 min (**Table 4.7**). However, the mixture became highly viscous. Surprisingly, none of the water-extracted, low-methylated NBPP formed LMP gel in the presence of Ca^{2+} (data not shown). Jordi *et al.* explained that the presence of neutral sugar and methoxyl groups in polygalaturonic chain could hinder the formation of egg-box structure at the junction zone (68). It was also possible that the amount of Ca^{2+} (2 mM) presenting in the tested gel mixtures was not high enough to form sufficient ionic interaction with free carboxylic groups to become egg-box structure that provide the LMP gel network. On the other hand, the concentration of pectin (1% w/v) in the gel mixture might be too low. It has been reported that syneresis of the mixture may occur when the calcium-to-pectin ratio is lower or higher than 35 mg Ca^{2+}/g pectin (130). Such ratio is much higher than that of the LMP gel mixtures in this study, of which the calcium-to-pectin ratio was 4 mg Ca^{2+}/g pectin. The LMP from Krueo Ma Noy (*Cissampelos pareira*) leaves were also reported not to from gel in the presence of Ca^{2+} at pH 5-8. It is believed that the stronger acidity would reduce of charge density of pectin chain that diminished electrostatic repulsion and promoted inter-chain interaction (131). The lack of gelation in LMP was also observed in water-extracted pectin from dragon fruit peel, which is reported to be due to the high ash content of the pectin (132).

Table 4.7 Gelling ability in high methoxy pectin gelling condition of NBPP from different extraction conditions

Extraction time (min)	HMP-gelling ability		
	pH 1.5	pH 2.3	pH 6.0
30	✓	✓	✓
60	✓	✓	✓
90	✓	✓	✓
120	✓	✓	×

✓ = Formed gel, × = did not form gel

4.1.3.3 Water- and oil-holding capacities

Only the NBPP extracted with HCl solution pH 1.5 and with DI water pH 6.0 for 60 min were selected for WHC and OHC determination in order to investigate their potential in being an emulsifier. Both NBPP were selected based on their extraction yield, and chemical and functional properties. The results are present in **Table 4.8**. WHC and OHC of the selected NBPP ranged from 4.2-5.9 g of water/ g of sample and 3.3-3.4 g of oil/ g of sample, respectively. The higher WHC of water-extracted NBPP indicated that water-extracted NBPP can be dissolved and adsorb more water than those obtained from the acidic extraction condition, although the difference was not statistically significant ($p>0.05$). There was also no significant difference between OHC values of NBPP obtained from water and acid extraction. WHC and OHC of acid-extracted NBPP were about similar; while WHC of water-extracted NBPP was about two times higher than OHC. The results suggested that NBPP are dissolvable in both oil and water, which is attributed to the presence of hydrophilic and hydrophobic parts in their structures. Therefore, NBPP were probably amphiphilic molecules which might be to provide emulsification effect to the mixture

of oil and aqueous phases. Although pectin is generally not considered to be an emulsifying agent, some of them, i.e., sugar beet and depolymerized citrus pectins, exhibit appreciable surface activity and have been proven to be effective emulsifiers (11, 72). Such ability is contributed from the pectin structure that contains a large amount of hydrophobic acetyl group and/or associated protein moiety (71). However, in this study the protein content of NBPP was not analyzed. A study of Sungpud reported that the WHC and OHC of pectin powder from Khruea-Ma-Noi (*Cissampelos Pereira* Linn.) leaves, analyzed using similar method, were 18.91 g of water/ g of dry matter and 2.25 g of oil/ g of dry matter, respectively (133). When compared to this study, NBPP can be absorbed less water, but can hold more oil content.

Table 4.8 Water-holding capacity and oil-holding capacity of NBPP extracted by HCl solution pH 1.5 and DI water pH 6.0 for 60 min at 90±5ºC

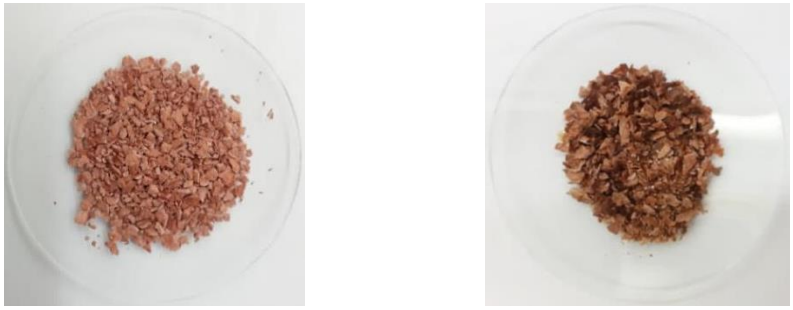
Extracting solution	WHC (g of water/ g of sample)[1]	OHC (g of oil/ g of sample)[1]
pH 1.5	4.18 ± 1.11[a]	3.37 ± 0.23[a]
pH 6.0	5.89 ± 1.11[a]	3.33 ± 0.28[a]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

