รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืช สำหรับอาหารที่มีอายุการเก็บรักษาสั้น

โดย สิริยุภา เนตรมัย และคณะ

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สัญญาเลขที่ MRG5980156

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

รหัสโครงการ: MRG5980156

ชื่อโครงการ: การพัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืช สำหรับอาหารที่มีอายุการเก็บรักษาสั้น

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ระยะเวลาโครงการ: 3 ปี

บทคัดย่อ:

บรรจุภัณฑ์อัจฉริยะ (Intelligent packaging) สามารถให้ข้อมูลของคุณภาพของผลิตภัณฑ์ในขณะนั้น ตั้งแต่คุณภาพด้านทางประสาทสัมผัสไปจนถึงคุณสมบัติทางจุลินทรีย์ โดยไม่จำเป็นต้องเปิดบรรจุภัณฑ์ออกสำรวจ ด้วยการใช้เครื่องมือซึ่งสามารถติดตามปริมาณของสารที่มีการเปลี่ยนแปลงปริมาณในระหว่างกระบวนการเสื่อมเสียของผลิตภัณฑ์อาหาร เทคโนโลยีใหม่นี้สามารถช่วยให้ผู้บริโภคสามารถตัดสินใจเลือกซื้อสินค้าได้ดีขึ้น และยังอาจช่วยประกันความปลอดภัยของผู้บริโภคได้อีกด้วย โครงการวิจัยนี้มุ่งที่จะพัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืชที่มีสารแอนโทไซยานินสูง เพื่อนำไปใช้กับระบบบรรจุภัณฑ์อัจฉริยะ สำหรับอาหารที่มีอายุการเก็บรักษาสั้น โดยเริ่มจากการทำการคัดเลือกพืชที่มีความสามารถในการเปลี่ยนสีในระดับที่มองเห็นได้ด้วยตาเปล่าเมื่ออยู่ในสภาวะความเป็นกรด-ด่างที่แตกต่างกัน จากการทดลองผสมสารสกัดของพืชนั้นๆ เข้ากับสารละลายบัฟเฟอร์ที่ค่าความเป็นกรด-ด่าง 3.0 5.0 และ 7.0 เมื่อได้พืชที่ผ่านการคัดเลือกแล้วจึงนำไปศึกษาหาเงื่อนไขการสกัดด้วยน้ำร้อนด้วยคลื่นไมโครเวฟ (Microwave assisted hot water extraction) ที่เหมาะสม โดยใช้เทคนิคพื้นผิวผลตอบ (Response surface methodology หรือ RSM) และการวิเคราะห์ความพึงพอใจ (Desirability analysis) พารามิเตอร์ที่ทำการศึกษา ได้แก่ สัดส่วนของตัวอย่างต่อน้ำ (1:5 – 1:3 กรัม/มิลลิลิตร) พลังงานที่ใช้ในการสกัด (480 – 800 วัตต์) และเวลาที่ใช้ในการสกัด (60 – 480 วินาที) เงื่อนไขที่เลือกใช้ควรให้สารสกัดที่มีค่าการดูดกลืนที่ค่าความยาวคลื่นที่มีค่าการดูดกลืนสูงสุด (λ_{max}) สูงสุด โดยไม่ส่งผลกระทบกับคุณภาพของสารสกัด เมื่อได้เงื่อนไขการสกัดสารสกัดจากพืชที่เหมาะสมแล้ว จึงใช้เงื่อนไขดังกล่าวในการสกัดสารที่จะใช้ในอินดิเคเตอร์ต่อไป ในขั้นตอนต่อไป ทำการศึกษาเพื่อระบุสูตรที่เหมาะสมของฟิล์มเปลี่ยนสีได้ ด้วยเทคนิคพื้นผิวผลตอบ และการวิเคราะห์ความพึงพอใจ สูตรที่เลือกใช้ควรให้ฟิล์มที่มีค่าความต่างของสี (ΔE) ที่ค่าความเป็นกรด-ด่างต่างกันสูง และมีค่าการละลายต่ำ จากนั้นทำการศึกษาหาระดับของ

สารละลายที่เหมาะสมที่จะใช้ในฟิล์มเปลี่ยนสี ด้วยการทดสอบทางประสาทสัมผัส ด้วยวิธีการทดสอบความแตกต่าง แบบ Two out of five กับผู้ทดสอบที่ไม่ผ่านการฝึกฝนจำนวน 50 คน เมื่อสูตรที่เหมาะสมแล้วจึงทำการผลิตฟิล์มเปลี่ยนสี แล้วนำไปวิเคราะห์คุณสมบัติด้านต่างๆ (ได้แก่ คุณสมบัติทางกายภาพ ลักษณะปรากฏ สัณฐานวิทยา เชิงกล และทางความร้อน) ศึกษากลไกการเปลี่ยนสี ประมาณอายุการเก็บรักษา ทดสอบความสามารถในการเปลี่ยนสีเมื่อความเป็นกรด-ด่าง หรือความเข้มข้นของก๊าซคาร์บอนไดออกไซด์ของระบบเปลี่ยนไป และประเมินประสิทธิภาพการใช้งานจริงกับผลิตภัณฑ์อาหาร โดยผลิตภัณฑ์อาหารที่มีอายุการเก็บรักษาสั้นที่เลือกใช้ ได้แก่ ผลไม้ และผลไม้ตัดแต่ง ปลาส้ม แหนมเห็ด และเต้าหู้ไข่ ในงานวิจัยนี้ได้ทำการเลือกกะหล่ำปลีม่วง (*Brassica oleraces* var. *capitate* f. *rubra*) ดอกกล้วยไม้สกุลหวาย (*Dendrobium* Sonia ‘Earsakul’) และดอกอัญชัน (*Clitoria ternatea* L.) มาใช้ในการพัฒนาฟิล์มเปลี่ยนสีได้ โดยเงื่อนไขการสกัดด้วยน้ำร้อนด้วยคลื่นไมโครเวฟที่เหมาะสมในการสกัดสารจากพืชดังกล่าว คือ สัดส่วนของตัวอย่างต่อน้ำ 1:3 กรัม/มิลลิลิตร พลังงานที่ใช้ในการสกัด 800 วัตต์ และเวลาที่ใช้ในการสกัดนาน 480 วินาที สำหรับกะหล่ำปลีม่วงและดอกกล้วยไม้สกุลหวาย และ 180 วินาที สำหรับดอกอัญชัน สูตรที่เหมาะสมสำหรับเตรียมฟิล์มเปลี่ยนสีได้ที่มีส่วนผสมของสารสกัดจากกะหล่ำปลีม่วงหรือดอกกล้วยไม้สกุลหวาย คือ คาราจีแนนร้อยละ 3 เพคตินร้อยละ 2 ผงเซลลูโลสร้อยละ 1 และสารสกัดร้อยละ 40 และสูตรที่เหมาะสมสำหรับฟิล์มที่มีสารสกัดจากดอกอัญชัน คือ CMC ร้อยละ 1.5 คาราจีแนนร้อยละ 1.5 เพคตินร้อยละ 1.5 ผงเซลลูโลสร้อยละ 1 และสารสกัดร้อยละ 6 จากการทดสอบความสามารถในการเปลี่ยนสีที่ค่าความเป็นกรด-ด่างต่างๆ พบว่า โดยรวม ฟิล์มให้ค่า ΔE ระหว่างคู่ความเป็นกรด-ด่างต่างกัน สูง โดยเฉพาะฟิล์มที่ประกอบด้วยสารสกัดจากดอกกล้วยไม้สกุลหวาย แต่พบว่า ฟิล์มที่มีส่วนผสมของสารสกัดจากกะหล่ำปลีม่วงและดอกอัญชันนั้น มีสีใกล้เคียงกันมากที่ค่าความเป็นกรด-ด่างในช่วง 4-6 ซึ่งทำให้ยากแก่การแยกแยะด้วยตาเปล่า ดังผลจากการทดสอบทางประสาทสัมผัส ซึ่งใช้ผู้ทดสอบที่ไม่ผ่านการฝึกฝน และพบว่า ฟิล์มที่พัฒนาได้ทั้งหมดมีความสามารถในการเปลี่ยนสีที่ความเข้มข้นของคาร์บอนไดออกไซด์ต่างกัน ต่ำ โดยสีของฟิล์มเปลี่ยนไปจนสังเกตเห็นได้เมื่ออยู่ในสภาวะที่มีก๊าซคาร์บอนไดออกไซด์เข้มข้นร้อยละ 75 หรือสูงกว่านั้น เมื่อนำฟิล์มเปลี่ยนสีได้ ไปทำการประกอบเป็นอินดิเคเตอร์แล้วทดลองใช้กับผลิตภัณฑ์อาหารที่มีอายุการเก็บรักษาสั้น พบว่า อินดิเคเตอร์ที่พัฒนาได้นั้นไม่เหมาะที่จะนำไปใช้เพื่อบ่งบอกความปลอดภัยของอาหาร เนื่องจากความสามารถในการเปลี่ยนสีของอินดิเคเตอร์ไม่สูงมากพอ แต่มีความเป็นไปได้ที่จะนำไปใช้บอกคุณภาพของอาหาร โดยเฉพาะอาหารประเภทอาหารหมัก นอกจากนั้น ยังพบว่าเมื่อนำอินดิเคเตอร์ไปใช้กับอาหารบางชนิด สีของอินดิเคเตอร์ซีดลงไปภายหลังจากการใช้ไปเพียง 2-3 วันอีกด้วย อินดิเคเตอร์ที่พัฒนาได้ สามารถเก็บไว้ในบรรจุภัณฑ์ที่ปิดสนิท ณ อุณหภูมิห้อง ได้นาน 2-4 สัปดาห์

คำหลัก : อินดิเคเตอร์วัดความเป็นกรด-ด่าง; บรรจุภัณฑ์อาหาร; แอนโทไซยานิน; กล้วยไม้สกุลหวาย; อัญชัน

Abstract

Project Code : **MRG5980156**

Project Title : **Development of Colorimetric Indicators for pH and CO_2 from Bio-based Materials and Plant Extract, for Short Shelf-life Foods**

Investigator : **Siriyupa Netramai, Ph.D. Faculty of Science, Mahidol University**

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Project Period : **3 years**

Abstract:

Intelligent packaging could inform users on current quality of its content, ranging from sensorial properties to microbiological status of the food, without having to open the package. By incorporating devices that could monitor levels of compounds that are declining or generating due to deteriorative changes continuously occurred in food, this novel technology could assist the consumers on their buying decision or ensure safety of the consumers. This study was aimed to develop bio-based colorimetric indicators for pH and CO_2 consisting of extract from anthocyanin-rich plant, to be used as part of intelligent packaging system for short shelf-life foods. Several plants were screened for their ability to visibly change color when exposed to different pH by mixing plant extract with pH buffer solution (pH 3.0, 5.0, or 7.0). The optimal conditions for microwave assisted hot water extraction (mHWE) for all selected plants were investigated using response surface methodology (RSM) and desirability analysis. The parameters studied were sample to water ratio (1:5 - 1:3 g/mL), extraction power (480 - 800 W), and extraction time (60 - 480 s). The extraction condition should give extract with maximum absorbance at λ_{max} without affecting quality of the extracts. The optimal mHWE conditions were then used to prepare plant extracts to be incorporated into colorimetric films. RSM and desirability analysis were used to optimize the colorimetric film formula. The formula should give the film with high color change in response to pH (ΔE) and low solubility. Most suitable amounts of plant extracts for the film were finalized using sensory testing (Difference test; Two out of five) by 50 untrained panelists. The developed colorimetric films were then characterized their physical-, optical-, morphological, mechanical-, and thermal properties; studied their color-changing mechanism; determined their shelf-lives; and tested for their sensitivity and performance as pH- and CO_2 indicator. Short shelf-life foods included in

the study were fruits and fresh-cut fruits, fermented fish and mushroom, and egg tofu. The plants selected for development of colorimetric layer were fresh red cabbage (*Brassica oleraces* var. *capitate* f. *rubra*) and Dendrobium orchid (*Dendrobium* Sonia 'Earsakul'), and dried butterfly pea (*Clitoria ternatea* L.). Optimal condition of mHWE were sample to water ratio of 1:30 g/mL and power of 800 W, and extraction time of 480 s for red cabbage and Dendrobium orchid, and 180 s for butterfly pea. The final formula for colorimetric layers with red cabbage or Dendrobium orchid extract consisted of 3% (w/w) of carrageenan, 2% (w/w) of pectin, 1% (w/w) of cellulose powder, and 40 % (v/w) of extract; and 1.5% (w/w) of CMC, 1.5% (w/w) of carrageenan, 1.5% (w/w) of pectin, 1% (w/w) of cellulose powder, and 6 % (v/w) of extract for colorimetric layer with butterfly pea. Regarding sensitivity to pH, it was found that the films generally gave high ΔE between different pH values, especially the film with Dendrobium orchid extract. However, for red cabbage and butterfly pea, the films' colors at pH 4-6 were difficult to distinguish, according to sensory evaluation by untrained panelists. All films had low sensitivity to CO_2. It was found that significant color changes occurred when exposed to CO_2 concentration of 75 or higher. As pH indicator for short shelf-life foods, it was found that the developed indicators was not suitable to be used as safety measure for food products, but might have potential use in informing the consumers about the foods' qualities, especially for fermented foods since the sensitivity of the indicators were not high enough. When used on some food samples, the indicators' colors were visibly fading after 2-3 days of use. The developed indicators could be kept for 2-4 weeks in air-tight container, at ambient storage.

Keywords : pH indicator; Food packaging; Anthocyanin; Dendrobium orchid; Butterfly pea

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We are immensely grateful for our families and friends for their patience, understanding, and care they have all been giving us during our times working on this project.

The research team

Table of Contents (สารบัญ)

Table of Figures (สารบัญภาพ)

Table of Schemes (สารบัญแผนภาพ)

Table of Tables (สารบัญตาราง)

Executive summary

โครงการ: การพัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืช สำหรับอาหารที่มีอายุการเก็บรักษาสั้น

วัตถุประสงค์ของการวิจัย:

โครงการวิจัยนี้มุ่งที่จะพัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืช เพื่อนำไปใช้เป็นส่วนประกอบในระบบบรรจุภัณฑ์อัจฉริยะ สำหรับอาหารที่มีอายุการเก็บรักษาสั้น โดยมีวัตถุประสงค์หลักและย่อย ดังนี้

1. พัฒนาอินดิเคเตอร์เปลี่ยนสีบ่งชี้ความเป็นกรด-ด่างและปริมาณก๊าซคาร์บอนไดออกไซด์จากวัสดุชีวภาพและสารสกัดจากพืช ซึ่งมีความคงตัวมากพอที่จะนำไปใช้ในระบบบรรจุภัณฑ์สำหรับอาหารที่มีอายุการเก็บรักษาสั้น
    1.1. คัดเลือกพืชที่มีรงควัตถุที่สามารถเปลี่ยนสีได้เมื่ออยู่ในสภาวะที่มีการเปลี่ยนแปลงของสภาพความเป็นกรด-ด่างของสิ่งแวดล้อม
    1.2. ระบุเงื่อนไขที่เหมาะสมของการสกัดด้วยน้ำร้อนด้วยคลื่นไมโครเวฟของพืชที่คัดเลือกมาทำการศึกษา
    1.3. ระบุสูตรที่เหมาะสำหรับใช้ในการผลิตฟิล์มเปลี่ยนสีได้ที่มีส่วนประกอบเป็นสารสกัดจากพืชที่คัดเลือกมาใช้
    1.4. วิเคราะห์คุณสมบัติทั่วไปของฟิล์มเปลี่ยนสีได้ และคุณสมบัติด้านความสามารถในการเปลี่ยนสีเมื่ออยู่ในสภาวะที่ความเป็นกรด-ด่าง หรือความเข้มข้นของก๊าซคาร์บอนไดออกไซด์ เปลี่ยนไป
    1.5. ศึกษากลไกการเปลี่ยนสีของฟิล์มเปลี่ยนสีได้
    1.6. ประเมินอายุการเก็บรักษาของอินดิเคเตอร์ที่มีส่วนประกอบของฟิล์มเปลี่ยนสีได้ที่พัฒนาขึ้น
2. ตรวจสอบความเป็นไปได้ในการนำอินดิเคเตอร์ที่พัฒนาได้ไปใช้เป็นส่วนหนึ่งของบรรจุภัณฑ์อาหาร

วิธีดำเนินการวิจัย:

เพื่อให้การวิจัยบรรลุตามจุดประสงค์ที่ได้วางไว้ การศึกษานี้ได้แบ่งการทดลองออกเป็นขั้นตอนดังนี้

1. **การคัดเลือกพืชเพื่อนำมาทำการสกัดสารสี** (จุดประสงค์ 1.1)

ทำการศึกษาหาข้อมูลในเบื้องต้นเกี่ยวกับพืชที่มีการรายงานว่า เคยมีการนำมาใช้เป็นอินดิเคเตอร์วัดความเป็นกรด-ด่าง และ/หรือ มีรงควัตถุประเภทแอนโทไซยานินอยู่เป็นจำนวนมาก แล้วทำการสกัดสารสีด้วยน้ำร้อน นำสารสีที่สกัดได้ไปปรับค่าความเป็นกรด-ด่าง ด้วยการผสมกับสารบัฟเฟอร์ที่มี

ค่าความเป็นดรด-ด่าง 3-7 จากนั้นทำการเปรียบเทียบด้วยตาเปล่าและวัดสีด้วยเครื่อง Colorimeter[1, 2] เพื่อเลือกพืชที่ให้สารสกัดที่มีการเปลี่ยนสีที่ชัดเจน สามารถสังเกตได้ด้วยตาเปล่า ในช่วงความเป็นกรด-ด่างดังกล่าว

2. **การระบุเงื่อนไขของการสกัดสารสกัดจากพืชด้วยน้ำร้อนด้วยคลื่นไมโครเวฟ** (จุดประสงค์ 1.2)

ทำการคัดเลือกเงื่อนไขของการสกัดสารสกัดจากพืชที่คัดเลือกได้จากขั้นตอนแรก ด้วยน้ำร้อนด้วยคลื่นไมโครเวฟ (Microwave-assisted hot water extraction หรือ mHWE) ที่มีประสิทธิภาพการสกัดสูงสุด คือ เป็นการสกัดที่ให้ค่าการสกัด (Yield) สูง และไม่ทำให้คุณสมบัติของสารสีที่สกัดได้เปลี่ยนไป โดยใช้การวางแผนการทดลองแบบ Response surface methodology (RSM) แบบ Box-Behnken และได้กำหนดค่าพารามิเตอร์ที่ทำการทดสอบ คือ 1) อัตราส่วนของพืชต่อน้ำ 2) ระดับพลังงานของเครื่องไมโครเวฟ และ 3) เวลาที่ใช้ในการสกัด ส่วนค่าตอบสนอง (Response) ที่ทำการตรวจวัด คือ ค่าการดูดกลืนแสงที่ λ_{max} ด้วยเครื่อง UV-Visible spectrophotometer ซึ่งใช้เป็นค่าบ่งชี้ค่าการสกัด แล้วทำการวิเคราะห์ความพึงพอใจ (Desirability analysis) เพื่อระบุเงื่อนไขที่เหมาะสม โดยต้องการเงื่อนไขการสกัดที่ให้ค่าการสกัดสูง และใช้การเปรียบเทียบ สเปกตรัมในช่วงความยาวคลื่น 400-700 nm ของสารสกัดที่สกัดด้วยเงื่อนไขแตกต่างกันในการการตรวจสอบคุณสมบัติด้านสีที่อาจเปลี่ยนไปของสารสกัด