4.2 Potential application of NBPP in food product

Based on the information on yield of the extraction and properties of NBPP from the previous part, NBPP extracted with HCl solution pH 1.5 and with DI water pH 6.0 for 60 min at 90±5°C were selected to evaluate their potential use as fat replacers in food product. Acid extraction at pH 1.5 gave NBPP with the highest yield, DM while maintaining its thickening and HMP-gelling abilities. Water extraction is the more environmental-friendly method that could produce NBPP at an appreciable yield with lower DM and higher M_v that resulted in greater thickening ability. The extraction time at 60 min was selected because it generated the highest pectin yield (**Table 4.1**).

Those pectins are referred to respectively as acid- and water-extracted NBPP for the rest of this study. The photographs of both NBPP are shown in **Figure 4.2**.



a **b**

Figure 4.2 NBPP extracted with HCl solution pH 1.5 (a) and DI water pH 6.0 (b) for 60 min at 90±5°C

Salad dressing was used as the food model in this study because it normally contains high amount of fat. The reduction of fat content in salad dressing could be an alternative choice to reduce risk of obesity and promote the consumption of vegetables and fruits. According to the functional properties of pectin in being thickening and gelling agents, previous studies have reported its possibilities to be used as a fat replacer to provide smooth and creamy mouthfeel as well as improve the texture in many reduced-fat products including mayonnaise and salad dressing (92, 134).

Preliminary experiments were conducted in order to determine the concentration of pectin solution to be used for oil substitution in salad dressing. Solutions (1-3% w/v in distilled water) of commercial pectin from citrus peel, (89% GalA, 54.3% DM) were prepared, measured their viscosities and used to substitute 30% of the soybean oil in the recipe of salad dressing in **Appendix B**. The results showed that the viscosity of commercial pectin solution was about two times higher than that of NBPP solution at the same concentration and the reduced fat salad dressing prepared with 1% (w/v) commercial pectin solution exhibited appropriate viscosity. Therefore, 2% (w/v) solution of NBPP was selected. pH and color values of the selected pectin at 2% (w/v) are shown as **Table 4.9**. Angpanitcharoen reported that the concentration of high ester pectin (Slendid™) generally used as fat replacer in salad dressing was 0.5-3% (w/w). However, at high levels of oil substitution with 3% pectin solution, the salad dressing was very slimy gel, lack of body and the color became dark due to the color of pectin powder (92).

Table 4.9 pH and color values of 2% (w/v) solution of NBPP extracted by HCl solution pH 1.5 and DI water pH 6.0 for 60 min for 60 min at 90±5ºC

Extracting solution	pH[1]	Color[1]		
		L*	a*	b*
pH 1.5	2.93 ± 0.02[b]	45.04 ± 0.22[a]	10.40 ± 0.14[b]	15.24 ± 0.29[a]
pH 6.0	6.70 ± 0.04[a]	32.62 ± 0.01[b]	10.89 ± 0.03[a]	12.31 ± 0.03[b]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

Further preliminary experiments were performed to screen the range of oil substitution level in salad dressing. The dressings with 10-40% reduced-oil were prepared by substituting the oil with 2% (w/v) NBPP solutions. The prepared dressings were evaluated and judged by experts, who are faculty members of the Food Science Unit at Institute of Nutrition, Mahidol University. The results showed that the thickness and appearance of reduced-fat salad dressing (RSD) with 10 and 20% oil

substitution did not differ from the control formula; while the formula with >40% oil substitution were unacceptable with very thin body and dark color. Therefore, 25-40% oil substitution levels were selected for further experiment. Such levels are in accordance to the specification of the Thailand's Food and Drug Administration, Ministry of Public Health on nutrient content claims, which states that the reduced fat product has to reduce at least 25% of the total fat content from the regular formula (101). The composition of RSD is listed in **Table 4.10**.

Table 4.10 Composition of salad dressings containing 2% (w/v) solution of NBPP as fat replacer

Ingredients	**Content (% w/w)**				
	Level of oil substitution				
	0% (Control)	**25%**	**30%**	**35%**	**40%**
Soybean oil	50	37.5	35	32.5	30
2% (w/v) NBPP solution	-	12.5	15	17.5	20
Sugar	18.5	18.5	18.5	18.5	18.5
Egg yolk	10.1	10.1	10.1	10.1	10.1
Vinegar	8.5	8.5	8.5	8.5	8.5
Water	7.2	7.2	7.2	7.2	7.2
Lemon juice	3.5	3.5	3.5	3.5	3.5
Salt	1.6	1.6	1.6	1.6	1.6
Mustard powder	0.6	0.6	0.6	0.6	0.6

4.3 Quality of salad dressing containing NBPP as fat replacer

Quality of the RSD containing NBPP as fat replacer at 25-40% oil substitution levels was determined and compared with the control formula. Photographs of the control salad dressing and the RSD prepared with acid- and water-extracted NBPP are shown in **Figure 4.3** and **Figure 4.4**, respectively. The key quality indices included pH, color, rheological properties, stability and sensory characteristics. Based on the results from quality determination, only RSD with appropriate level of oil substitution were selected for consumer acceptability test

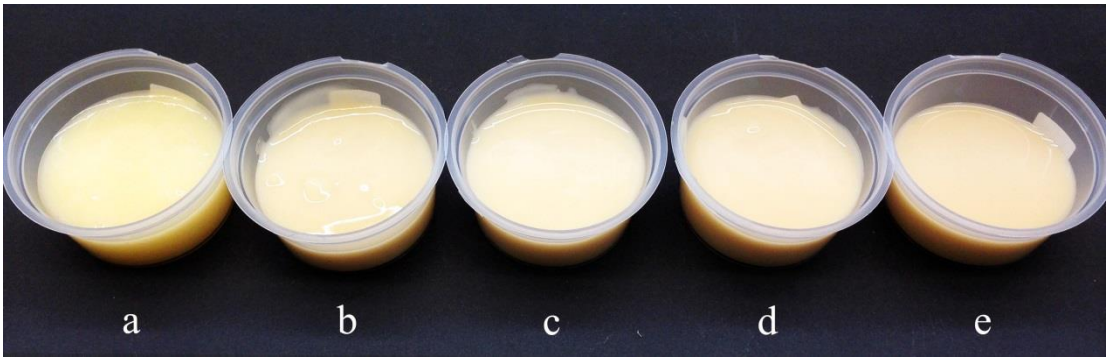


Figure 4.3 Salad dressings containing 2% (w/v) solution of NBPP extracted with HCl solution pH 1.5 for 60 min at 90±5°C as fat replacer at 0% (a), 25% (b), 30% (c), 35% (d) and 40% (e) oil substitution levels

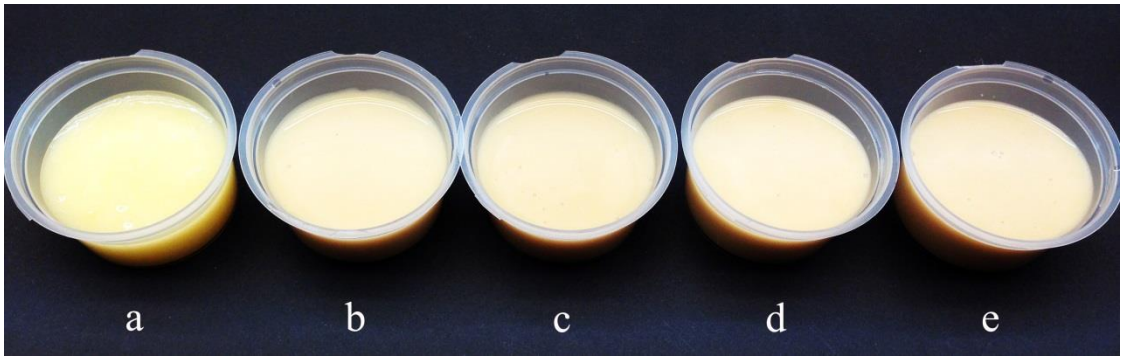


Figure 4.4 Salad dressings containing 2% (w/v) solution of NBPP extracted with DI water pH 6.0 for 60 min at 90±5°C as fat replacer at 0% (a), 25% (b), 30% (c), 35% (d) and 40% (e) oil substitution levels