3. **การพัฒนาสูตรของฟิล์มเปลี่ยนสีได้ที่มีส่วนประกอบของสารสกัดจากพืช** (จุดประสงค์ 1.3)

ทำการพัฒนาชั้นฟิล์มเปลี่ยนสีได้ (Colorimetric layer) โดยเริ่มจากการทำการทดลองเบื้องต้นเพื่อหาช่วงของส่วนประกอบที่จะใช้ในการทดลอง ซึ่งส่วนประกอบของฟิล์มที่ทดลองประกอบด้วย Carboxymethyl cellulose (CMC) คาราจีแนน เพคติน แป้งกล้วย ผงเส้นใยเซลลูโลส และสารสีที่สกัดจากพืช เมื่อได้ช่วงของส่วนประกอบของฟิล์มที่จะทำการศึกษา จึงนำไปออกแบบการทดลองด้วยการใช้ RSM ในการวางแผนการทดลอง จากนั้นทำการเตรียมตัวอย่างฟิล์มเปลี่ยนสีได้ มาทำการวิเคราะห์ 1) ค่าความสามารถในการเปลี่ยนสี (ΔE) ที่ค่าความเป็นกรด-ด่าง 3 5 และ 7[1] 2) ค่าการละลายของฟิล์ม[3] และ 3) ค่าการพองตัว เพื่อนำไปวิเคราะห์ความพึงพอใจ ระบุสูตรที่เหมาะสมที่สุดของชั้นฟิล์มเปลี่ยนสีได้สำหรับสารสกัดจากพืชแต่ละชนิด โดยต้องการให้ฟิล์มมีค่า ΔE ที่ค่าความเป็นกรด-ด่างต่างๆ กันสูง และค่าการละลายและค่าการพองตัวต่ำ

จากนั้นทำการทดลองเพื่อปรับปริมาณสารสกัดที่ใช้ในการเตรียมฟิล์มเปลี่ยนสีได้อีกครั้ง โดยทำการเตรียมฟิล์มจากสูตรที่เหมาะสมที่สุด แต่ผสมสารสกัดลงในฟิล์มในปริมาณที่แตกต่างกัน (ช่วงของปริมาณสารสกัดนี้ได้ทำการทดลองเบื้องต้นไว้ก่อนแล้ว แต่ไม่ได้นำไปเป็นตัวแปรหนึ่งในการทดสอบหาสูตรที่เหมาะสม เนื่องจากต้องการศึกษาตัวแปรของสารก่อเจลหลายชนิดเป็นหลัก จึงได้เลือกใช้สารสกัดในปริมาณที่อยู่ในช่วงที่จะศึกษาเพียงค่าเดียวก่อน) จากนั้นทำการวัดค่า ΔE ที่ค่าความเป็นกรด-ด่าง 3 5 และ 7 แล้วเลือกปริมาณสารสกัดที่ให้ค่า ΔE สูงที่สุด[4]

4. **การวิเคราะห์คุณสมบัติของฟิล์มเปลี่ยนสีได้ และคุณสมบัติด้านความสามารถในการเปลี่ยนสีตามค่าความเป็นกรด-ด่าง หรือปริมาณของก๊าซคาร์บอนไดออกไซด์ที่เปลี่ยนแปลงไป** (จุดประสงค์ 1.4)

นำฟิล์มเปลี่ยนสีได้ที่พัฒนาได้ มาวิเคราะห์คุณสมบัติทางกายภาพ สัณฐานวิทยา ทางกล และทางการเปลี่ยนแปลงอุณหภูมิ โดยเปรียบเทียบกับฟิล์มที่เตรียมจากสูตรที่เหมาะสมที่สุดแต่ไม่มีสารสกัด

ในการตรวจสอบความสามารถในการเปลี่ยนสีตามค่าความเป็นกรด-ด่างของฟิล์มนั้น ทำการจุ่มฟิล์มที่พัฒนาได้ลงในสารละลายบัฟเฟอร์ที่มีค่าความเป็นกรด-ด่าง 2 3 4 5 6 และ 7 แล้วทำการวัดสีของฟิล์มด้วยเครื่อง Colorimeter[1] จากนั้นนำมาคำนวณค่า ΔE ของคู่ฟิล์ม ณ ค่าความเป็นกรด-ด่างที่ต่างกัน โดยคู่ฟิล์มที่มี ค่า ΔE ต่ำกว่า 12.0[4] ถูกนำไปทำการทดสอบทางประสาทสัมผัสแบบการทดสอบความแตกต่าง 'Two out of five' กับผู้เข้าร่วมการทดสอบที่ไม่ผ่านการอบรม (Untrained panelist) จำนวน 50 คน เป็นการเพิ่มเติม เพื่อตรวจสอบว่า ผู้บริโภคจะสามารถแยกแยะสีของฟิล์มที่ค่าความเป็นกรด-ด่างคู่ดังกล่าวออกจากกันได้หรือไม่

ในการตรวจสอบความสามารถในการเปลี่ยนตามปริมาณก๊าซคอร์บอนไดออกไซด์ของฟิล์มนั้น มีขั้นตอนการทดลองเช่นเดียวกับกรณีของการศึกษาการเปลี่ยนสีตามค่าความเป็นกรด-ด่าง แต่ในขั้นตอนเตรียมตัวอย่างฟิล์มใช้การอบฟิล์มไว้ในบรรยากาศที่มีก๊าซ CO_2 เข้มข้นร้อยละ 0 25 50 75 และ 100 แทน

5. **การศึกษากลไกการเปลี่ยนสี** (จุดประสงค์ 1.5)

ทำการศึกษากลไกการเปลี่ยนสีตามค่าความเป็นกรด-ด่างที่เปลี่ยนไปของฟิล์มเปลี่ยนสีได้ ด้วยการนำฟิล์มที่พัฒนาได้มาจุ่มลงในสารละลายบัฟเฟอร์ที่มีค่าความเป็นกรด-ด่าง 2 3 5 และ 7 นำไปทำให้แห้งด้วยการทำแห้งด้วยการแช่เยือกแข็ง (Freeze drying) แล้วเตรียมตัวอย่างเพื่อเข้าเครื่อง Fourier transformed infrared (FTIR) spectrophotometer[5, 6] จากนั้นศึกษา IR สเปกตรัมที่ได้ เปรียบเทียบกับกลไกการเปลี่ยนสีตามการเปลี่ยนค่าความเป็นกรด-ด่างของรงควัตถุแอนโทไซยานิน

6. **การตรวจสอบการใช้งานจริงของอินดิเคเตอร์ที่พัฒนาได้ กับผลิตภัณฑ์อาหาร** (จุดประสงค์ 2)

นำฟิล์มเปลี่ยนสีได้ที่พัฒนาขึ้น มาเตรียมเป็นอินดิเคเตอร์ ด้วยการนำไปหุ้มด้วยฟิล์มเซลโลเฟน (Cellophane) และแผ่นพอลิเมอร์ประเภท Polylactide (PLA) เจาะรอยปรุ และมีแผ่นพลาสติกสีขาวทึบรองอีกชั้น เพื่อให้สามารถสังเกตสีของฟิล์มได้ชัดเจน จากนั้นนำอินดิเคเตอร์ที่เตรียมได้มาแปะไว้บนพื้นผิวของอาหาร ทำการตรวจติดตามการเปลี่ยนสีของอินดิเคเตอร์อย่างสม่ำเสมอ (วัดสีด้วยเครื่อง Colorimeter) พร้อมกับบันทึกค่าความเป็นกรด-ด่าง และปริมาณจุลินทรีย์ที่มีชีวิตทั้งหมด (Aerobic plate count หรือ APC) ของอาหารที่เปลี่ยนไปตามระยะการเก็บรักษา[7] โดยตัวอย่างผลิตภัณฑ์อาหารที่ใช้ ได้แก่ ปลาสัม แหนมเห็ด เต้าหู้ไข่ ผลไม้สด และผลไม้ตัดแต่ง

7. **การประเมินอายุการเก็บรักษาของอินดิเคเตอร์ที่พัฒนาได้** (จุดประสงค์ 1.6)

นำอินดิเคเตอร์ที่พัฒนาได้ไปบรรจุในบรรจุภัณฑ์แบบสุญญากาศ (Vacuum packaging) เก็บไว้ ณ อุณหภูมิห้อง (25±1ºC) ทุก 1-2 สัปดาห์ ทำการสุ่มตัวอย่างอินดิเคเตอร์ออกมา สังเกตลักษณะ

ปรากฏ แล้วจุ่มลงในสารละลายบัฟเฟอร์ที่มีค่าความเป็นกรด-ด่าง 3 5 และ 7 แล้วนำไปวัดสีด้วยเครื่อง Colorimeter[1] เพื่อทำการวิเคราะห์ประสิทธิภาพของการเปลี่ยนสีของอินดิเคเตอร์

ผลการวิจัย:

ผลการวิจัย รายงานตามแต่ละจุดประสงค์ที่ได้วางไว้ เป็นดังนี้

1. **พืชที่จะนำมาทำการสกัดสารสี** (จุดประสงค์ 1.1)

พืชที่นำมาทดลองคัดเลือกสารสกัด ได้แก่ กะหล่ำปลีม่วง (*Brassica oleraces* var. *capitate* f. *rubra*) ดอกกล้วยไม้สกุลหวายสีม่วง (*Dendrobium* Sonia 'Earsakul') ดอกอัญชัน (*Clitoria ternatea* L.) และกุหลาบ (*Rosa* L.) และจากผลการทดลองพบว่า สารสกัดที่สกัดได้ด้วยน้ำร้อนจากกะหล่ำปลีม่วง ดอกกล้วยไม้สกุลหวาย และดอกอัญชันแห้ง มีการเปลี่ยนสีอย่างเห็นได้ชัด สามารถสังเกตได้ด้วยตาเปล่า เมื่อมีค่าความเป็นกรด-ด่างต่างกัน (3 5 หรือ 7) และเมื่อทำการเปรียบเทียบค่าสี (L* a* b*) ของสารสกัดชนิดเดียวกันที่มีค่าความเป็นกรด-ด่างต่างกัน พบว่า สารสกัดเดียวกันที่ค่าความเป็นกรด-ด่างต่างกันมีค่า a* (สีเขียว-สีแดง) และ/หรือ b* (สีฟ้า-สีเหลือง) ที่แตกต่างกันอย่างมีนัยสำคัญ

2. **เงื่อนไขที่เหมาะสมของการสกัดสารสกัดจากพืชด้วยน้ำร้อนด้วยคลื่นไมโครเวฟ** (จุดประสงค์ 1.2)

เมื่อนำค่าการดูดกลืนแสงที่วัดได้ และค่าพารามิเตอร์ที่ทำการทดลอง มาสร้างแบบจำลองเชิงพยากรณ์ (Predictive model) พบว่า พารามิเตอร์ที่มีผลกระทบทางบวก (Positive correlation) เป็นอย่างมากต่อประสิทธิภาพของการสกัดด้วยน้ำร้อนแบบใช้ไมโครเวฟนั้น ได้แก่ อัตราส่วนของพืชต่อน้ำ และเวลาที่ใช้ในการสกัด และเงื่อนไขการสกัดด้วยน้ำร้อนด้วยคลื่นไมโครเวฟที่เหมาะสมในการสกัดสารจากพืชที่คัดเลือก คือ อัตราส่วนของพืชต่อน้ำ 1:3 กรัม/มิลลิลิตร ระดับพลังงานของเครื่องไมโครเวฟ 800 วัตต์ และเวลาที่ใช้ในการสกัด 480 วินาที สำหรับกะหล่ำปลีม่วงและดอกกล้วยไม้สกุลหวาย และอัตราส่วนของพืชต่อน้ำ 1:30 กรัม/มิลลิลิตร สำหรับดอกอัญชัน ระดับพลังงานของเครื่องไมโครเวฟ 800 วัตต์ และเวลาที่ใช้ในการสกัด 180 วินาที สำหรับดอกอัญชัน โดยเงื่อนไขดังกล่าวให้ค่าการสกัดสูง และไม่ทำให้คุณสมบัติของสารสกัดที่ได้เปลี่ยนไป

3. **สูตรที่เหมาะสมของฟิล์มเปลี่ยนสีได้ที่มีส่วนประกอบของสารสกัดจากพืช** (จุดประสงค์ 1.3)

จากผลการวิเคราะห์ความพึงพอใจ พบว่า สูตรของฟิล์มเปลี่ยนสีได้ที่เหมาะสม คือ CMC ร้อยละ 1.5 คาราจีแนนร้อยละ 1.5 เพคตินร้อยละ 1.5 ผงเส้นใยเซลลูโลสร้อยละ 1 และสารสกัดร้อยละ 6 สำหรับฟิล์มเปลี่ยนสีได้ที่มีส่วนผสมของสารสกัดจากดอกอัญชัน ส่วนสูตรฟิล์มที่เหมาะสมสำหรับฟิล์มที่มีสารสกัดจากกะหล่ำปลีม่วงและดอกกล้วยไม้สกุลหวายนั้นเหมือนกัน คือ คาราจีแนนร้อยละ 3 เพคตินร้อยละ 2 ผงเส้นใยเซลลูโลสร้อยละ 1 และสารสกัดร้อยละ 40 โดยสูตรดังกล่าวจะให้ฟิล์มเปลี่ยนสีได้ที่มีค่า ΔE สูงสุด และมีค่าการละลายต่ำ[1, 3]

<u>หมายเหตุ</u>: จากผลการทดลอง พบว่า ฟิล์มสูตรต่างๆ มีค่าการพองตัวไม่แตกต่างกันมากนัก จึงไม่ได้นำค่าการพองตัวมาใช้ในการคัดเลือกสูตรที่เหมาะสม ใช้เพียงค่า ΔE และค่าการละลายของฟิล์มเท่านั้น

4. คุณสมบัติด้านต่าง ๆ ของฟิล์มเปลี่ยนสีได้ (จุดประสงค์ 1.4)

จากการเปรียบเทียบคุณสมบัติของฟิล์มเปลี่ยนสีได้ที่มีส่วนประกอบของสารสกัดชนิดต่างๆ และฟิล์มที่เตรียมจากสูตรที่เหมาะสมแต่ไม่มีสารสกัด (Base film) พบว่า นอกจากคุณสมบัติทางด้านสีของฟิล์มแต่ละชนิดที่มีค่าแตกต่างกันอย่างมีนัยสำคัญ คุณสมบัติอื่นๆ ของฟิล์มที่พัฒนาได้ ไม่มีความแตกต่างอย่างมีนัยสำคัญ กล่าวคือ การเติมสารสกัดจากพืชลงไปไม่มีผลกระทบอย่างมีนัยสำคัญกับคุณสมบัติด้านอื่นๆ ของฟิล์ม) และยังพบว่า ฟิล์มที่พัฒนาได้มีคุณสมบัติทางกล คือ แรงต้านทางการดึงยืด (Tensile strength) ต่ำกว่าฟิล์มที่ทำจากคาร์โบไฮเดรตทั่วไป[8, 9] ซึ่งอาจเป็นเพราะมีการเติมผงเส้นใยเซลลูโลส ทำให้โครงสร้างไม่เรียบเนียน เกิดเป็นจุดความเค้นรวมศูนย์ (Stress concentrator)[10]

จากการทดสอบความสามารถในการเปลี่ยนสีที่ค่าความเป็นกรด-ด่างต่างๆ ผลโดยรวม พบว่า ฟิล์มที่พัฒนาได้สามารถเปลี่ยนสีตามค่าความเป็นกรด-ด่างได้ แต่ในบางคู่ของค่าความเป็นกรด-ด่าง โดยเฉพาะในช่วง 4-6 ฟิล์มจะมีสีใกล้เคียงกันมากจนอาจจะยากต่อการแยกแยะด้วยตาเปล่า (อิงผลทั้งจากค่า ΔE ที่ไม่สูงนัก และผลจากการทดสอบทางประสาทสัมผัส) โดยเฉพาะฟิล์มที่มีสารสกัดจากกะหล่ำปลีม่วงและดอกอัญชัน แต่ฟิล์มที่มีสารสกัดจากดอกกล้วยไม้สกุลหวายนั้นจะมีความสามารถในการเปลี่ยนสีที่ค่าความเป็นกรด-ด่างต่างกันสูงกว่าฟิล์มอีก 2 ชนิด

จากการทดสอบความสามารถในการเปลี่ยนสีที่ค่าความเข้มข้นของก๊าซคาร์บอนไดออกไซด์ต่างกัน ผลโดยรวม พบว่า ฟิล์มที่พัฒนาได้ทั้งหมดมีความสามารถในการเปลี่ยนสีต่ำ กล่าวคือ ฟิล์มที่พัฒนาได้ที่มีส่วนผสมของสารสกัดจากกะหล่ำปลีม่วง ดอกกล้วยไม้สกุลหวายและดอกอัญชันจะเปลี่ยนสีไปจนสังเกตเห็นได้ด้วยตาเปล่า เมื่อบรรยากาศมีความเข้มข้นของก๊าซคาร์บอนไดออกไซด์ร้อยละ 75 100 และ 75 ขึ้นไป ตามลำดับ

5. กลไกการเปลี่ยนสีของฟิล์มเปลี่ยนสีได้ (จุดประสงค์ 1.5)

การใช้การสังเกต IR สเปกตรัม เพื่อยืนยันกลไกการเปลี่ยนสีของฟิล์มที่มาจากการเปลี่ยนรูปของแอนโทไซยานินเมื่ออยู่ในสภาวะความเป็นกรด-ด่างต่างกันนั้น สามารถยืนยันการเปลี่ยนแปลงรูปของรงควัตถุแอนโทไซยานินประเภทไซยานิดินได้ดี ซึ่งไซยานิดินเป็นแอนโทไซยานินประเภทที่พบมากในกะหล่ำปลีม่วงและดอกกล้วยไม้สกุลหวาย โดยคาดว่า ที่ค่าความเป็นกรด-ด่างประมาณ 2-3 4-5 และ 6-7 ไซยานิดินจะอยู่ในรูป Flavylium cation Hemiketal และ Quinoidal base ตามลำดับ จึงทำให้ฟิล์มเปลี่ยนสีจากสีแดงส้ม เป็นสีม่วง เมื่อค่าความเป็นกรด-ด่างสูงขึ้น[11]

ส่วนกลไกการเปลี่ยนสีของแอนโทไซยานินในสารสกัดจากดอกอัญชันนั้น เป็นสารแอนโทไซยานินประเภทเดลฟินิดิน โดยคาดว่า สารดังกล่าวจะเปลี่ยนรูปจาก Flavylium cation ไปเป็น Anhydrobase และ Anhydrobase anion เมื่อสิ่งแวดล้อมมีค่าความเป็นกรด-ด่าง 2 3-5 และ 7 ตามลำดับ และทำให้ฟิล์มเปลี่ยนสีจากสีม่วงแดง เป็นสีม่วง และเป็นสีฟ้า เมื่อค่าความเป็นกรด-ด่างสูงขึ้น[12] ทั้งนี้การคาดเดากลไกการเปลี่ยนสีของสารเดลฟินิดินนั้น ต้องใช้การสังเกตสีของฟิล์มด้วยตาเปล่าร่วมด้วย

6. การใช้งานจริงของอินดิเคเตอร์ที่พัฒนาได้ กับผลิตภัณฑ์อาหาร (จุดประสงค์ 2)

จากการทดลองนำอินดิเคเตอร์มาใช้กับผลิตภัณฑ์อาหารที่มีอายุการเก็บรักษาสั้น พบว่า อินดิเคเตอร์เปลี่ยนสีเมื่ออาหารมีค่าความเป็นกรด-ด่างเปลี่ยนไป และโดยรวม สีที่แสดงมีความใกล้เคียงกับสีที่

ได้จากการนำฟิล์มเปลี่ยนสีได้ไปจุ่มในสารละลายบัฟเฟอร์ที่มีค่าความเป็นกรด-ด่างใกล้กับอาหารนั้นๆ แต่สีของอินดิเคเตอร์ที่เปลี่ยนไปไม่มีความละเอียดมากพอที่จะบอกความแตกต่างเมื่อค่าความเป็นกรด-ด่างของอาหารไม่แตกต่างกันมากนักได้ อีกทั้ง ในบางกรณี สีที่เปลี่ยนไปของอินดิเคเตอร์ไม่เหมือนกับสีของอินดิเคเตอร์ที่จุ่มลงในสารละลายบัฟเฟอร์ หรือเมื่อนำอินดิเคเตอร์ไปใช้กับอาหารบางชนิด สีของอินดิเคเตอร์ซีดลงไปภายหลังจากการใช้ไปเพียง 2-3 วันอีกด้วย (ปัญหาที่พบ เมื่อใช้กับผลิตภัณฑ์ประเภทผลไม้ตัดแต่งที่มีของเหลวปริมาณมาก และ/หรือมีค่าความเป็นกรด-ด่างใกล้เคียง 7) อาจเนื่องมาจากการที่อินดิเคเตอร์ต้องสัมผัสกับผลิตภัณฑ์อาหารโดยตรง จึงอาจได้รับผลกระทบจากหลายปัจจัย เช่น การเกิดสารประกอบหรือการเกิดโคพิกเมนต์เทชั่น (Copigmentation) หรือการได้รับผลกระทบจากโลหะหรือเอนไซม์ เป็นต้น[13, 14] อินดิเคเตอร์ที่พัฒนาจึงไม่เหมาะที่จะนำไปใช้กับงานที่เกี่ยวข้องกับความปลอดภัยของอาหาร แต่มีความเป็นไปได้ในการนำไปใช้บอกคุณภาพ (ในด้านที่เกี่ยวข้องกับค่าความเป็นกรด-ด่าง) ของอาหาร โดยพบว่า อินดิเคเตอร์สามารถเปลี่ยนสีและมีความคงตัวของสีดีเมื่อใช้กับอาหารประเภทอาหารหมัก อาจจะเนื่องมาจากสารแอนโทไซยานินจะมีความคงตัวสูงขึ้นในสภาวะที่เป็นกรด[11, 15]

<u>หมายเหตุ</u>: การตรวจสอบการใช้งานจริงของอินดิเคเตอร์สำหรับก๊าซคาร์บอนไดออกไซด์ที่พัฒนาได้กับผลิตภัณฑ์อาหารนั้น ไม่ได้ทำการทดลอง เนื่องจากฟิล์มที่พัฒนาได้มีความสามารถในการเปลี่ยนสีตามปริมาณก๊าซคาร์บอนไดออกไซด์ต่ำ

7. **อายุการเก็บรักษาของอินดิเคเตอร์ที่พัฒนาได้** (จุดประสงค์ 1.6)

อินดิเคเตอร์ที่มีส่วนประกอบของสารสกัดจากกะหล่ำปลีม่วง ดอกกล้วยไม้สกุลหวาย และดอกอัญชันที่พัฒนาได้ เมื่อเก็บไว้ในบรรจุภัณฑ์สุญญากาศ ณ อุณหภูมิห้อง สามารถเก็บไว้ได้นาน 2 4 และ 4 สัปดาห์ ตามลำดับ หากเก็บไว้นานกว่านั้น เมื่อนำไปทดสอบความสามารถในการเปลี่ยนสีที่ค่าความเป็นกรด-ด่างต่างๆ จะมีสีเพี้ยนไปจนสังเกตได้ (ค่า ΔE ต่างกัน 4.0 ขึ้นไป[4]) ไม่เหมือนสีเดิม ณ ค่าความเป็นกรด-ด่างนั้นๆ โดยการเปลี่ยนแปลงที่เกิดขึ้น อาจจะมีจากการเสื่อมสลายหรือการเกิดปฏิกิริยาสีน้ำตาลของสารแอนโทไซยานินที่มีความคงตัวไม่สูงมากนัก โดยเฉพาะสารแอนโทไซยานินชนิด ไซยานิดิน[11] ซึ่งเป็นแอนโทไซยานินชนิดหลักในกะหล่ำปลีม่วงและดอกกล้วยไม้สกุลหวาย[16, 17]

ข้อเสนอแนะการนำไปใช้และการวิจัยต่อยอด:

อินดิเคเตอร์ที่พัฒนาขึ้นนั้น อยู่ในขั้นการทดสอบความเป็นไปได้ (Proof of concept) ซึ่งพบว่ายังต้องมีการพัฒนาเพิ่มเติม ก่อนจะสามารถนำไปใช้ในระบบบรรจุภัณฑ์อาหารสำหรับอาหารที่มีอายุการเก็บรักษาสั้น ได้จริง โดยด้านที่ควรทำการพัฒนาต่อไป คือ การหาเทคนิคที่จะช่วยทำให้รงควัตถุแอนโทไซยานินมีความคงตัวสูงขึ้น ดังจะเห็นได้จากผลการวิจัยว่า เมื่อนำไปใช้กับผลิตภัณฑ์อาหารจริง บางกรณีพบว่า อินดิเคเตอร์มีสีซีดลงอย่างรวดเร็ว ซึ่งอาจเป็นผลจากการเสื่อมสลายของสารรงควัตถุดังกล่าว หรือมีการซึมของสารสีออกจากอินดิเคเตอร์ เข้าไปในผลิตภัณฑ์อาหาร เทคโนโลยีที่สามารถนำมาวิจัยเพื่อแก้ไขปัญหาดังกล่าว ยกตัวอย่างเช่น การใช้เทคนิคโคพิกเมนต์เทชั่นกับสารอินทรีย์หรือรงควัตถุชนิดอื่นเกิดเป็นสารที่มีความเสถียรสูงกว่าเดิม เช่น กรดฟีนอลิก กรดอะมิโน หรือรงควัตถุแอน

โทไซยานินด้วยกันเอง[18, 19] หรือใช้โลหะ เช่น Fe^{+3} หรือ Zn^{2+}[12, 20, 21] ทั้งนี้ เทคนิคนี้อาจจะส่งผลให้แอนโทไซยานินมีความคงตัวมากขึ้น แต่ในขณะเดียวกันมักจะมีผลต่อคุณสมบัติความสามารถในการเปลี่ยนสี[18, 19] จึงควรต้องทำการศึกษาในด้านดังกล่าวควบคู่กันไป หากสามารถทำการพัฒนาให้แอนโทไซยานินมีความคงตัวสูงขึ้นแล้ว อายุการเก็บรักษาของอินดิเคเตอร์ที่ได้จะยาวนานขึ้นอีกด้วย

การเลือกใช้สารก่อเจล (สารขึ้นรูปฟิล์ม) ชนิดอื่น อาจจะสามารถแก้ปัญหาด้านความคงตัวของฟิล์มและความเสถียรของรงควัตถุได้ในคราวเดียวกัน ยกตัวอย่างเช่น สารก่อเจลประเภทเวย์โปรตีน (Whey protein) สามารถเกิดโคพิกเมนต์เทชั่นได้ในระดับที่สูงกว่าสารก่อเจลประเภทเพคติน[22] และฟิล์มที่เตรียมได้ซึ่งจัดเป็นฟิล์มประเภทฟิล์มโปรตีน (Protein-based film) ยังมีคุณสมบัติมีค่าการละลายน้ำต่ำอีกด้วย[23] ทั้งนี้ การศึกษาการใช้เวย์โปรตีนควรมีการศึกษาเรื่องผลกระทบของการสูญเสียสภาพทางธรรมชาติ (Denaturation) โพลิเมอร์ก่อเจลชนิดอื่นๆ ที่สามารถนำมาทดลองผลิตอินดิเคเตอร์ ได้แก่ ไคโตซาน และ โปรตีนซีน (Zein protein) หรือใช้โพลิเมอร์หลายชนิดผสมกัน[3, 23]

ถึงแม้จะมีการพัฒนาอินดิเคเตอร์ที่มีสารสกัดจากพืชที่มีรงควัตถุหลักเป็นแอนโทไซยานินเพิ่มเติมแล้ว มีความเป็นไปได้สูงมากว่า อินดิเคเตอร์ดังกล่าวจะไม่มีความละเอียดมากพอที่จะบอกการเปลี่ยนแปลงของค่าความเป็นกรด-ด่างของผลิตภัณฑ์อาหาร เพื่อเพิ่มความปลอดภัยของอาหารได้ แต่อินดิเคเตอร์ดังกล่าว สามารถที่จะนำไปประยุกต์ใช้ในบรรจุภัณฑ์อัจฉริยะ ที่ทำหน้าที่บอกการเปลี่ยนแปลงของค่าความเป็นกรด-ด่างของอาหารในแง่ที่เกี่ยวข้องกับคุณภาพในการรับประทานได้ ยกตัวอย่างเช่น อินดิเคเตอร์สามารถที่จะบอกผู้บริโภคผลิตภัณฑ์ปลาส้มหรือแหนมเห็ดได้เมื่อถึงเวลาที่จะย้ายการเก็บผลิตภัณฑ์ดังกล่าวจากอุณหภูมิห้องไปไว้ในตู้เย็น (อุณหภูมิ 4 องศาเซลเซียส) เป็นต้น การมุ่งใช้อินดิเคเตอร์ดังกล่าว ในอาหารหมัก ยังมีผลเพิ่มความคงตัวของแอนโทไซยานินซึ่งมีความเสถียรมากกว่าเมื่ออยู่ในสภาวะที่มีค่าความเป็นกรด-ด่างต่ำ[15]

Objectives (วัตถุประสงค์)

Packaging is a necessary tool to maintain the product's safety and quality. Packaging for food products, if used appropriately, could help to protect the content inside from extrinsic factors, such as moisture and gases, mechanical forces, etc. that could potentially damage the product's quality and/or reduce the product's shelf-life, during storage and distribution. Well-designed packaging system could communicate (at least) crucial information of the product to the end-users[24, 25]. The introduction of intelligent packaging technologies, which incorporate additional devices, e.g. time-temperature indicator (TTI), pH-, or ethylene indicator, increase the ability of the package to communicate the product's current quality to the users, without having to open the package. Packaging equipped with pH indicator could give information on sensorial quality of food content to potential consumers. It could also indicate the state of microbiological quality of the products; similar to the use of CO_2 indicator; to notify if the content is safe for consumption. Therefore these novel packaging systems could help the consumers in their decision making step, reduce food waste, and, most importantly, reduce the risks of foodborne disease by warning of possible problems[26-28].

Globally, the use of novel packaging technologies like intelligent-, active packaging, as well as, modified atmosphere packaging (MAP) reached around 17 billion in 2008, with the compound annual growth rate of 6.9%[27]. On the other hand, the use of packaging, especially when over-packaged, also generate significant amount of solid waste. Currently, packaging waste makes up around 30% of global solid waste composition[29]. Thus, due to increasing environmental concerns, as well as, the global trend in sustainability, convergent changes towards packaging from renewable sources and/or packaging made from biodegradable materials are growing. These new packaging technologies, giving proper waste management, would not only perform required packaging functions, but would also reduce packaging waste[28].

Therefore, this project was aimed to develop pH- and CO_2 indicators from bio-based, biodegradable materials, and natural acid/base indicator extracted from plants to be used as part of intelligent packaging for RTE- and short shelf-life food products. Specific objectives, along with corresponding methodology and outcome are listed below:

1. To develop colorimetric indicators for pH and CO_2 from bio-based materials and plant extract that are stable enough to be used in packaging system for short shelf-life foods
    1.1. To select plant that contain pigment which can change color according to the change in pH of the environment (Section 2 of Materials and Methods; Section 1 of Results and Discussion)

1.2. To optimize microwave assisted hot water extraction of selected plant (Section 3 of Materials and Methods; Section 2 of Results and Discussion)

1.3. To identify suitable formula for colorimetric layer consisted of plant extract (Section 4 and 5 of Materials and Methods; Section 3 and 4 of Results and Discussion)

1.4. To characterize properties of the developed colorimetric layer and test for the film's sensitivity to changes in pH and CO_2 concentration (Section 6 and 7 of Materials and Methods; Section 5, 6, and 8 of Results and Discussion)

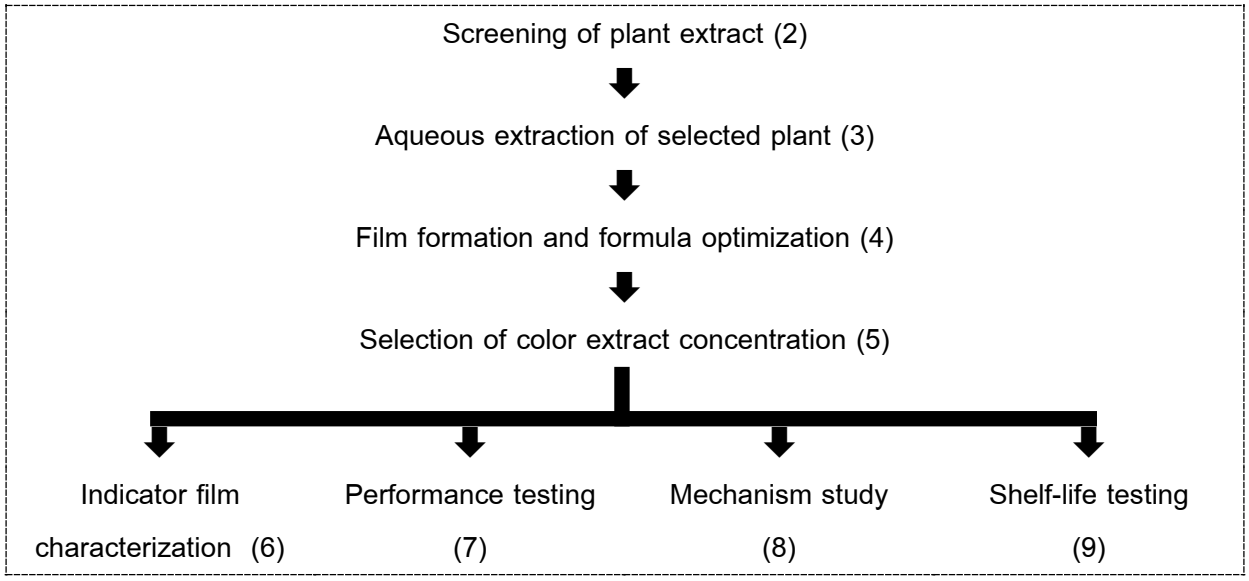
1.5. To study color-changing mechanism of the developed colorimetric layer (Section 8 of Materials and Methods; Section 9 of Results and Discussion)

1.6. To estimate shelf-life of the developed indicator (Section 9 Materials and Methods; Section 10 of Results and Discussion)

2. To investigate the potential uses of both indicators in food packaging applications (Section 7 Materials and Methods; Section 7 of Results and Discussion)

Materials and Methods (วิธีทดลอง)

The research methodology for developing feasible pH- and CO_2 indicators is outlined in Scheme 1; each research phase is described below. Briefly, several plants were screened for their color-changing ability when exposed to different pH (2). The optimal extraction condition for each selected plant was investigated using response surface methodology (RSM) (3). The plant extracts obtained from using determined, optimal extract conditions were used to prepare film samples, and the optimal film formula was determined using RSM (4). The suitable color extract concentration for each plant type to be used in the indicators was then determined (5). The developed colorimetric films were characterized (6), tested for their performance as pH- or CO_2 indicator (7), studied their color-changing mechanisms (8), and determined their shelf-lives (9).



Scheme 1. Development of pH- and CO_2 indicators from biodegradable materials and plant extract; and their utilization

1. Materials

Fresh red cabbage (*Brassica oleraces* var. *capitate* f. *rubra*)[2, 30], rose (*Rosa* L.)[31, 32], and Dendrobium orchid (*Dendrobium* Sonia 'Earsakul')[33], and dry butterfly pea (*Clitoria ternatea* L.)[2, 34] were purchased from local supermarkets in Bangkok and Kanchanaburi, Thailand. Fresh plant samples were stored at 4±1ºC; and dry plant sample was kept, in desiccator, at room temperature, until used. Cultivated banana (*Musa* ABB cv. Kluai 'Namwa') was purchased from local markets in Kanchanaburi, Thailand. Fermented fish and fermented mushroom were prepared in the

laboratory[35, 36] using ingredients purchased from local supermarket in Bangkok, Thailand. Other food samples were purchased from local supermarkets in Bangkok and Kanchanaburi, Thailand

Colorless buffers of pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 (Reagecon, Munster, Ireland) were purchased from Apex Chemicals Co., Ltd., Bangkok, Thailand. Carboxylmethyl cellulose (CMC), carrageenan, pectin, and cellulose powder were purchased from Chemipan Corporation Co., Ltd., Bangkok, Thailand. Disposable plastic cuvette (Bibby Scientific Ltd., Staffordshire, UK) was used in UV-Vis spectroscopy experiment. Paraffin film (Bemis Co., Ltd., Neenah, WI, USA) and paraffin wax and were purchased from Chemipan Corporation Co., Ltd., Bangkok, Thailand; cellophane film was purchased from Gammaco (Thailand) Co., Ltd., Nonthaburi, Thailand; cellophane tape (3M 610 cellophane tape) was purchased from D&B quality store Co., Ltd., Bangkok, Thailand; and polylactide (PLA) sheet was purchased from Brownie Points Co., Ltd., Bangkok, Thailand.