4.3.1 pH

The pH values of RSD with acid- and water-extracted NBPP are shown in **Table 4.11** and **Table 4.12**, respectively. The pH value of control formula was 3.43. RSD containing acid-extracted NBPP solution as fat replacer had slightly lower pH than the control formula since the pH of the pectin solution is lower than that of the soybean oil. The decrease in the pH of RSD was more observable with increasing oil substitution level by acid-extracted NBPP. On the other hand, the RSD of which the fat content was substituted by water-extracted NBPP solution exhibited slightly higher pH than the control formula. However, no significant difference in the pH was found among the four RSD containing different amounts of water-extracted NBPP solution. When comparing the pH values of RSD incorporated with acid- and water- extracted NBPP solution at the same level of oil substitution, significant differences were observed at all levels. The pH of RSD containing acid-extracted NBPP solution was lower than that containing the solution of water-extracted NBPP at all levels of oil substitution. This could result from the lower pH of acid-extracted NBPP solution than that of water-extracted NBPP (**Table 4.9**). It should be noted that the pH of all RSD in this study did not exceed 3.5 which is in accordance with the Thai Industrial Standard for mayonnaise and salad dressing, that the pH must be lower than 4.1 (100). This suggested that both NBPP could be applied in salad dressing at oil substitution levels of up to 40% without deviation of the pH from the standard.

Table 4.11 pH and color values of salad dressings containing 2% (w/v) solution of NBPP extracted with HCl solution pH 1.5 for 60 min at 90±5°C as fat replacer

Level of oil substitution	pH[1]	Color[1]			
		L*	a*	b*	ΔE*
0%	3.43 ± 0.01[a]	81.24 ± 0.01[a]	5.59 ± 0.01[e]	40.51 ± 0.04[a]	-
25%	3.31 ± 0.01[d]	77.59 ± 0.02[c]	6.44 ± 0.01[d]	28.75 ± 0.01[e]	9.54 ± 0.02[c]
30%	3.32 ±0.01[cd]	77.70 ± 0.01[b]	6.67 ± 0.01[c]	31.84 ± 0.01[c]	9.42 ± 0.03[d]
35%	3.34 ± 0.01[c]	76.41 ± 0.01[d]	7.37 ± 0.01[a]	32.22 ± 0.01[b]	9.76 ± 0.03[b]
40%	3.37 ± 0.03[b]	75.62 ± 0.01[e]	7.31 ± 0.01[b]	31.78 ± 0.01[d]	10.52 ± 0.03[a]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

Table 4.12 pH and color values of salad dressings containing 2% (w/v) solution of NBPP extracted with DI water pH 6.0 for 60 min at 90±5°C as fat replacer

Level of oil substitution	pH[1]	Color[1]			
		L*	a*	b*	ΔE*
0%	3.43 ± 0.01[a]	81.24 ± 0.01[a]	5.59 ± 0.01[e]	40.51 ± 0.04[a]	-
25%	3.50 ± 0.01[a]	73.22 ± 0.04[b]	6.25 ± 0.01[c]	29.64 ± 0.01[d]	13.52 ± 0.03[c]
30%	3.49 ± 0.01[a]	72.32 ± 0.07[c]	6.26 ± 0.03[c]	30.53 ± 0.01[b]	13.40 ± 0.00[d]
35%	3.51 ± 0.01[a]	70.91 ± 0.14[d]	7.08 ± 0.06[a]	30.46 ± 0.01[c]	14.48 ± 0.11[b]
40%	3.51 ± 0.02[a]	70.94 ± 0.06[d]	6.84 ± 0.03[b]	29.29 ± 0.02[e]	15.28 ± 0.07[a]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

4.3.2 Color

The L*, a* and b* color values indicate the lightness, redness/greenness, and yellowness/blueness, respectively. Color difference is defined as the difference of L*, a* and b* values between two samples and was used to determine the extent of color difference between RSD and control formula. According to **Table 4.11**, the color of RSD containing acid-extracted NBPP tended to be darker, redder and less yellow when compared to the control formula. Such differences were more observed in the RSD with higher levels of oil substitution, which contained larger amounts of NBPP solution (**Table 4.11** and **Figure 4.3**). For RSD using water-extracted NBPP solution as fat replacer, the significant differences in the color values of the control and all RSD formulas were in a similar manner to those of the RSD containing acid-extracted NBPP solution but to a greater extent, as indicated by the higher ΔE* value (**Table 4.12** and **Figure 4.4)**. At the same level of oil reduction, the L*, a* and b* values of all RSD prepared with acid-extracted NBPP solution were higher than those containing water-extracted NBPP solution, except for the yellowness of both RSD at 25% oil substitution. The ΔE* value of RSD containing NBPP suggested that their color was different from the control formula. The increase in amount of both NBPP significantly increased the color difference. The major reason for the changes in the color of RSD is the reddish brown color of NBPP solutions, especially the red color of acid-extracted NBPP. The lower in lightness of water-extracted NBPP solution led RSD tend to be darker (**Table 4.9** and **Figure 4.2**).