Colorless buffer solutions of pH 1.0 and 4.5 used in determination of total monomeric anthocyanin pigment content were prepared from potassium chloride (KCl, Ajax Finechem, New South Wales, Australia) and sodium acetate ($CH_3CO_2Na{\cdot}3H_2O$, Ajax Finechem, New South Wales, Australia), respectively. The buffer solutions were adjusted their final pH with hydrochloric acid (HCl, Fisher Scientific, MA, USA)[37]. Banana flour was prepared by drying unripe cultivated banana flesh and ground into powder[38].

2. Screening of plant extract

The first phase was to screen for potential plant extracts to be used in indicator film, by preparing plant extracts from several plants reported to be 1) used as acid/base indicators; and 2) rich in anthocyanins, using hot water extraction (HWE) method. For the screening phase, the extract condition applied should result in extract with visible color; color intensity of extract should be sufficient for visual comparison if its color changes.

2.1. Plant sample preparation

For rose (open flower or OF stage)[39], Dendrobium orchid (growth stage 5)[40], and dry butterfly pea, only flower petals were used. For red cabbage, the leaves were used and the core was discarded (Figure 1). All plant samples were cut into small pieces; and used within 1 h of preparation[1, 2].

2.2. Hot water extraction

Each cut sample was extracted, using hot water extraction (HWE) method. Briefly, known amount of plant sample was submersed for 30 min, in 300 mL of distilled water that had been

installed in waterbath (Memmert Waterbath WNE 22, Schwabach, Germany), set at 80^{O}C. Sample to hot water ratios used in the experiment were listed in Table 1. The heated mixture was filtered using cheesecloth, the residue was discarded, and the color extract was left to cool to room temperature before further testing[1, 2]. All experiments were performed in triplicate.

Rose (*Rosa* L.) | Dendrobium orchid (*Dendrobium* Sonia 'Earsakul') | Red cabbage (*Brassica oleraces* var. *capitate* f. *rubra*) | Dry butterfly pea (*Clitoria ternatea* L.)

Figure 1. Plant samples used in hot water extraction

Table 1: Plant sample to water ratio used in hot water extraction

Plant Type	Weight (g)	Hot Water (mL)	Extraction Ratio (g/mL)
Red cabbage	100	300	1:3
Rose	100	300	1:3
Dendrobium orchid	100	300	1:3
Dried butterfly pea	10	300	1:30

2.3. Color comparison of color extracts

Each obtained plant extract was mixed with colorless pH buffer of pH 3.0, 5.0, or 7.0. Visual comparisons were made on colors of extract at different pH points[1]. The extracts were also measured their color values, i.e. L*, a*, and b* values using handheld colorimeter (CR-400 Chroma Meter, Konica Minolta Inc., Osaka, Japan). Twenty mL of extract was used and the measurements were randomly performed at 5 different locations of the same extract sample.

3. Aqueous extraction of selected plant

This step involved identifying optimal extract condition of microwave assisted hot water extraction (mHWE) for each selected plant to obtain maximum extraction yield, and still maintain the quality of the extract. The determination of optimal mHWE condition for each plant was carried out according to RSM. The investigating parameters were 1) microwave power (Watt), 2) extraction time (second), and 3) plant to water ratio (g/mL); and the response was absorbance at λ_{max} read by UV-Visible spectrophotometer (LAMBDA^{TM} 35 UV/Vis Spectrophotometer, PerkinElmer Inc., MA, USA)[37]. The conditions that gave highest absorbance, without altering the properties of the obtained extract, i.e. did not significant decrease total anthocyanin content, did not burn the mixture, or did not alter the visible spectrum of the extract, would be used for color extraction. The amount of total monomeric anthocyanin pigment content in extracted solutions were also determined, using pH differential method[37].

The obtained plant extracts prepared using optimal condition were kept in airtight container wrapped with aluminum foil, at 4ºC, and used in further steps within 24 hours of extraction. The extract was measured its absorbance at λ_{max} before used, and adjusted its concentration level to ensure that the optical properties of the plant extract were consistent for every batch of film prepared[1].

3.1. Plant sample preparation

See Step 2.1.

3.2. Hot water extraction

Table 2 shows extraction conditions used in the experiment. Pre-determined amount of plant sample was submersed in 300 mL, 80^{O}C distilled water installed in waterbath. The extractions were carried out at 80˚C, for 10 to 180 min (increment of 10 min). After the extraction, the heated mixture was filtered using cheesecloth; the aliquot was collected as color extract and left to cool to room temperature before further testing[1, 2]. All experiments were performed in triplicate.

3.3. Microwave assisted hot water extraction

To determine the range of sample to water ratio, and extraction power and time, preliminary experiments were conducted. The selected levels of all testing parameters, listed in Table 3, were sufficient to yield extracts with visible color and did not cause significant violent boiling.

Table 2: Conditions used in hot water extraction

Plant Type	Sample to Water Ratio (g/mL)	Extraction Temperature	Extraction Time (min)
Red cabbage	1:3	80	10-180 (10-min interval)
Dendrobium orchid	1:3		
Butterfly pea	1:30		

Table 3: Testing parameters for microwave assisted hot water extraction

Parameter	Plant Type	Effective Range		Code	
			-	0	+
Sample to water ratio (g/mL)	Red cabbage Dendrobium orchid	1:5 – 1:3	1:5	1:4	1:3
	Butterfly pea	1:50 – 1:30	1:50	1:40	1:30
Extraction power (W)	Red cabbage Dendrobium orchid Butterfly pea	480 - 800	480	640	800
Extract time (s)	Red cabbage Dendrobium orchid	120 - 480	120	300	480
	Butterfly pea	60 - 180	60	120	180

Table 4 and 5 show 15 extraction treatments conducted in random order according to response surface methodology (RSM) – Box-Behnken design. All treatments listed were conducted in triplicate. For each treatment, known amount of plant sample was submersed in 300 mL of distilled water at room temperature, for 60 s to ensure thorough submersion, before starting the microwave heating process using household microwave oven (LG MG-3937C Microwave Oven, LG Electronics, Bangkok, Thailand). After the treatment, the mixture was filtered, and plant extract was collected and left to cool to room temperature[41, 42].

3.4. UV-Vis spectroscopy

The visible spectra and λ_{max} of plant extracts, from hot water extraction (HWE) and microwave assisted hot water extraction (mHWE), were obtained using UV-Vis spectrophotometer.

Table 4: Conditions for microwave assisted hot water extraction of red cabbage and Dendrobium orchid

Treatment	Code	Sample to Water Ratio (g/mL)	Extract Power (W)	Extraction Time (s)
1	0 0 0	1:4	640	300
2	0 - +	1:4	480	480
3	- - 0	1:5	480	300
4	0 0 0	1:4	640	300
5	- 0 -	1:5	640	120
6	0 0 0	1:4	640	300
7	+ - 0	1:3	480	300
8	+ + 0	1:3	800	300
9	+ 0 -	1:3	640	120
10	0 - -	1:4	480	120
11	0 + -	1:4	800	120
12	0 + +	1:4	800	480
13	- + 0	1:5	800	300
14	+ 0 +	1:3	640	480
15	- 0 +	1:5	640	480

Table 5: Conditions for microwave assisted hot water extraction of butterfly pea

Treatment	Code	Sample to Water Ratio (g/mL)	Extract Power (W)	Extraction Time (s)
1	0 0 0	1:40	640	120
2	0 - +	1:40	480	180
3	- - 0	1:50	480	120
4	0 0 0	1:40	640	120
5	- 0 -	1:50	640	60
6	0 0 0	1:40	640	120
7	+ - 0	1:30	480	120
8	+ + 0	1:30	800	120
9	+ 0 -	1:30	640	60
10	0 - -	1:40	480	60

Treatment	Code	Sample to Water Ratio (g/mL)	Extract Power (W)	Extraction Time (s)
11	0 + -	1:40	800	60
12	0 + +	1:40	800	180
13	- + 0	1:50	800	120
14	+ 0 +	1:30	640	180
15	- 0 +	1:50	640	180

3.5. Determination of total monomeric anthocyanin pigment content

The amount of total monomeric anthocyanin pigment in extracted solution was determined, using pH differential method[37]. Briefly, the extract was mixed with pH 1.0 or pH 4.5 buffers (final concentration of the extract in the solution should not exceed 20% vol/vol). The solutions were then measured their absorbance at 520 and 700 nm, using UV-Vis spectrophotometer. Amount of anthocyanin pigment was calculated using Equation 1 and 2.

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5} \quad (1)$$

Total monomeric anthocyanin (cyanidin-3-glucoside equivalents, mg/L) =

$$\frac{A \times M_w \times DF \times 10^3}{\varepsilon \times 1} \quad (2)$$

where M_w is molecular weight of cyanidin-3-glucoside (cyd-3-glu) = 449.2 g/mol; DF is dilution factor; 1 represents pathlength in cm; ε is molar extinction coefficient for cyd-3-glu = 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$.

3.6. Data analysis for response surface modeling

To obtain mathematical models of mHWE of all selected plants, the response, *i.e.* absorbance at λ_{max} of the extracts were analyzed using JMP 8.0 program (SAS Institute Inc., Cary, NC, USA). Equation 3 describes 2nd-order polynomial equation used to develop a predictive model for mHWE of selected plants:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \varepsilon \quad (3)$$

where y is absorbance at λ_{max} or total monomeric anthocyanin content; x_1, x_2, and x_3 is coded values of sample to water ratio (g/mL), extraction power (W), and extraction time (s), respectively; β_0 is intercept; β_1, β_2, and β_3 are linear effects of sample to water ratio, extraction power, and extraction time, respectively; β_{11}, β_{22}, and β_{33} are quadratic effects of sample to water ratio, extraction power, and extraction time, respectively; β_{12}, β_{13}, and β_{23} are interaction effects of sample to water ratio and extraction power, sample to water ratio and extraction time, and extraction power and extraction time, respectively; and ε is residual error.

4. Film formation and formula optimization

The colorimetric films consisted of plant extracts were prepared using casting method. The optimal formula of each colorimetric layer was identified using RSM. The responses were color change in response to pH of the films (ΔE), film solubility, and swelling ratio[(1)]. The colorimetric films were then measured their properties.

4.1. Plant extract preparation

Table 6 shows extraction conditions that were used for each plant sample. All extracts were individually prepared for each batch of film.

Table 6: Extraction conditions used in microwave assisted hot water extraction of selected plants

Plant type	Sample to Water Ratio (g/mL)	Extraction Power (W)	Extraction Time (min)
Red cabbage	1:3	800	8
Dendrobium orchid	1:3	800	8
Dry butterfly pea	1:30	800	3

4.2. Film formation

Materials used for casting of colorimetric layer of indicator film were listed in Table 7. Briefly, film solution was prepared by mixing the ingredients together with water at 80^{O}C, until the solution was homogeneous, and then degassed using vacuum pump[(1)]. The film solution was then casted on to a flat surface, and air-dried for predetermined time. The obtained films were stored in airtight container at ambient temperature for further testing. Three batches of films were prepared for each formula. The concentration range of each material used was determined, in preliminary experiments. RSM – Mixture design was implemented for formulation optimization of

colorimetric layers with red cabbage or Dendrobium orchid extracts; and RSM – Box-Behnken was implemented for formulation optimization of colorimetric layer with dry butterfly pea extract.

Table 7. Film formula of colorimetric layer (based on 100 mL of film solution)

Plant type	Amount used (mL)	Materials	Amount range (g)*
Red cabbage	40 mL	CMC	0-3 g
		Carrageenan	0-3 g
		Banana flour	0-3 g
		Pectin	2 g (fixed)
		Cellulose powder	1 g (fixed)
Dendrobium orchid	40 mL	CMC	0-3 g
		Carrageenan	0-3 g
		Banana flour	0-3 g
		Pectin	2 g (fixed)
		Cellulose powder	1 g (fixed)
Dry butterfly pea	6 mL	CMC	0-2 g
		Carrageenan	0-2 g
		Pectin	1-4 G
		Cellulose powder	1 g (fixed)

*25% (w/w), based on gelling agents' total dry weight, of sorbitol was added to all formula

4.3. Film characterization

The prepared film samples were measured important properties, i.e. color change in response to pH, swelling ratio, and film solubility. All testing was conducted in triplicates.

4.3.1. Color change in response to pH

The prepared films were immersed in pH buffers of pH 3.0, 5.0, and 7.0. After predetermined time, the films were measured their color values (L*, a*, and b*), using handheld colorimeter. Color values of each replication were calculated from 5 different locations of the same film. The color values of the films at different pH were used to calculate ΔE values for color change in response to pH at 3 and 7 ($\Delta E_{3,7}$)[2].

$$\Delta E^*_{xy} = \sqrt{\left(L^*_y - L^*_x\right)^2 + \left(a^*_y - a^*_x\right)^2 + \left(b^*_y - b^*_x\right)^2} \quad (4)$$

where, in color space, L^* indicates lightness; a^* (green-red) and b^* (blue-yellow) are chromaticity coordinates.

4.3.2. Determination of film solubility

The prepared film samples were determined their solubility using method outlined by Hosseini *et al* (2009). Briefly, the film sample was dried at 105ºC before immersing in 100 mL of distilled water, with constant stirring, for 1 h, and then the mixture was filtered through filter paper. Used filter paper, along with remaining film sample, was dried at 105ºC before being weigh. The film solubility could be calculated using Equation 5[3].

$$\%\, Solubility = \frac{w_1 - (a_2 - a_1)}{w_1} \times 100 \quad (5)$$

where w_1 is initial weight of film sample; a_1 is initial weight of filter paper; and a_2 is final weight of used filter paper after drying.

4.3.3. Swelling ratio

Two types of swelling ration were measured for each film formula, i.e. mass swell ratio and volume swell ratio[43]. First, the prepared films were measure their dry weight and volume. The film samples were then submerged in phosphate buffer of pH 3.0 and 7.0 (to determine swelling degree of the films in the environment of pH 3.0 and 7.0, respectively), until the equilibrium swelling was reached. The swollen samples were then measure their weights and volumes. The film's swelling ratio could be calculated using Equation 6 and 7.

$$Mass\ swell\ ratio = \frac{(Mass\ of\ swollen\ film - Mass\ of\ dry\ film)}{Mass\ of\ dry\ film} \quad (6)$$

$$Volume\ swell\ ratio = \frac{(Volume\ of\ swollen\ film - Volume\ of\ dry\ film)}{Volume\ of\ dry\ film} \quad (7)$$

4.4. Identification of suitable formula for colorimetric layer

Obtained data of important properties listed in 4.3. were later used as response for RSM to identify suitable formula for each colorimetric film through desirability analysis. Generally, the formula that gives low solubility and swelling ratio, and high degree of color change (ΔE) in response to pH is preferred.

5. Selection of color extract's concentration

To identify the appropriate concentration of each plant extract to be included in the colorimetric film, the suitable film formula from Step 4 was used to prepared indicator films at various plant extract concentrations, and then subjected to sensory testing.

5.1. Film formation

In this study, the concentrations of plant extracts (prepared according to Step 4.1) used were varied to identify the most suitable concentration for each colorimetric film type. The film samples with red cabbage, Dendrobium orchid, and butterfly pea extracts were prepared as outlined in Step 4.2 according to formula listed in Table 8 below.

Table 8: Film formula of colorimetric layers (based on 100 mL of film solution)

Plant type	CMC (g)	Carrageenan (g)	Pectin (g)	Cellulose powder (g)	Extract (mL)
Red cabbage	0	3	2	1	20-50 mL (increment of 10)
Dendrobium orchid	0	3	2	1	20-50 mL (increment of 10)
Dry butterfly pea	1.5	1.5	1.5	1	4-10 mL (increment of 2)

5.2. Film characterization

The prepared film samples were measured their color change in response to pH. Briefly, film samples were immersed in pH buffers of pH 3, 5, and 7, for predetermined time, the films were measured their color values (L*, a*, and b*), using handheld colorimeter. Color values of each replication were calculated from 5 different locations of the same film. The color values of the films at different pH were used to calculate ΔE and ΔH values for color change in response to pH at 3 and 7 ($\Delta E_{3,7}$ and $\Delta H_{3,7}$)[(2)]. Testing was conducted in triplicate. ΔH was calculated according to Equation 8-10.

$$\Delta H^*_{xy} = \sqrt{\Delta E^{*2}_{xy} - \left(L^*_y - L^*_x\right)^2 - \Delta C^{*2}_{xy}} \quad (8)$$

$$\Delta C^{*2}_{xy} = \left(C^*_y - C^*_x\right)^2 \quad (9)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (10)$$

where, in color space, L^* indicates lightness; a^* (green-red) and b^* (blue-yellow) are chromaticity coordinates.

6. Indicator film characterization

The developed indicator films with selected plant extract concentration, along with the base films prepared from suitable formula without the plant extract, were characterized their physical-, optical-, morphological, mechanical-, and thermal properties.

6.1 Film preparation

Film samples and base films were prepared as outlined in Step 4.2 according to formula listed in Table 9.

Table 9: Film formula of colorimetric films and base films (based on 100 mL of film solution)

Film type	CMC (g)	Carrageenan (g)	Pectin (g)	Cellulose powder (g)	Extract (mL)
Base film for film with red cabbage or Dendrobium orchid extract	0	3	2	1	-
Base film for film with butterfly pea extract	1.5	1.5	1.5	1	-
Film with red cabbage extract	0	3	2	1	40 mL
Film with Dendrobium orchid extract	0	3	2	1	40 mL
Film with butterfly pea extract	1.5	1.5	1.5	1	6 mL

6.2. Physical properties

a. Thickness, using micrometer (M120-25, Mitutoyo, Kawasaki, Japan)

b. Water activity (a_w), using water activity meter (3TE Decagon devices, Aqua Lab, Corona, CA, USA)

c. Moisture content[44]

d. Solubility[3]

e. Volume swelling ratio[45]

6.2. **Optical properties;** the prepared indicators were measured their natural color values (L*, a*, and b*), using handheld colorimeter[2].

6.3. **Morphological properties;** the indicators' surfaces were observed their morphologies, using scanning electron microscopy (SEM; JSM-7610F, JEOL Ltd., Akishima, Tokyo, Japan and X-MaxN 20, Oxford Instruments, Abingdon, UK).

6.4. **Mechanical properties;** tensile strength and % elongation of the indicators were assessed using universal testing machine (H10 KM, Hounsfield Test Equipment Ltd., Redhill, UK), according to ASTM D882-02 standard[46]

6.5. **Thermal properties;** glass transition temperature (T_g) and decomposition temperature (if applicable) were measured using simultaneous thermal analyzer (TGA/DSC 3+, Mettler-Toledo (Thailand) Ltd., Bangkok, Thailand), according to ASTM D3418-03 standard[47]

7. Performance testing

7.1. Colorimetric film formation

See Step 6.1.

7.2. Determination of the film's pH sensitivity

The sensitivity and limitation of the colorimetric layers was investigated by immersing the films in pH buffers of pH 2 to 7 (increment by 1) for 20 minutes. The colors values of the immersed films were then measured using handheld colorimeter, and used to calculate ΔE values. Color values of each replication were calculated from 5 different locations of the same film. Based on preliminary experiment, the pairs of films at different pH that have ΔE values of less than 12.0 were subjected to sensory evaluation[4, 48], by 50 untrained panelists to represent consumers' perception of the film's colors, i.e. to determine if the color difference could be distinguished by the naked eye. The difference test used was 'Two out of five' test[1, 49-51]

7.3. Performance testing as pH indicator

7.3.1. Fabrication of pH indicator

The final designs of the indicator consisted of colorimetric layer wrapped with layer of cellophane film and perforated PLA sheet (Figure 2). The previous designs of the indicator, along with their descriptions and problems encountered when subjected to early stage of performance testing with food products were listed in Table 10.

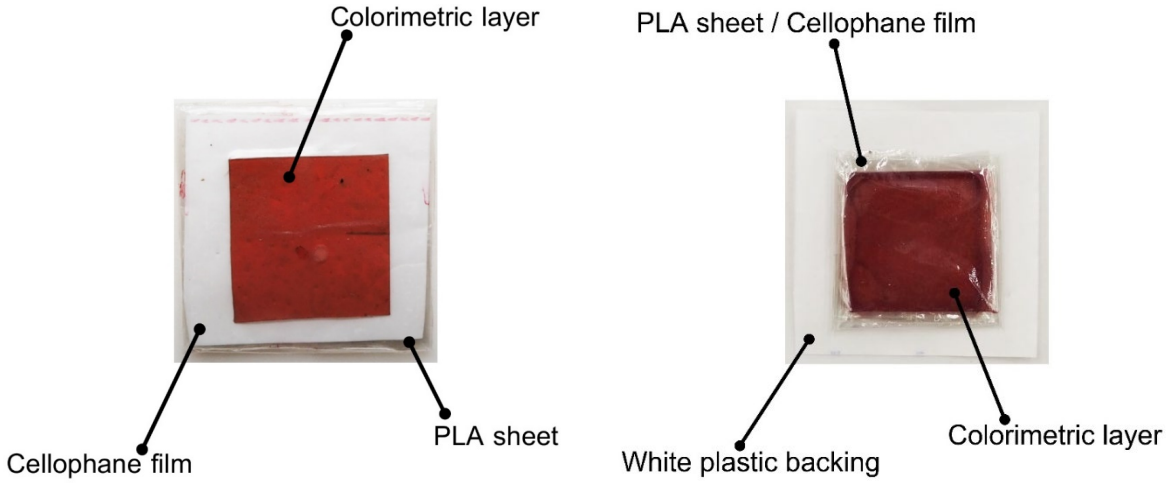


Figure 2. Indicator prototype design no. 4 (Left) and no. 5 (Right)

7.3.2. Performance testing

The selected food products were fermented fish and mushroom, and egg tofu. Indicators were placed directly on food product's surface to evaluate the performance of the films as pH indicator. Periodically, the attached indicators were measured their color values using handheld colorimeter, and the food product were sampled for pH determination, using handheld digital pH meter (PH-200 HM Digital Handheld pH Meter, HM Digital, Inc., Redondo Beach, CA, USA)[44]. The obtained indicators' color values and pH values of food samples were then compared with color values obtained from immersing colorimetric layers in buffer solutions. Some additional fruits were also included in the test to determine the validity of developed pH indicators.

7.4. Determination of the film's sensitivity to CO_2

To investigate the sensitivity and limitation of colorimetric layers, the film samples were placed in the closed container (with volume of 1 L) that flushed with gas mixtures between CO_2 and N_2. The selected concentrations of gas mixtures were 0, 25, 50, 75, and 100% of CO_2. The colors of the films were measured after predetermined exposure time by handheld colorimeter, and used to calculate ΔE values. Color values of each replication were calculated from 5 different locations of the same film. Color values of pairs of films at different CO_2 concentrations were compared. Some particular pairs of films at different CO_2 concentrations that have ΔE values of less than 12.0 were subjected to sensory evaluation[4], by 50 untrained panelists. The difference test used was 'Two out of five' test[1, 49-51].

Table 10: Different designs of pH indicator

Design no.	Description	Problem
1	Colorimetric layer with cellophane film (backing layer), coated with paraffin wax (protective layer); the purposed structure in approved proposal	Molten wax, when solidified, formed the coating layer, but did not attach to cellophane film.
2	Colorimetric layer with cellophane film and paraffin film. Outer layers were heat-sealed to enclose the colorimetric layer inside.	The seal of outer layers held well before subjected to performance test, but the structure fell apart after exposed to liquid in food samples.
3	Colorimetric layer wrapped in cellophane film	The structure held well before and during performance test, but there was noticeable amount of plant extract leaked out after exposed to liquid in food samples for more than 24 hours.
4; Final	Colorimetric layer, with white polyethylene (PE)* backing film, wrapped in cellophane film and perforated PLA sheet (outer layer)	The structure held well before and during performance test.
5; Final	Colorimetric layer wrapped in cellophane film and perforated PLA sheet (outer layer), and attached to white PE film*.	The structure held well before and during performance test.

*PE film provided white background for better visibility. It can be substituted with other white polymeric film.

7.3.2. Performance testing

The selected food products were fermented fish and mushroom, and egg tofu. Indicators were placed directly on food product's surface to evaluate the performance of the films as pH indicator. Periodically, the attached indicators were measured their color values using handheld colorimeter, and the food product were sampled for pH determination, using handheld digital pH meter (PH-200 HM Digital Handheld pH Meter, HM Digital, Inc., Redondo Beach, CA, USA)[44]. The obtained indicators' color values and pH values of food samples were then compared with color values obtained from immersing colorimetric layers in buffer solutions. Some additional fruits were also included in the test to determine the validity of developed pH indicators.

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7.5. Performance testing as CO_2 indicator

The performance testing as CO_2 indicator of the film samples was not performed since the sensitivity of the film samples were too low (see Section 8 of Results and Discussion for more detail).

8. Mechanism study

This step involved studying color-changing mechanism of the developed indicator films in which majority of the pigments responsible for the changes are anthocyanins[12, 17, 30, 42].

8.1. Colorimetric film formation

See Step 6.1.

8.2. Mechanism study of pH indicator film

To study the color-changing mechanism, in response to pH of the developed indicators, the films were immersed in pH buffer of pH 3.0, 5.0, and 7.0 for 20 minutes, and then freeze-dried, ground, and kept in airtight container at 4^{O}C until further testing. The powdered film samples were prepared according to methods modified from Ahmed *et al.* (2015) and Espinosa-Morales *et al.* (2012) for characterization by Fourier transformed infrared (FTIR) spectroscopy. The obtained IR spectrum of the films, at various pH were compared and studied for the mechanism of colorimetric indicator for pH[5, 6]. The base films prepared from suitable formula without the plant extracts were also included in the testing (Table 9).

9. Shelf-life testing

The developed indicators were packaged and determined their shelf-lives[1].

9.1. Indicator preparation

See Step 7.3.1.

9.2. Mechanism study of pH indicator film

To study the indicators' storage stability, the indicators were kept in vacuum packages, and stored at ambient temperature (25±1ºC). Every 1-2 weeks, the film samples were taken out to measure their color change in response to pH (at pH 3, 5, and 7). The indicator films were considered as at the end of their shelf-life if 1) the ΔE value of any pair of films at different pH points (same storage period) and/or at different storage period (same pH value) was higher than 4.0[4], or decrease more than 75% of the original ΔE value; or 2) the integrity of the film is significantly altered, for example, the film is broken, has noticeable mold growth, etc.

10. Statistical analysis

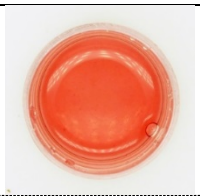
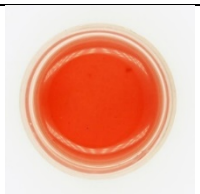
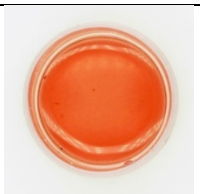
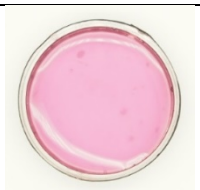
Each experimental treatment or film testing was performed in triplicates, except those that specified otherwise. Statistical analysis for RSM on optimal extraction condition and suitable film formula were described in section 3.6 and 4.4, respectively. All data obtained from the study were statistically analyzed by analysis of variance (ANOVA) using JMP 8.0 program (SAS Institute Inc., Cary, NC, USA) at the confidence level of 95% (α = 0.05) with Tukey's adjustment for comparison of the means.

Results and Discussion (ผลการทดลอง และวิจารณ์ผลการทดลอง)

1. Selected plant extract, and their visible spectra and λ_{max}

Table 11 shows pictures of plant extracts obtained from HWE and their corresponding color values, at different pH conditions. While rose extract showed no significant change in color from pH 3 to 7, the color changes observed in extracts from red cabbage, Dendrobium orchid, and butterfly pea were noticeable to the naked eye. At different pH values, all 3 extracts had either a* (green to red hues) or b* (blue to yellow hues) values that were significantly different. The differences along green to red or blue to yellow planes could be visually intensified by increasing the concentrations of the extracts, i.e. lower their L* values.[4, 52]

Table 11: Visual appearance of plant extracts at different pH values and their corresponding color values*,**

Plant Type		pH 3.0	pH 5.0	pH 7.0
Red cabbage				
Color values	L*	68.21 ± 4.42^{b}	81.32 ± 1.32^{a}	76.78 ± 3.80^{ab}
	a*	43.45 ± 6.12^{a}	13.89 ± 1.83^{b}	3.06 ± 0.57^{c}
	b*	-3.94 ± 2.91^{a}	-8.70 ± 0.38^{b}	-10.75 ± 2.05^{b}
Rose				
Color values	L*	57.96 ± 2.58^{a}	55.35 ± 3.90^{a}	57.66 ± 3.00^{a}
	a*	60.35 ± 4.57^{a}	60.91 ± 11.21^{a}	56.33 ± 5.21^{a}
	b*	52.12 ± 0.55^{a}	58.06 ± 7.12^{a}	59.81 ± 3.72^{a}
Dendrobium orchid				
Color values	L*	66.93 ± 4.81^{b}	81.08 ± 0.89^{a}	63.84 ± 6.91^{b}
	a*	53.23 ± 5.30^{a}	26.48 ± 1.54^{b}	25.37 ± 2.66^{b}
	b*	-28.99 ± 1.76^{b}	-4.11 ± 1.13^{a}	-29.66 ± 3.53^{b}

Plant Type		pH 3.0	pH 5.0	pH 7.0
Butterfly pea				
Color values	L*	43.66 ± 3.89[a]	46.03 ± 5.24[a]	48.47 ± 3.69[a]
	a*	53.94 ± 6.71[a]	29.36 ± 6.32[b]	-14.29 ± 1.45[c]
	b*	-35.95 ± 5.79[a]	-66.55 ± 8.26[b]	-34.09 ± 5.09[a]

L, a*, and b* values were expressed in mean ± standard deviation, based on three replications. Color values of each replication were calculated from 5 different locations of the same solution.
**Values with similar superscript letter, within the same row, are not significantly different at α of 0.05.

Since the ability to visibly change color according to pH is a crucial characteristic for pH indicator (and CO_2 indicator that requires significant amount of CO_2 gas to dissolve in the liquid layer of indicator to change the solution's pH value)[26], rose extract was excluded as plant extract that would be used to develop colorimetric layer for pH- and CO_2 indicator.

All plants included in this study are reported to have anthocyanins, water-soluble plant pigments, as major pigments in their flowers. Important anthocyanins often found in flowers include pelargonidin-, cyanidin-, peonidin-, and delphinidin-based anthocyanins (Figure 3), which are responsible in giving various hues to flowers. Different anthocyanins and/or their different forms (according to pH value of the surrounding) give different colors, depending on their current chemical structures. For example, blue hue depends largely on the number of hydroxyl (-OH) groups on the B-ring; and O-methylation or glycosylation of the structure results in red hue. Cyanidin glycoside is a major anthocyanin in red cabbage, giving it purple/violet color[30], while butterfly pea is rich in delphinidin glycoside, which responsible for the blue color of the flower[53]; and purple Dendrobium orchid has high amount of cyanidin glycoside and Peonidin glycoside, derivative of cyanidin (with peonidin glycoside contributed between 1 to 11%, depending on Dendrobium species and type of hybrids)[17].

Figure 4 shows visible spectra (400-700 nm) of red cabbage, Dendrobium orchid, and butterfly pea extracts obtained through mHWE method; corresponding λ_{max} of each extracts are listed in Table 12. The absorbance at these λ_{max} of the color extracts obtained through different extraction methods and/or extract conditions were measured and later used as an indication of extraction efficacy (see Section 2.2 of Results and Discussion for more detail).

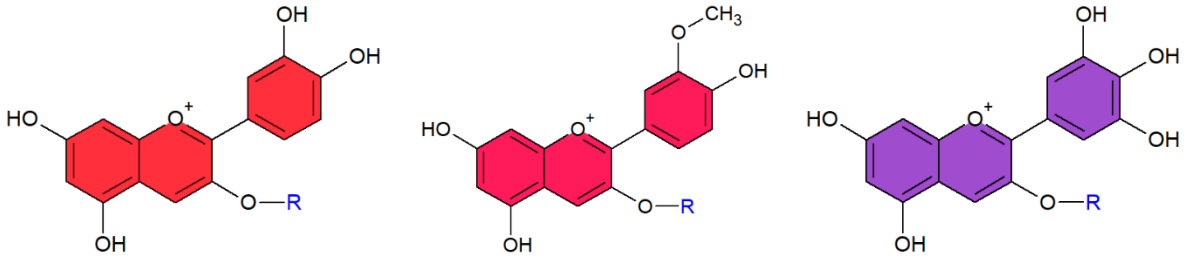


Figure 3. Some anthocyanin structures and their respective colors[54]

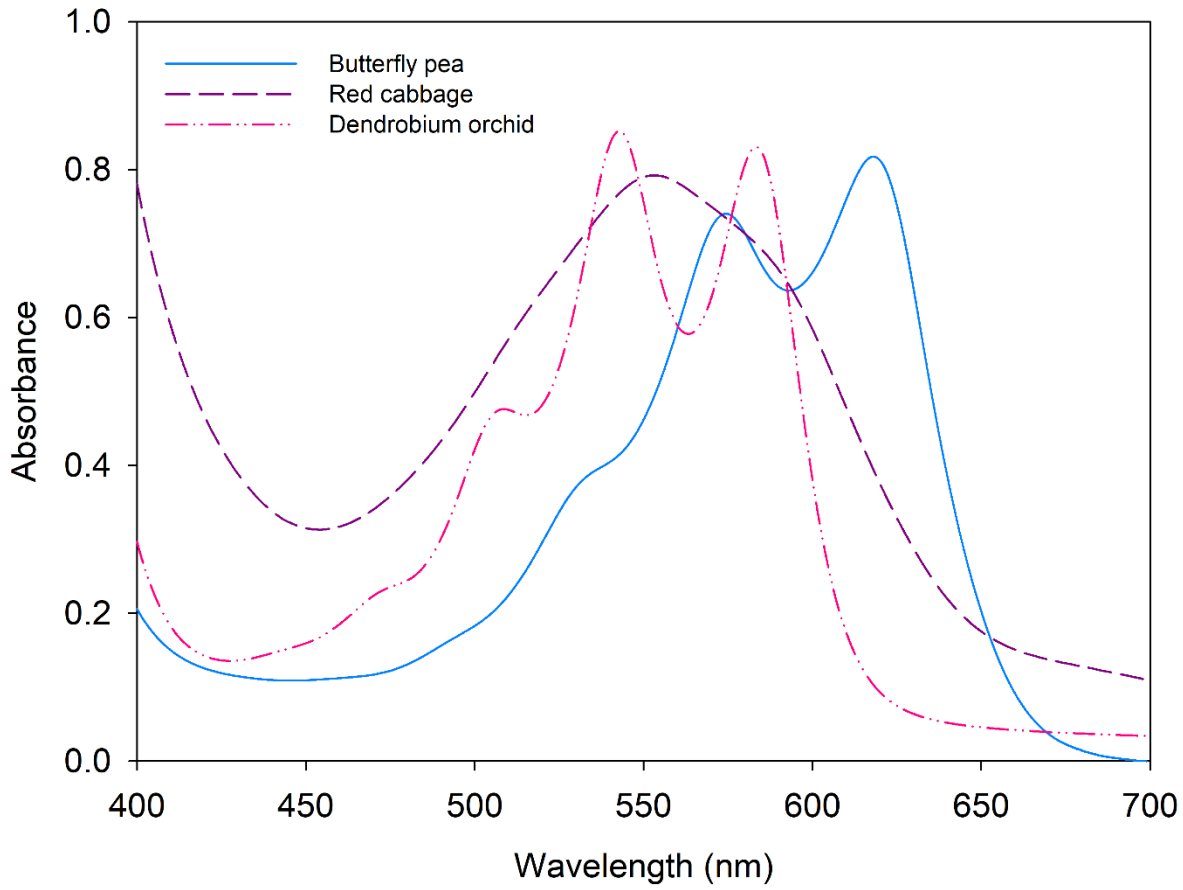


Figure 4. Visible spectrum (400-700 nm) of red cabbage, dendrobium, and butterfly pea extracts

Table 12: pH value and λ_{max} of plant extracts

Plant Type	pH of plant extract	λ_{max} (nm)
Red cabbage	6.82±0.02	553
Dendrobium orchid	6.84±0.01	543 and 583
Butterfly pea	6.84±0.01	574 and 618

2. Microwave assisted hot water extraction of plant extracts

To study the effects of mHWE on absorbance at λ_{max} of selected plant extracts, mathematical models were constructed. The response (y) was absorbance at λ_{max} of the extract and the studied parameters were sample to water ratio (x_1), extraction power (x_2), and extraction time (x_3). The developed predictive models for mHWE of selected plants are listed in Table 13.