The effect of pectin on color of other reduced-fat products was also found in baked products. For cookies which use pectin-enrich material from apple pomace as a fat replacer, the yellowness of the product decreased with the increase of added pectin material (6). The changes in color of reduced-fat cake and cookies also found when their fat content was substituted by pectin. The added pectin reflected to increase the lightness and yellowness of both baked products, while the redness was decreased (135).

4.3.3 Rheological properties

Rheological properties are related to the thickness, consistency, mouthfeel and creaminess which are the important characteristics of salad dressing. The flow curves of RSD with acid- and water-extracted NBPP are given in **Appendix G**. Apparent viscosities measured at the shear rate of 28 s^{-1}, flow behavior and consistency indices and yield stress of RSD with acid- and water-extracted NBPP are listed in **Table 4.13** and **Table 4.14**, respectively.

Table 4.13 Rheological properties of salad dressings containing 2% (w/v) solution of NBPP extracted with HCl solution pH 1.5 for 60 min at 90±5°C as fat replacer

Level of oil substitution	Apparent viscosity at 28 s^{-1} (cP)[1]	Flow behavior index[1]	Consistency index ($Pa \cdot s^n$)[1]	Yield stress (Pa)[1]
0%	2377 ± 15.28[a]	0.35 ± 0.01[a]	200.72 ± 5.94[b]	87.85 ± 3.87[b]
25%	1623 ± 15.28[b]	0.23 ± 0.01[b]	210.91 ± 7.16[a]	121.10 ± 4.38[a]
30%	1127 ± 11.55[c]	0.22 ± 0.01[b]	151.67 ± 2.64[c]	86.87 ± 5.58[b]
35%	892 ± 7.64[d]	0.23 ± 0.01[b]	114.17 ± 3.87[d]	69.10 ± 3.08[c]
40%	788 ± 7.64[e]	0.23 ± 0.01[b]	102.97 ± 4.38[e]	58.85 ± 4.42[d]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

Table 4.14 Rheological properties of salad dressings containing 2% (w/v) solution of NBPP extracted with DI water pH 6.0 for 60 min at 90±5°C as fat replacer

Level of oil substitution	Apparent viscosity at 28 s^{-1} (cP)[1]	Flow behavior index[1]	Consistency index ($Pa \cdot s^n$)[1]	Yield stress (Pa)[1]
0%	2377 ± 15.28[a]	0.35 ± 0.01[ab]	200.72 ± 5.94[a]	87.85 ± 3.87[a]
25%	1720 ± 10.00[b]	0.30 ± 0.00[d]	175.89 ± 0.29[b]	82.96 ± 1.24[b]
30%	1413 ± 5.77[c]	0.32 ± 0.01[c]	135.50 ± 2.09[c]	59.96 ± 2.24[c]
35%	1190 ± 10.00[d]	0.35 ± 0.01[b]	106.24 ± 2.10[d]	37.64 ± 3.58[d]
40%	1160 ± 17.32[e]	0.37 ± 0.00[a]	99.23 ± 1.95[e]	33.32 ± 1.21[d]

[1]Means ± standard deviation of triplicate samples
[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

The highest viscosity was observed in the control formula. For RSD with acid-extracted NBPP solution, the apparent viscosity significantly decreased when vegetable oil was replaced by NBPP solution (**Table 4.13**). Viscosity of RSD with 40% oil reduction by acid-extracted NBPP was about 3 times lower than that of the control sample. The apparent viscosity of RSD containing water-extracted NBPP as fat replacer also decreased with the increasing level of fat substitution (**Table 4.14**) but the change was at a lesser extent than that of the acid-extracted NBPP (**Table 4.13**). This is probably due to the higher viscosity of water-extracted NBPP solution (**Table 4.6**). The decrease in viscosity of RSD was majorly caused by the decreased fat content, which conduces to the body (viscosity, thickness and cling) and texture (creamy and smooth mouthfeel) of salad dressing (102).

Flow behavior index is the value which indicates the flow behavior of the polymer. Newtonian fluids have flow behavior index of 1; while the values of less than 1 indicate that the fluids are non-Newtonian, of which the viscosity decreased with increasing shear rates (115). Consistency index of a fluid relates directly to its viscosity (69). A viscous fluid thus has higher consistency index than the thinner ones.