For all plant samples, both sample to water ratio and extraction time had strong positive linear effects on amount of color compounds being extracted through mHWE, as were indicated by the increases in absorbance at λ_{max}, with sample to water ratio being the most prominent factor for extraction of red cabbage (Table 14); and extraction time being the most influential parameter in extraction of Dendrobium orchid and butterfly pea. The synergistic effects of both factors (x_1x_3), in extraction of Dendrobium orchid and butterfly pea, were also observed (P < 0.001) (Table 15 and 16). Note that; the negative quadratic effects of water to sample ratio (x_1^2) in extraction of Dendrobium orchid and butterfly pea (Table 15 and 16) were significant (P < 0.05) and indicated that the amount of color compounds extracted increased, at slower degrees, as the sample to water ratio increased (see Figure 6 and 7 for graphical descriptions). *For example of full reports of RSM – Box-Behnken design and desirability analysis, see* ***Appendix 2***.

Prediction profilers (Figure 5-7) indicated that even at the conditions where highest level of every testing parameters were applied, it might be possible to obtain higher yield if the levels were raised beyond the testing conditions, as could be implied by the on-going positive trends in the profilers. However, due to the risks of violent boiling from applying higher extraction power and/or longer extraction time, the mHWE conditions that should be used to extract the color compounds from selected plants are the highest levels of every parameter listed in Table 3. Additionally, at these extract conditions the visible spectra and λ_{max} of the obtained extracts (data not shown) were similar to those of extracts yielded from extractions at less severe conditions, indicating that the qualities of the color extracts were not significantly affected.

Table 13: Predictive models for mHWE of red cabbage, Dendrobium orchid, and butterfly pea

Plant Type	λ_{max} (nm)	Predictive Model	R^2
Red cabbage	553	$y = -0.7618 + 0.2064x_1 + 0.0011x_2 + 0.1513x_3 + 0.0001x_1x_2 + 0.0374x_1x_3 + 0.0005x_2x_3 + 0.0129x_1^2 + 0.000005x_2^2 + 0.0003x_3^2 + \varepsilon$	0.8066
Dendrobium orchid	543	$y = -3.9022 + 07689x_1 + 0.0050x_2 + 0.8396x_3 + 0.0001x_1x_2 + 0.28464x_1x_3 + 0.0021x_2x_3 - 0.6138x_1^2 - 0.000003x_2^2 + 0.0149x_3^2 + \varepsilon$	0.9026
	583	$y = -3.7666 + 0.7331x_1 + 0.0048x_2 + 0.8079x_3 + 0.0001x_1x_2 + 0.27054x_1x_3 + 0.0020x_2x_3 - 0.5998x_1^2 - 0.000002x_2^2 + 0.0138x_3^2 + \varepsilon$	0.9007
Butterfly pea	574	$y = -14.5093 + 3.1542x_1 + 0.0156x_2 + 5.7261x_3 + 0.0093x_1x_2 + 2.9138x_1x_3 + 0.0164x_2x_3 - 1.1323x_1^2 + 0.00001x_2^2 + 1.5721x_3^2 + \varepsilon$	0.9432
	618	$y = -14.1379 + 3.0658x_1 + 0.0152x_2 + 5.6576x_3 + 0.0089x_1x_2 + 2.8227x_1x_3 + 0.0160x_2x_3 - 1.1290x_1^2 + 0.000002x_2^2 + 1.5482x_3^2 + \varepsilon$	0.9407

Table 14. Parameter estimates of RSM equation for mHWE of red cabbage

Parameter	λ_{max} = 553 nm			
	Estimate	**Standard Error**	**T Ratio**	**Prob > | t |**
Intercept	-0.761812	0.218043	-3.49	0.0013*
Water to sample ratio	0.2064417	0.047085	4.38	0.0001*
Extraction power	0.0010759	0.000294	3.66	0.0008*
Extraction time	0.1512792	0.015695	9.64	<.0001*
Ratio*Power	8.1667e-5	0.000416	0.20	0.8456
Ratio*Time	0.0373611	0.022196	1.68	0.1012
Power*Time	0.0005313	0.000139	3.83	0.0005*
Ratio*Ratio	0.0128542	0.069308	0.19	0.8539
Power*Power	4.6086e-6	2.707e-6	1.70	0.0976
Time*Time	0.0003032	0.007701	0.04	0.9688

* indicates significance of the effects at type I error (α) of 0.05.

Table 15. Parameter estimates of RSM equation for mHWE of Dendrobium orchid

Parameter	λ_{max} = 543 nm				λ_{max} = 583 nm			
	Estimate	Standard Error	T Ratio	Prob > | t |	Estimate	Standard Error	T Ratio	Prob > | t |
Intercept	-3.902207	0.755463	-5.17	<.0001*	-3.766574	0.734417	-5.13	<.0001*
Water to sample ratio	0.7689375	0.163138	4.71	<.0001*	0.7331167	0.158593	4.62	<.0001*
Extraction power	0.0049633	0.00102	4.87	<.0001*	0.0048103	0.000991	4.85	<.0001*
Extraction time	0.8395569	0.054379	15.44	<.0001*	0.8079181	0.052864	15.28	<.0001*
Ratio*Power	0.0001494	0.001442	0.10	0.9181	0.0001043	0.001402	0.07	0.9411
Ratio*Time	0.2846306	0.076904	3.70	0.0007*	0.2705056	0.074762	3.62	0.0009*
Power*Time	0.0020987	0.000481	4.37	0.0001*	0.0020262	0.000467	4.34	0.0001*
Ratio*Ratio	-0.613793	0.240133	-2.56	0.0151*	-0.599779	0.233443	-2.57	0.0146*
Power*Power	-2.656e-6	9.38e-6	-0.28	0.7788	-1.928e-6	9.119e-6	-0.21	0.8338
Time*Time	0.0149045	0.026681	0.56	0.5800	0.013844	0.025938	0.53	0.5969

* indicates significance of the effects at type I error (α) of 0.05.

Table 16. Parameter estimates of RSM equation for microwave assisted hot water extraction of butterfly pea

Parameter	λ_{max} = 574 nm				λ_{max} = 618 nm			
	Estimate	Standard Error	T Ratio	Prob > | t |	Estimate	Standard Error	T Ratio	Prob > | t |
Intercept	-14.50932	1.524857	-9.52	<.0001*	-14.1379	1.53129	-9.23	<.0001*
Water to sample ratio	3.15425	0.320284	9.85	<.0001*	3.0657708	0.321635	9.53	<.0001*
Extraction power	0.0156074	0.002002	7.80	<.0001*	0.0151935	0.00201	7.56	<.0001*
Extraction time	5.7260625	0.320284	17.88	<.0001*	5.6576458	0.321635	17.59	<.0001*
Ratio*Power	0.0093172	0.002831	3.29	0.0023*	0.0088565	0.002843	3.12	0.0037*
Ratio*Time	2.91375	0.45295	6.43	<.0001*	2.8226667	0.454861	6.21	<.0001*
Power*Time	0.0163701	0.002831	5.78	<.0001*	0.0160164	0.002843	5.63	<.0001*
Ratio*Ratio	-1.13234	0.471445	-2.40	0.0218*	-1.129028	0.473434	-2.38	0.0226*
Power*Power	0.0000117	1.842e-5	0.64	0.5296	1.1859e-5	1.849e-5	0.64	0.5255
Time*Time	1.5721181	0.471445	3.33	0.0020*	1.5482222	0.473434	3.27	0.0024*

* indicates significance of the effects at type I error (α) of 0.05.

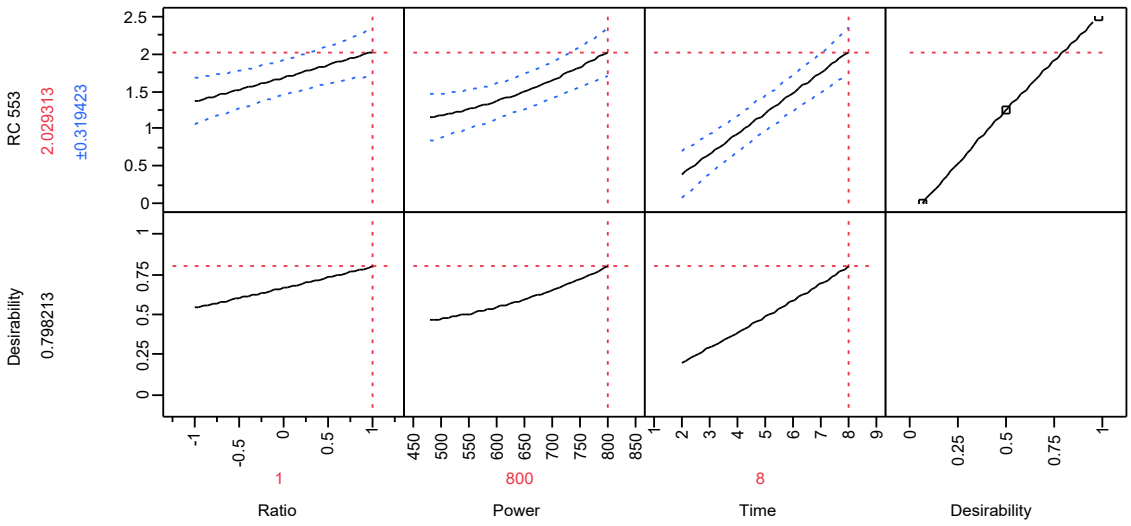


Figure 5. Prediction profiler of red cabbage extraction; range of each parameter are 1:5 (-1) to 1:3 (1) g/mL for sample to water ratio; 480-800 W for extraction power; and 120-480 s for extract time

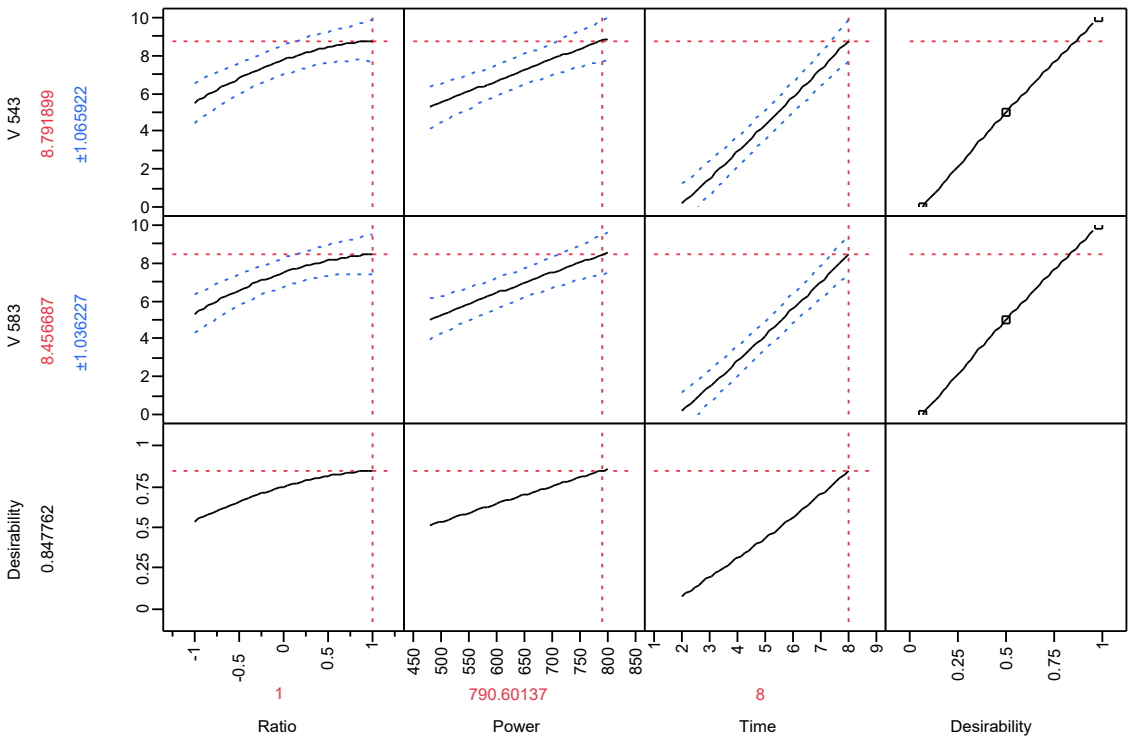


Figure 6. Prediction profiler of Dendrobium orchid extraction; range of each parameter are 1:5 (-1) to 1:3 (1) g/mL for sample to water ratio; 480-800 W for extraction power; and 120-480 s for extract time

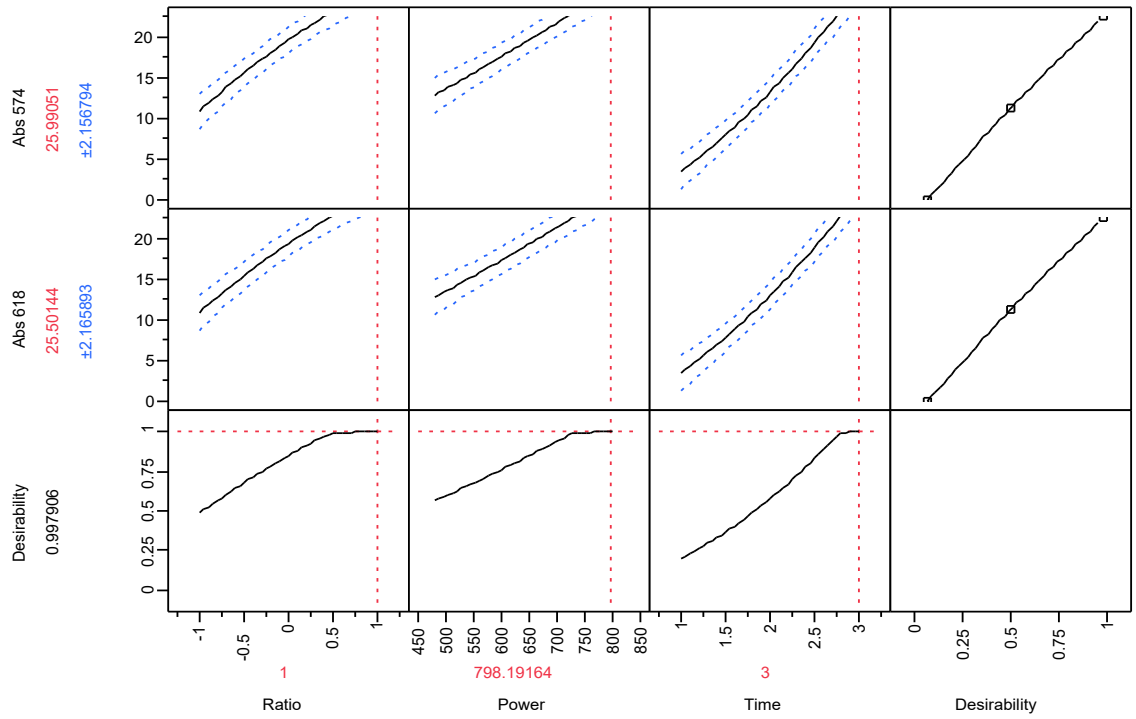


Figure 7. Prediction profiler of butterfly pea extraction; range of each parameter are 1:50 (-1) to 1:30 (1) g/mL for sample to water ratio; 480-800 W for extraction power; and 60-180 s for extract time

2.1. Hot water extraction of the plant extracts

In comparison to mHWE, Figure 8 show absorbance at λ_{max} of red cabbage, Dendrobium orchid, and butterfly pea obtained from hot water extraction (HWE) at constant 80ºC. The sample to water ratio used was similar to the highest ratio selected in mHWE of each plant (Table 3). Based on absorbance data, the extraction time of 60, 120, and 40 min gave maximum yields for HWE of red cabbage, Dendrobium orchid, and butterfly pea, respectively. After such extraction times, the absorbance at λ_{max} decreased, in all extracts. However, even at the maximum absorbance of each plant extract using HWE, the absorbance obtained were significantly lower than those obtained through mHWE, i.e. 1.88 ± 0.36 at 1:4 g/mL, 800 W, for 480 s, 8.60 ± 0.99 at 1:4 g/mL, 800 W, for 480 s (λ_{543nm}), and 21.04 ± 1.80 at 1:4 g/mL, 800 W, for 180 s (λ_{574nm}), in extracts of red cabbage, Dendrobium orchid, and butterfly pea, respectively.

Both HWE and mHWE are high-temperature extraction techniques which generally result in increased extraction yield due to increases in solubility and/or mobility of compounds, or better accessibility from disruptions of cell or structure. However, prolonged exposure of anthocyanins,

which are heat-sensitive compounds to high extraction temperature, as in the case of HWE, can cause significant degradation of anthocyanins[55, 56].

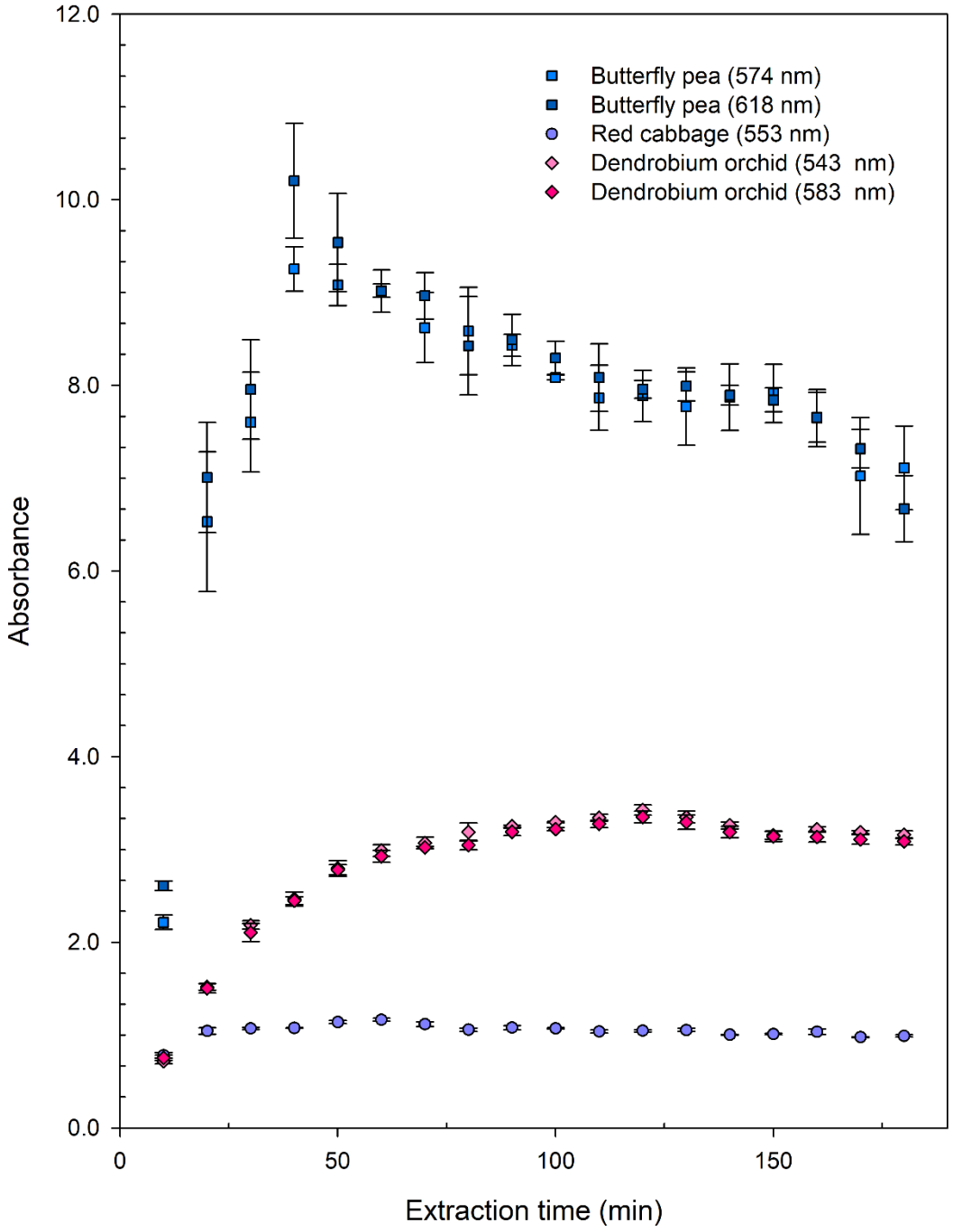


Figure 8. Absorbance at λ_{max} of red cabbage, Dendrobium orchid, and butterfly pea obtained from hot water extraction at 80ºC and various extraction time

2.2. Total monomeric anthocyanin pigment content

All obtained extracts from both extraction methods were determined their total monomeric anthocyanin content[37]. Figure 9-11 show positive correlation between absorbance at λ_{max} and the corresponding total monomeric anthocyanin pigment content of the extract samples. Thus, absorbance value at λ_{max} of color extract was used as an indication of extraction yield.