In this study, the flow behavior and consistency indices of RSD were determined by measuring the viscosity of the samples at shear rates varied from 0.14 to 28 s^{-1} and fitted the data with the power law equation. The coefficient of determination (R^2) of all data-fitting models ranged from 0.9862-0.9996. All RSD are non-Newtonian fluids with shear-thinning behavior, i.e., pseudoplastic fluid (**Table 4.13** and **Table 4.14**). Flow behavior index of the control formula was significantly higher than the RSD prepared with acid-extracted NBPP solution but no significant difference was observed among four formulas of RSD (**Table 4.13**). For RSD containing water-extracted NBPP solution, there were significant differences in the flow behavior indices of the control salad dressing and RSD at 25 and 30% oil substitution level (**Table 4.14**). However, the flow behavior indices of RSD with higher amounts of water-extracted NBPP (35 and 40% oil substitution) did not significantly different from the control. The decrease in flow behavior index has also been reported in low-fat mayonnaise when the incorporating level of guar gum increased (136). At the same level of oil substitution, flow behavior index of all RSD prepared with NBPP solution from acid extraction was lower than those from water extraction, suggesting the more shear-thinning behavior of RSD. This may be due to the lower M_v of acid-extracted NBPP (**Table 4.4**) which leads to the more lacking of mutual entanglements in the sample (102).

The consistency index of RSD significantly decreased with the increasing level of fat substitution by NBPP solutions. The changes follow the similar manner and corresponded to the viscosity (**Table 4.13**). It should be noted that the consistency indices of RSD containing acid-extracted NBPP were higher than water-extracted NBPP, although their viscosity were lower. It was probably due to the high apparent viscosity at the lowest shear rate (**Figure H.2**) that led to the high consistency index of RSD containing acid-extracted NBPP, especially of the formula with 25% oil reduction. However, the apparent viscosity of those RSD sharply decreased with the increasing shear rate due to their lower flow behavior indices and more shear thinning behavior than the RSD of water-extracted NBPP. This resulted in the lower apparent viscosity, which were measured at the shear rate of 0.14 s^{-1}. Moreover, acid-extracted NBPP might form gel during RSD preparation because the vinegar and sugar in the recipe created the condition that is suitable for HMP gelation. The formed gel of acid-

extracted NBPP thus contributed to the stronger network structure that resulted in the higher viscosity of the respective RSD at low shear rates. There might also be the interaction between the pectin and oil granules in RSD. The study of Liu *et al.* suggested that the interaction between LMP weak-gel and oil granules could provide the structure and fat-like texture to low-fat mayonnaise (134).

Yield stress is the minimum shear stress required to initiate the fluid to flow. It implies the ability of salad dressing to retain its adherence to the surface of salad (137) and also the ease of salad dressing in being poured from its container. The yield stress of the samples usually determined from the rheologram according to the Herschel–Buckley model (69). The yield stress of all salad dressings varied from 33-121 Pa (**Table 4.13** and **Table 4.14**). Incorporations of both NBPP in RSD resulted in the lower yield stress compared to the control formula, except that the RSD containing acid-extracted NBPP solution at 25% oil substitution had the highest yield stress. This phenomenon could be explained by the same argument for consistency index as mentioned earlier. Yield stress of all RSD tended to decrease with increasing oil substitution level. At the same level of oil substitution, RSD prepared with water-extracted NBPP solution exhibited lower yield stress than the RSD with acid-extracted NBPP. Ma and Boye reviewed that due to the fact that salad dressing is a gel-like material, the decrease in yield stress of salad dressing indicated the weakening gel structure of the dressing emulsion which resulted in the more ease to pour or flow (102).

4.3.4 Stability

Stability of salad dressing was evaluated by observing phase separation in RSD stored at room temperature during 2 wk. There was no observed separation of cream layer or oil phase in all salad dressing within 2 wk of the stability test (data not presented). This is due to the high viscosity of the aqueous phase of RSD which is contributed by the NBPP solution (**Table 4.13** and **Table 4.14**). Although the viscosities of all RSD were lower than that of the control formula, the aqueous phases of RSD were still viscous enough to provide the stability to the emulsion. The addition of thickening agent such as gums or starch to the aqueous phase of low-fat product is known to provide the stability to the emulsion. Mun *et al.* suggested that the addition

of thickening agent such as gums or starch to the aqueous phase of low-fat product can slow down the droplet movement and prevent creaming of the emulsion. In addition, a reduced-fat mayonnaise containing modified rice starch and xanthan gum was stable after 1 mo storage at room temperature due to its resistant to droplet coalescence which causes the instability of emulsion (138). Another probable reason is that since NBPP were able to adsorb both water and oil (**Table 4.8**), they thus could be able to provide emulsification effect by aligning itself at the oil-water interface and stabilize the emulsion (139).