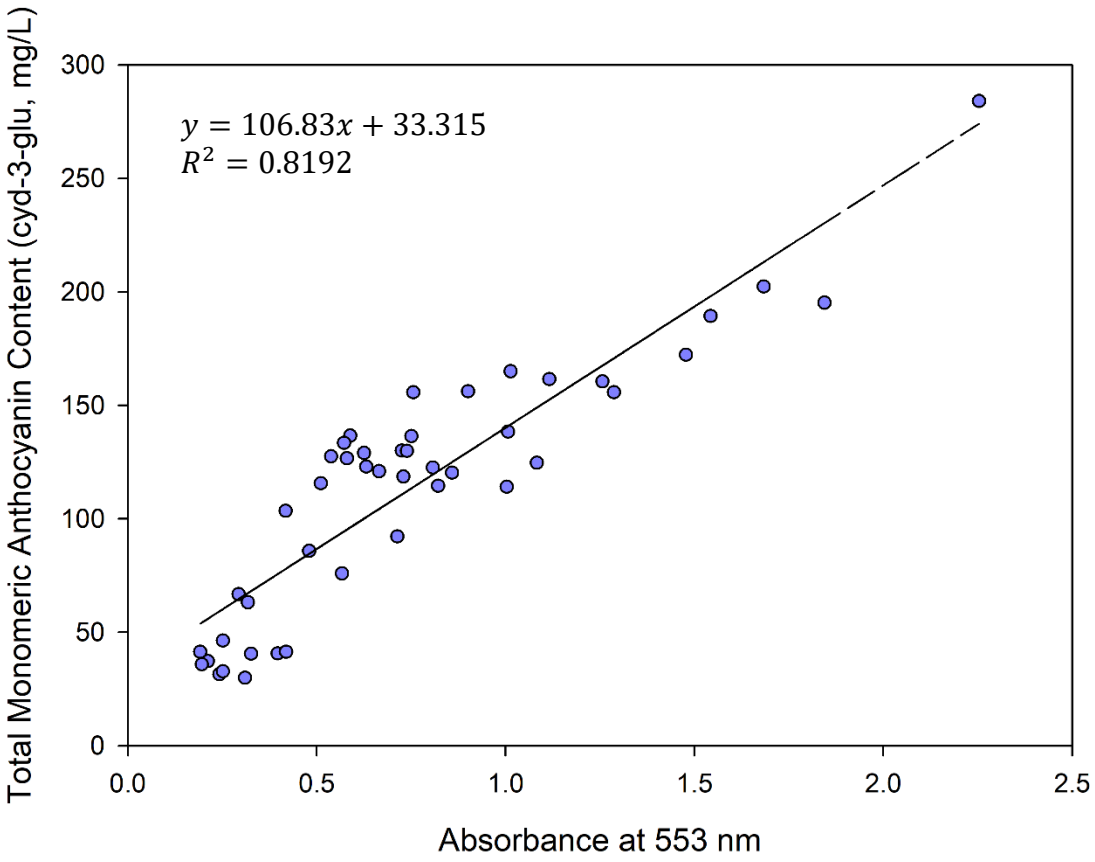


Figure 9. Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L) and absorbance at λ_{max} of red cabbage extracts

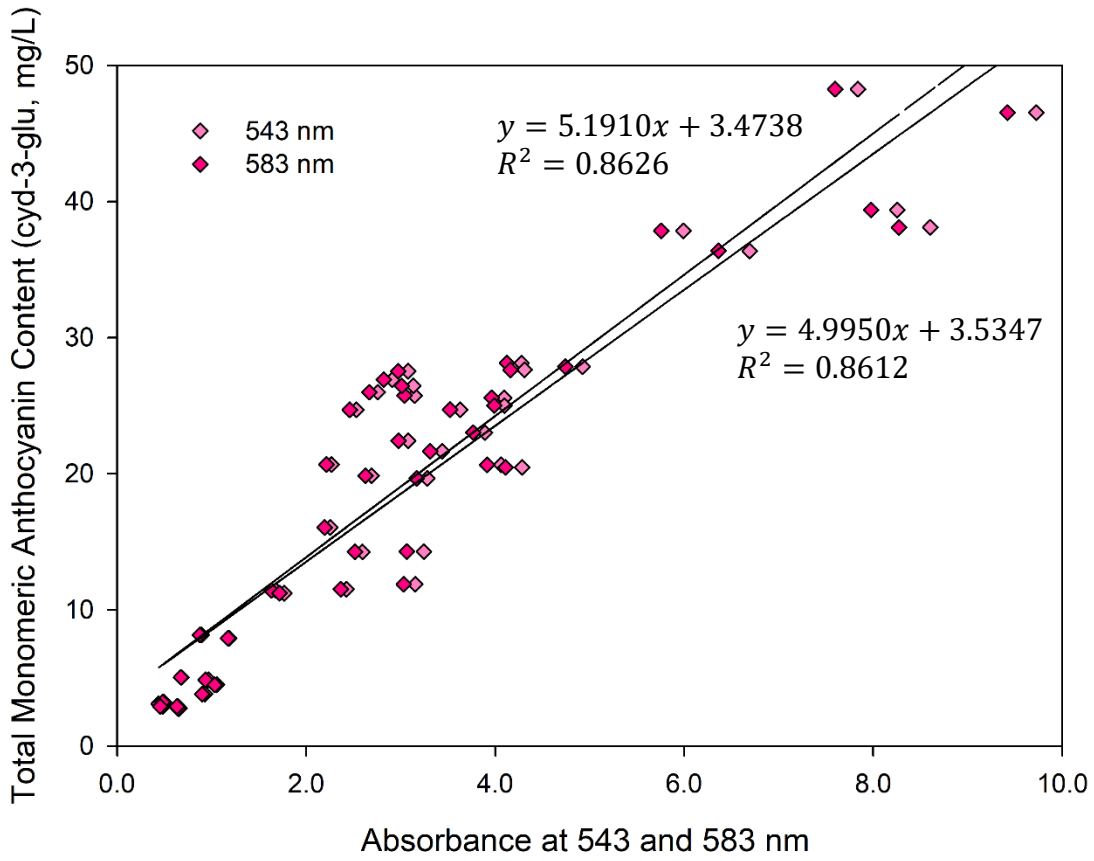


Figure 10. Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L) and absorbance at λ_{max} of Dendrobium orchid extracts

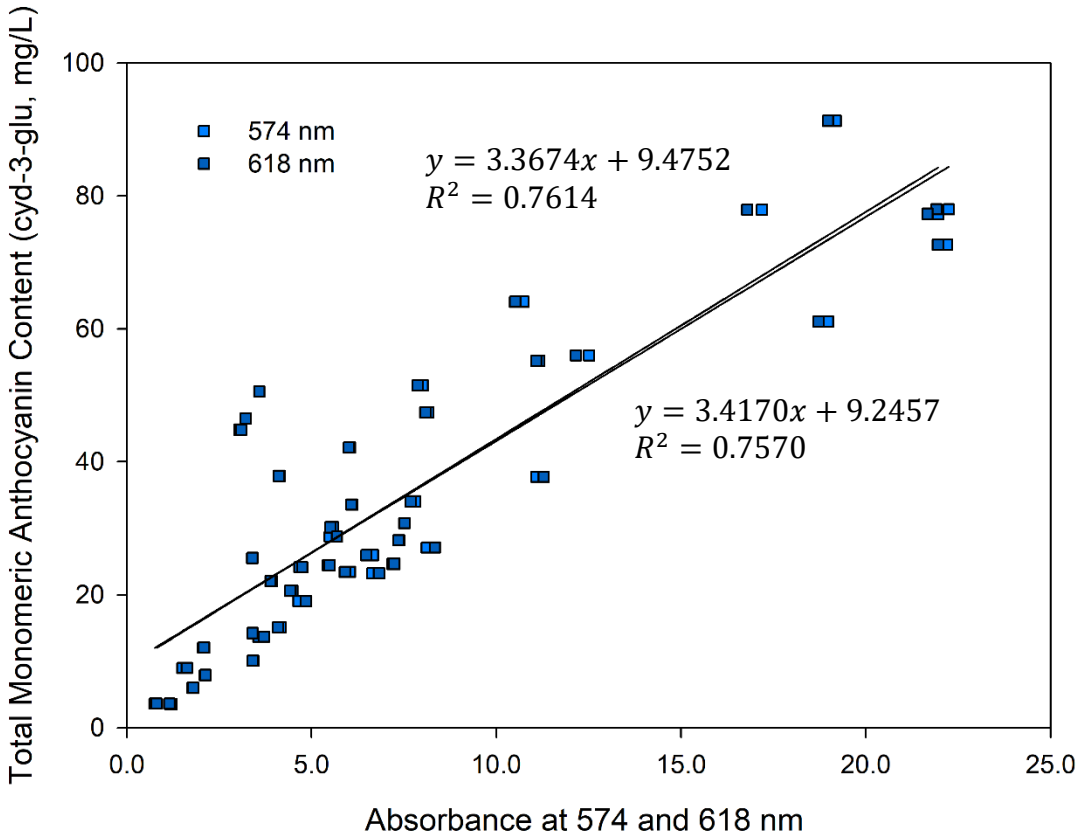


Figure 11. Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L) and absorbance at λ_{max} of butterfly pea extracts

3. Optimal base formula of colorimetric film

Based on preliminary experiments, both mass- and volume swell ratios of all film formulas (Table 7) were between 2.07±0.57 and 1.88±0.56, respectively; the differences of either values between all formulas, while not negligible, also not crucial enough to be included as one of the responses for RSM. Thus, only color change in response to pH and film solubility values were used to identify suitable formula for each colorimetric film.

3.1. Characterization of colorimetric layer with red cabbage extract

Table 17 shows ΔE and solubility of film sample with red cabbage extract. Increasing amount of carrageenan significantly increased color change according to pH and reduced the films' solubility. On the other hand, increasing banana flour and/or CMC content decrease ΔE and increased solubility of the samples.

Table 17: Color change in response to pH at 3 and 7 ($\Delta E_{RC3,7}$) and solubility of colorimetric films with red cabbage*

Treatment	Code	$\Delta E_{RC3,7}$	Solubility (%)
1	0.167,0.167,0.667	4.97±0.81	39.14±2.77
2	0,1,0	6.56±1.06	47.27±14.47
3	0.167,0.667,0.167	6.29±1.42	37.73±8.40
4	0.333,0.333,0.333	4.85±0.56	56.96±38.31
5	0.5,0.5,0	4.50±3.22	34.50±4.03
6	0.667,1.67,0.167	6.00±3.26	34.35±4.99
7	1,0,0	5.49±0.68	33.52±1.46
8	0,0.5,0.5	7.06±1.25	68.91±78.71
9	0.5,0,0.5	10.50±1.54	17.41±21.46
10	0,0,1	4.18±1.42	33.34±5.62

3.2. Characterization of colorimetric layer with Dendrobium orchid extract

Table 18 shows ΔE and solubility of film sample with Dendrobium orchid extract. Similar to the films with red cabbage extract, ΔE increased and the films' solubility decreased when amount of carrageenan was increased. However, the presence banana flour, while still increased solubility of the film, did not significantly influence the ability of the film to change color when exposed to different pH (Figure 12).

Table 18: Color change in response to pH at 3 and 7 ($\Delta E_{O3,7}$) and solubility of colorimetric films with Dendrobium orchid*

Treatment	Code	$\Delta E_{O3,7}$	Solubility (%)
1	0.167,0.167,0.667	4.91±1.75	28.75±7.17
2	0,1,0	7.82±1.16	23.01±6.27
3	0.167,0.667,0.167	800±1.25	22.92±5.62
4	0.333,0.333,0.333	5.94±0.75	24.39±2.76
5	0.5,0.5,0	8.67±3.32	24.95±5.41
6	0.667,1.67,0.167	5.66±0.08	27.19±3.82
7	1,0,0	4.29±1.25	23.31±2.59
8	0,0.5,0.5	7.92±1.40	21.50±15.38
9	0.5,0,0.5	8.07±1.74	23.62±6.92
10	0,0,1	8.07±3.17	26.18±5.09

3.3. Characterization of colorimetric layer with butterfly pea extract

Table 19 shows color change in response to pH at 3 and 7 ($\Delta E_{BP3,7}$) and solubility of film samples with butterfly pea extract. The presence of banana flour and CMC both increased the films' solubility, increasing carrageenan amount did not significantly affect ΔE; increasing CMC content slightly lowered the film's ability to change colors according to pH.

Table 19: Color change in response to pH at 3 and 7 ($\Delta E_{BP3,7}$) and solubility of colorimetric films with butterfly pea*

Treatment	Code	$\Delta E_{BP3,7}$	Solubility (%)
1	+ + 0	7.11±0.38	100.64±1.78
2	- - 0	17.24±1.81	72.49±3.80
3	0 - +	7.85±0.73	53.09±2.22
4	0 0 0	11.32±1.09	101.27±2.54
5	0 - -	5.38±0.41	61.73±3.52
6	+ 0 -	7.97±0.33	54.72±3.11
7	- 0 +	6.57±0.71	58.91±1.90
8	- 0 -	18.25±1.22	57.10±5.00
9	0 + +	16.58±1.73	72.29±3.89
10	0 + -	8.59±1.77	48.33±2.82
11	+ - 0	23.52±0.54	101.73±0.70
12	+ 0 +	9.19±0.53	56.35±2.63
13	0 0 0	4.87±1.54	53.77±2.00
14	0 0 0	7.66±1.06	63.10±2.37
15	- + 0	11.03±1.45	100.75±0.14

3.4. Identification of suitable formula for colorimetric layers

Most suitable formula for colorimetric layers with red cabbage, Dendrobium orchid, and butterfly pea had been identified and listed in Table 20. Based on desirability analysis, the selected formulas should produce films with low solubility and high ΔE. For colorimetric layers with red cabbage and Dendrobium orchid extracts, the presence of banana flour increased film samples' solubility and did not significantly affect the films' color change in response to pH, while increasing carrageenan content (first increased, and then) reduced the films' solubility and increased ΔE (Figure 12). *For example of full reports of RSM – Mixture design and desirability analysis, see* ***Appendix 4***.

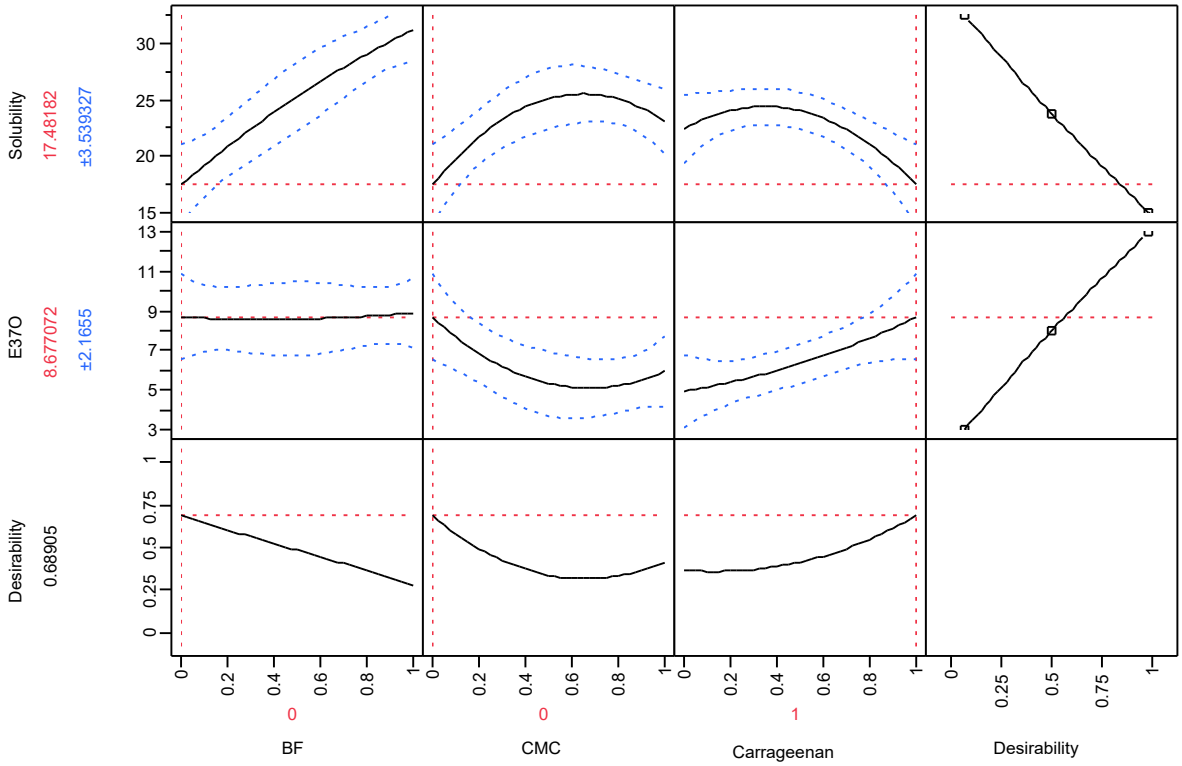


Figure 12: Prediction profiler of formula for colorimetric layer with Dendrobium orchid extract

Table 20: Most suitable film formula of colorimetric layers (based on 100 mL of film solution)

Plant type	CMC (g)	Carrageenan (g)	Banana flour (g)	Pectin (g)	Cellulose powder (g)
Red cabbage	0	3	0	2	1
Dendrobium orchid	0	3	0	2	1
Dry butterfly pea	1.5	1.5	0	1.5	1

4. Optimal plant extract concentration for colorimetric film

Table 21-23 shows ΔE and ΔH of colorimetric layers at pH 3 and 7, with red cabbage, Dendrobium orchid, and butterfly pea, respectively. High color change in response to pH indicated that there is higher chance that observers could distinguish color difference of colorimetric film at different pH. Generally, significant color difference for ΔE is approximately 4.0[(4)]; based on ΔE determined at pH 3 and 7 of every film sample, the changes of all films' colors should be noticeable to the naked eyes. Thus, the selection of extract volume for each colorimetric layer was based on ΔE and sensory evaluation was not performed.