4.3.5 Sensory characteristics

The key factor in evaluating the potential of a fat replacer is its ability to mimic the functional properties of fat in food products. Therefore, fat reduction can be achieved while the sensory characteristics and consumer acceptability of the reduced-fat product are maintained. The effect of oil substitution with NBPP on the sensory quality of salad dressing was investigated. RSD containing NBPP at different oil substitution levels were evaluated for their color, thickness, smoothness and taste by 20 untrained panelists using five-point just-about-right scale. The distribution of rating from just-about-right test of RSD prepared with acid- and water-extracted NBPP are listed in **Table 4.15** and **Table 4.16**, respectively.

Table 4.15 indicated that the highest frequency of "just-about-right" on color attribute was observed in the control salad dressing (0% oil substitution). The color of RSD prepared with acid-extracted NBPP at 25 and 30% oil substitution were perceived by 50 and 55% of panelist as "just-about-right". The rating of the majority of panelists on color of RSD changed from "just-about-right" to "too dark" when the oil substation level was further increased to up to 40%. This corresponded to the decrease in L* and increase in ΔE* values of those samples (**Table 4.11**) and indicated that such difference in color was detectable by the panelists. Substantial change was observed in the thickness score. Oil substitution seemed to improve the thickness of the RSD since 55% of the panelist rated the control formula as "much too thick" while those with 25 and 30% oil substitution were rated to have appropriate thickness by more than one-third of the panelist. At higher levels of oil substitution, the thickness of RSD was majorly rated as "too thin". The change in thickness score of the RSD

containing acid-extracted NBPP followed the decreases in viscosity and other rheological parameters when the oil substitution level increased (**Table 4.13**). The results suggested that the decrease in viscosity of RSD due to the oil substitution by acid-extracted NBPP was detectable by the panelists and that the appropriate viscosity of the RSD was in the range of 1000-1600 cP. The major distribution of ratings on smoothness and taste of all RSD was at just-about-right.

Table 4.15 Distribution of rating on just-about-right scale of salad dressings containing 2% (w/v) solution of NBPP extracted with HCl solution pH 1.5 for 60 min at 90±5°C as fat replacer

Sensory attribute	Level of oil substitution	Distribution[1]				
		Much too weak	Too weak	Just-about-right	Too strong	Much too strong
Color	0%	-	5(25)	12(60)	2(10)	1(5)
	25%	-	1(5)	11(55)	8(40)	-
	30%	-	1(5)	10(50)	9(45)	-
	35%	-	-	9(45)	10(50)	1(5)
	40%	2(10)	2(10)	7(35)	9(45)	-
Thickness	0%	-	-	5(25)	4(20)	11(55)
	25%	1(5)	1(5)	15(75)	3(15)	-
	30%	-	4(20)	13(65)	3(15)	-
	35%	4(20)	12(60)	3(15)	-	1(5)
	40%	10(10)	15(75)	3(15)	-	-
Smoothness	0%	-	2(10)	18(90)	-	-
	25%	-	2(10)	17(85)	1(5)	-
	30%	-	-	20(100)	-	-
	35%	-	1(5)	17(85)	2(10)	-
	40%	-	1(5)	14(70)	4(20)	1(5)
Taste	0%	-	2(10)	11(55)	7(35)	-
	25%	-	1(5)	8(40)	9(45)	2(10)
	30%	-	-	8(40)	9(45)	3(15)
	35%	1(5)	2(10)	9(45)	8(40)	-
	40%	1(5)	2(10)	12(60)	4(20)	1(5)

[1] Frequency from 20 panelists with %distribution in parenthesis

For RSD prepared with water-extracted NBPP, the effect of oil substitution on the score distribution was closed to RSD containing acid-extracted NBPP (**Table 4.16)**. Majority of color rating changed from "just-about-right" in the control recipe to "too dark", when 25-40% of oil in the formula was substituted with water-extracted NBPP. Such difference was more observable than that of the RSD prepared from acid-extracted NBPP (**Table 4.15**). This corresponded well with the lower L* and higher ΔE* values of the RSD with water-extracted NBPP than that of RSD containing acid-extracted NBPP at the same level of oil substitution (**Table 4.11** and **Table 4.12**). The appropriateness level of oil substitution on thickness attribute was 25 and 35% which their RSD were rated as "just-about-right" by a greater number of panelists (50 and 60%, respectively). The decrease in thickness of the RSD containing water-extracted NBPP when the oil substitution level increased was less perceived by the panelist. At 35 and 40% oil substitution levels, the distribution of just-about-right rating on the thickness were 20-25% which was higher than those of RSD containing water-extracted NBPP (**Table 4.14**). At all levels of oil substitution, water-extracted NBPP gave RSD with the greater distribution of thickness score of 3 than acid-extracted NBPP because their higher viscosity (**Table 4.13** and **Table 4.14**). The results of smoothness and taste indicated that all developed RSD were majorly perceived as "just-about-right".

Table 4.16 Distribution of rating on just-about-right scale of salad dressings containing 2% (w/v) solution of NBPP extracted with DI water pH 6.0 for 60 min at 90±5°C as fat replacer

Sensory attribute	Level of oil substitution	Distribution[1]				
		Much too weak	Too weak	Just-about-right	Too strong	Much too strong
Color	0%	1(5)	5(25)	13(65)	1(5)	-
	25%	-	-	8(40)	12(60)	-
	30%	-	-	7(35)	13(65)	-
	35%	-	-	6(30)	13(65)	1(5)
	40%	-	-	2(10)	14(70)	4(20)
Thickness	0%	-	-	3(15)	4(20)	13(65)
	25%	-	6(30)	10(50)	4(20)	-
	30%	-	7(35)	12(60)	1(5)	-
	35%	3(15)	11(55)	4(20)	1(5)	1(5)
	40%	2(10)	12(60)	5(25)	-	1(5)
Smoothness	0%	-	2(10)	14(70)	3(15)	1(5)
	25%	-	1(5)	13(65)	4(20)	2(10)
	30%	-	-	13(65)	4(20)	3(15)
	35%	-	-	12(60)	4(20)	4(20)
	40%	-	-	12(60)	5(25)	3(15)
Taste	0%	-	-	15(75)	5(25)	-
	25%	-	3(15)	13(65)	3(15)	1(5)
	30%	1(5)	1(5)	11(55)	6(30)	1(5)
	35%	-	3(15)	9(45)	7(35)	1(5)
	40%	-	2(10)	11(55)	7(35)	-

[1] Frequency from 20 panelists with %distribution in parenthesis

Based on the results from sensory evaluation, 30% oil substitution level seemed to be suitable for the application of NBPP as fat replacer in salad dressing because all sensory attributes of RSD at this level of oil substitution was majorly rated as “just-about-right”. Therefore, the RSD with 30% oil substitution level were selected for the acceptability test by 50 panelists. The test was conducted in order to determine whether the change in the characteristics of salad dressing, which was caused by the substitution of oil with NBPP, affect the consumer’s preference on RSD.

The acceptability scores of the control formula and the RSD were presented in **Table 4.17**. Significant differences were found only in the appearance and color scores. Substitution of 30% oil with any NBPP resulted in a slight decrease in the hedonic score of appearance from 6.5, which was between “like slightly” to “like moderately”, to 5.8 which was closer to “like slightly”. Similarly, the color acceptability score also decreased from “light moderately” to about “like slightly” when 30% oil in the control formula was substituted with NBPP. The lower acceptability in the color of RSD corresponded with their ratings as “too dark” (**Table 4.15** and **Table 4.16**), owing to the darker color of RSD as compared with the full-fat dressings (**Table 4.11** and **Table 4.12**). It is noteworthy that the lower viscosity of both RSD (**Table 4.13** and **Table 4.14**) did not affect their hedonic scores on thickness and overall acceptability, although the thickness of RSD prepared with water-extracted NBPP as fat replacer at 30% fat substitution level was rated between “too thin” and “just-about-right” (**Table 4.16**). The results suggested that the both RSD were acceptable by the consumer. Therefore, both of water- and acid-extracted NBPP have potential to use as fat replacers in salad dressing at oil substitution level of up to 30%.

Table 4.17 Sensory acceptability scores of control and reduced-fat salad dressings containing 2% (w/v) NBPP solutions as a fat replacer at 30% oil substitution level

NBPP solution	Level of oil substitution	Appearance[1,2]	Color[1,2]	Thickness[1,2]	Smoothness[1,2]	Taste[1,2]	Overall acceptability[1,2]
-	0% (Control)	6.56 ± 1.39[a]	7.02 ± 1.10[a]	5.44 ± 1.74[a]	6.62 ± 1.35[a]	6.60 ± 1.40[a]	6.74 ± 1.23[a]
Acid-extracted	30%	5.84 ± 1.58[b]	5.74 ± 1.60[b]	5.98 ± 1.41[a]	6.66 ± 1.49[a]	6.62 ± 1.61[a]	6.56 ± 1.61[a]
Water-extracted	30%	5.86 ± 1.47[b]	6.04 ± 1.35[b]	5.82 ± 1.52[a]	6.52 ± 1.34[a]	6.46 ± 1.75[a]	6.48 ± 1.62[a]

[1] Rated on 9-point hedonic scale: 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely

[2] Means ± standard deviation from 50 panelists

[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)