For colorimetric layer with red cabbage extract, ΔE of all films with 20-50% (v/v) extracts were not significantly different, but samples with 30 and 40% gave higher ΔH as compared to films with 20 and 50% extract. The extraction volume of 40 mL/100 mL of film solution was then selected due to 1) the ease during film preparation; and 2) higher anthocyanin content. Including more anthocyanin pigments in the film structure could result in increased stability of the pigments due to higher chance of copigmentation[19].

Table 21: Color change in response to pH at 3 and 7, $\Delta E_{RC3,7}$ and $\Delta H_{RC3,7}$ of colorimetric films with red cabbage*

Extract volume (mL)	$\Delta E_{RC3,7}$	$\Delta H_{RC3,7}$
20	10.94±3.51^{a}	2.95±0.92^{b}
30	8.75±0.65^{a}	4.88±1.28^{ab}
40	8.70±0.57^{a}	5.07±0.87^{a}
50	8.51±1.40^{a}	3.89±2.87^{ab}

*Values were expressed in mean ± standard deviation, based on three replications; values with similar superscript letter, within the same column, are not significantly different at α of 0.05.

The same amount of extract was selected for colorimetric layer with Dendrobium orchid extract based on similar reasons.

Table 22: Color change in response to pH at 3 and 7, $\Delta E_{O3,7}$ and $\Delta H_{O3,7}$ of colorimetric films with Dendrobium orchid*

Extract volume (mL)	$\Delta E_{O3,7}$	$\Delta H_{O3,7}$
20	6.82±0.44^{b}	6.16±0.77^{b}
30	9.51±1.14^{a}	8.95±0.96^{a}
40	8.48±0.92^{a}	7.37±0.69^{ab}
50	8.56±2.56^{ab}	6.64±0.37^{b}

*Values were expressed in mean ± standard deviation, based on three replications; values with similar superscript letter, within the same column, are not significantly different at α of 0.05.

ΔE of all films with butterfly pea were high, but the results in Table 23 indicated that increasing extract volume could significantly reduce visibility of color change in response to pH. Increasing level of color extract in the film could decrease the film's lightness and increase color saturation which could result in films at different pH environments becoming less

distinguishable[57]. The extraction volume of 6 mL/100 mL of film solution was selected to add maximum amount of anthocyanin without reducing the color changing capacity of the film.

Table 23: Color change in response to pH at 3 and 7, $\Delta E_{BP3,7}$ and $\Delta H_{BP3,7}$ of colorimetric films with butterfly pea*

Extract volume (mL)	$\Delta E_{BP3,7}$	$\Delta H_{BP3,7}$
4	17.24±1.44[a]	15.67±1.93[ab]
6	18.03±1.05[a]	15.35±0.84[a]
8	15.00±0.63[b]	12.92±0.88[b]
10	14.86±0.77[b]	11.18±0.72[c]

*Values were expressed in mean ± standard deviation, based on three replications; values with similar superscript letter, within the same column, are not significantly different at α of 0.05.

5. Colorimetric film characterization

Table 24 shows the base film's and developed indicators' thickness, water activity (a_w), moisture content, solubility, volume swelling ratio, natural color values, tensile strength, % elongation, and glass transition temperature (T_g), while Figure 13 shows scanning electron microscopy (SEM) images of indicators with and without cellulose powder.

In this study, addition of extracts from red cabbage, Dendrobium orchid, and butterfly pea, into base film did not significantly affect the properties of indicator films.

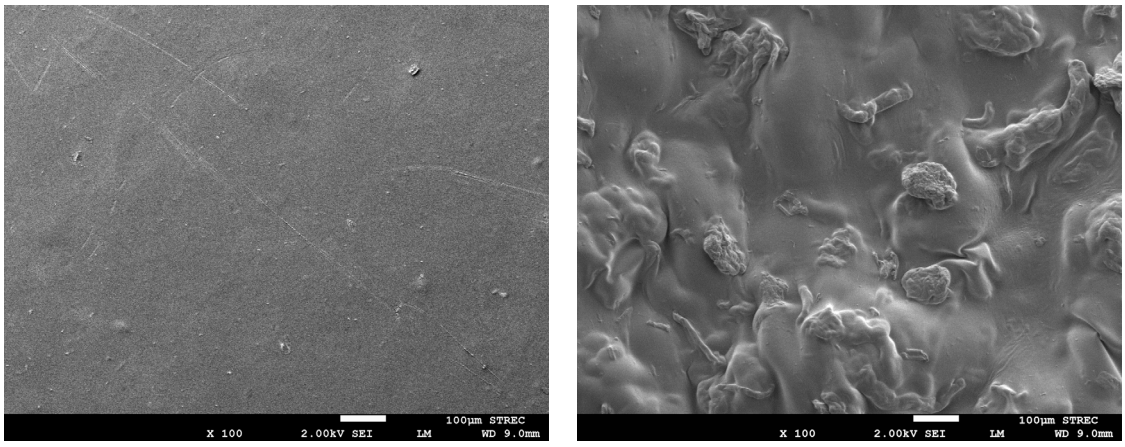


Figure 13. Film samples; Left - indicator film without cellulose powder; and Right – indicator film, with butterfly pea extract

Table 24. Properties of developed indicators with red cabbage, Dendrobium orchid, and butterfly pea extracts

Properties*	Base film		Indicator type		
	For cabbage/orchid	For butterfly pea	Red cabbage	Dendrobium orchid	Butterfly pea
Thickness (mm)	0.58±0.06[a]	0.57±0.07[a]	0.59±0.07[a]	0.62±0.10[a]	0.55±0.08[a]
a_w	0.40±0.02[a]	0.41±0.03[a]	0.41±0.04[a]	0.41±0.01[a]	0.41±0.04[a]
Moisture content (%)	9.95±0.60[a]	10.03±0.62[a]	10.42±0.79[a]	10.89±1.36[a]	10.13±0.58[a]
Solubility (%)	15.14±1.53[a]	16.97±1.91[a]	18.66±2.65[a]	17.05±0.76[a]	14.54±2.04[a]
Volume swelling ratio	1.46±0.39[a]	1.59±0.48[a]	1.55±0.21[a]	1.34±0.41[a]	1.66±0.32[a]
Color value:					
L*	n/a	n/a	30.15±0.45	34.21±0.88	42.09±1.52
a*	(translucent, whitish)	(translucent, whitish)	42.86±0.32	45.57±1.87	6.24±0.68
a*			11.45±0.83	2.62±0.65	-42.39±0.41
Tensile strength (MPa)	0.43±0.08[a]	0.40±0.07[a]	0.37±0.10[a]	0.33±0.06[a]	0.36±0.07[a]
Elongation (%)	92.81±5.45[a]	91.14±5.35[a]	87.49±4.10[a]	87.23±3.66[a]	94.83±5.89[a]
T_g (ºC)	77.90±2.96[a]	75.39±3.06[a]	77.04±2.71[a]	75.61±3.39[a]	74.89±4.06[a]
Decomposition temperature (ºC)	200.35±2.87[a]	198.71±3.53[a]	199.02±3.62[a]	202.06±7.02[a]	198.77±5.51[a]

*Values were expressed in mean ± standard deviation, based on 3 replications (except for mechanical properties which were based on 12 replicates); values with similar superscript letter, within the same roll, are not significantly different at α of 0.05.

However, based on previous studies on mechanical properties of edible film prepared from pectin and/or other polysaccharides, the prepared indicators had comparatively lower tensile strength[8, 9]. The decrease in tensile strength could be due to addition of cellulose powder which caused irregularity in the film (Figure 13), acting as stress concentrator[10]. The presence of carrageenan increased elongation of the developed indicators[58].

6. pH sensitivity of colorimetric films

Figure 14 shows colorimetric layers at various pH; and Table 25-27 show ΔE and ΔH of colorimetric layers at pH 2 to 7, with red cabbage, Dendrobium orchid, and butterfly pea, respectively. Originally, only the pairs with ΔE of less than 4.0, i.e. lower limit of 'significant color difference'[4] were to be subjected to sensory evaluation, but the actual difference test was conducted on all pairs with ΔE of less than 12.0 instead, based on the finding from preliminary experiment. Theoretically, >50% of group of untrained panelists should be able to distinguish the pair samples with ΔE 9.0 (upper limit of 'strong color difference') or more[4, 48], however, the minimum ΔE for sensory testing was raised to 12.0 to include the pH pairs that some 'consumers' might not be able to distinguish, and assess the actual percentage of the 'consumers' that might fail to notice the difference; ΔE of 12.0 was from preliminary experiment performed during consumer survey interview.

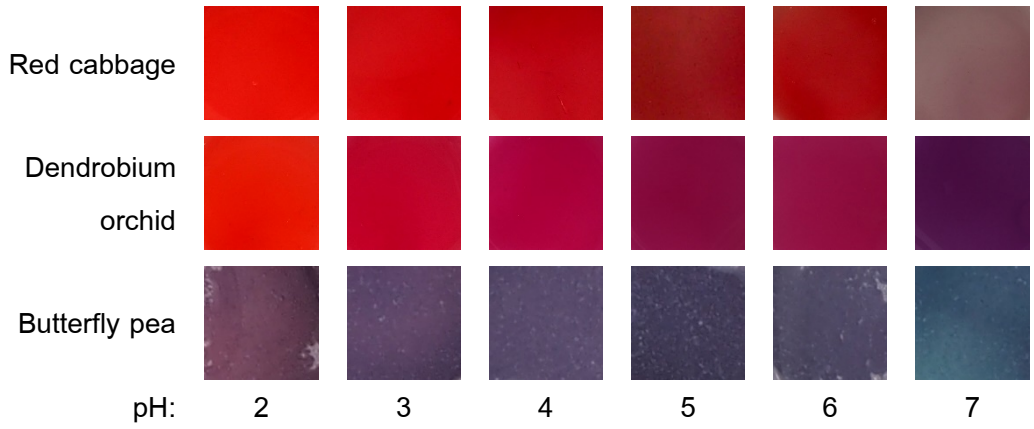


Figure 14. Colorimetric layers with different plant extracts at pH from 2 to 7

Table 25: Color change in response to pH at 2 to 7 of colorimetric films with red cabbage

pH	L*	a*	b*	pH	ΔE	ΔH	Further test
2	36.72±1.11	54.56±1.07	32.72±0.74	3	12.15	9.79	-
				4	26.93	14.67	-
				5	38.02	25.72	-
				6	27.98	14.08	-
				7	46.56	27.27	-
3	35.47±1.06	52.58±0.67	20.80±0.28	4	16.54	5.99	-
				5	27.00	17.11	-
				6	17.92	5.50	-
				7	36.50	19.74	-
4	34.44±0.69	39.85±0.17	10.28±0.45	5	11.35	9.90	Difference test
				6	4.69	0.37	Difference test
				7	20.16	12.90	-
5	35.21±0.94	35.66±0.38	-0.24±0.63	6	12.46	10.16	-
				7	10.80	4.20	Difference test
6	29.81±0.98	39.07±0.45	10.46±0.51	7	20.87	13.07	-
7	37.05±0.27	25.61±0.25	-3.74±0.39	-	-	-	-

Table 26: Color change in response to pH at 2 to 7 of colorimetric films with Dendrobium orchid

pH	L*	a*	b*	pH	ΔE	ΔH	Further test
2	43.79±0.93	64.97±0.61	45.55±0.18	3	14.08	10.75	-
				4	22.55	13.85	-
				5	32.93	17.43	-
				6	45.31	32.34	-
				7	65.93	42.04	-
3	42.15±0.72	62.85±0.93	31.73±0.77	4	9.90	3.46	Difference test
				5	20.77	7.56	-
				6	31.85	22.16	-
				7	53.59	33.00	-
4	36.32±0.28	57.81±0.46	25.51±0.67	5	10.96	4.12	Difference test
				6	22.91	18.12	-
				7	43.94	28.96	-
5	31.50±1.43	51.06±3.39	18.35±5.81	6	13.95	13.17	-
				7	33.11	23.92	-
6	32.32±0.14	49.55±1.19	4.51±0.92	7	23.37	12.62	-
7	19.82±0.61	33.77±0.31	-7.36±0.54	-	-	-	-

Table 27: Color change in response to pH at 2 to 7 of colorimetric films with butterfly pea

pH	L*	a*	b*	pH	ΔE	ΔH	Further test
2	32.58±1.09	20.98±0.89	-5.00±0.22	3	11.33	11.33	Difference test
				4	15.30	15.05	-
				5	17.45	16.07	-
				6	16.42	15.81	-
				7	28.59	28.17	-
3	33.41±0.45	15.58±0.68	-14.92±0.56	4	4.68	4.23	Difference test
				5	9.75	6.23	Difference test
				6	7.49	6.06	Difference test
				7	20.67	20.17	-
4	35.13±0.36	11.69±0.03	-16.88±0.22	5	8.87	2.29	Difference test
				6	4.42	2.16	Difference test
				7	16.60	16.32	-
5	27.10±1.21	8.18±0.26	-15.51±0.26	6	6.11	0.10	Difference test
				7	15.52	13.15	-
6	33.20±0.41	8.10±0.44	-15.16±0.34	7	13.29	13.11	-
7	35.34±1.42	-4.91±0.05	-16.82±0.34	-	-	-	-

Table 28 shows results of 'Two out of five' difference test of colorimetric layers with red cabbage, Dendrobium orchid, and butterfly pea. While ≥50% of panelists could distinguish the difference of colorimetric films' colors at different pH pairs included in sensory evaluation[4]. The results indicated the developed colorimetric films' limitations, i.e. large percentage of consumers might have problem distinguish indicators with red cabbage extract, showing pH 4 or 6, and 5 or 7 from one another. Similar problem could occur with Dendrobium orchid indicators at pH 4 or 5, as well as, indicators with butterfly pea extract at pH 3 or 4, 4 or 6, and 5 or 6.

Table 28: Results of 'Two out of five' difference test

Plant type	pH pair	Corrected answer (panelist)	Total (panelist)	Probability of a correct response (%)
Red cabbage	4,5	38	50	76%
	4,6	30		60%
	5,7	30		60%
Dendrobium orchid	3,4	39		78%
	4,5	27		54%
Butterfly pea	2,3	43		86%
	3,4	32		64%
	3,5	46		92%
	3,6	46		92%
	4,5	39		78%
	4,6	26		52%
	5,6	25		50%

In comparison to previous work, such as those reported by Veiga-Santos *et al.* (2011) and Bento *et al.* (2015) which incorporated plant extracts from Merlot grape and red cabbage, respectively, into biodegradable polymer matrix, this work provided pH indicator with higher sensitivity[59, 60].

7. Performance of colorimetric film as pH indicator

7.1. Performance testing on fermented fish and egg tofu

Figure 15 shows placement of indicators on fermented fish (Pla-som) and egg tofu; and Table 29 shows obtained pictures, and pH and color values of indicators; and aerobic plate count

(APC) of food sample as the fermentation progressed. Fermented fish is typically made from fermentation of Java barb (*Barbonymus gonionotus*) fish, with cooked rice or -sticky rice, salt, and garlic. The process often lasts less than a week and the finished fish product has a dominant sour flavor due to presence of lactic acid produced by lactic acid bacteria, such as *Lactobacillus plantarum* and *L. brevis*. The changes of the fermented fish's pH helps selecting the types of bacteria that will be metabolically active during the fermentation process (อังคณา และคณะ, 2553). The initial pH of homemade fermented fish was 6.62±0.31, and decreased to 4.55±0.28 and 3.68±0.30, at day 3 and 5, respectively, of the fermentation (Table 29). According to the Thai Community Product Standard (TCPS), fermented fish must have pH between 4.0-6.0[61], and most fermented fish products available in the market were reported to have pH values within the range. In small scale productions, the food processors/sellers are often prepared the products and stored them at room temperature, and then put the fermented fish on sale on day 3 or 4 of fermentation process[28, 62].

Table 30 shows obtained pictures, and pH and color values of indicators; and APC of tofu. The initial pH of purchased egg tofu was 7.75±0.19 and dropped to 6.19±0.46 on day 7 of storage (Table 29). According to the Thai Community Product Standard (TCPS), egg tofu must have pH between 7.0-9.0[63].

Figure 15. Placement of pH indicator with Dendrobium orchid on fermented fish (Left); and pH indicator with butterfly pea extract on egg tofu (Right)

The pH range associated with fermented fish was where color values of all developed indicators, especially indicators with red cabbage and butterfly pea were similar to the adjacent pH values, i.e. had small ΔE (Table 25, 27, and 28). However, color changes of indicators with Dendrobium extract, and, to a lesser extent, the indicators with butterfly pea, were noticeable

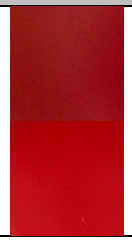
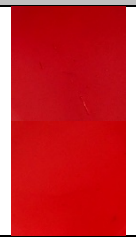
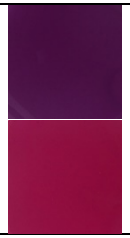
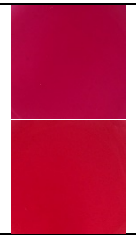
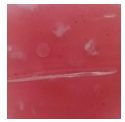
when the food's pH value changed from ~7 (original pH of fermented fish before fermentation begins) to ~4.5 (pH of fermented fish at the 3rd day of fermentation). This indicated that both pH indicators could be used to notify the sellers/consumers on when might be the right consumption period of this product.

The use of indicators, especially ones with red cabbage and Dendrobium orchid, with egg tofu was more promising since the colors of indicators at this pH range, i.e. color at pH 7.0 and 6.0, were more different (Table 25 and 26).

In both cases, even though all developed indicators visibly changed colors when exposed to food products, i.e. fermented fish and egg tofu, some of their actual colors were different from colors of indicators immersed in pH buffers. This could be due to interaction between anthocyanins and other small molecules in the food matrix as color change and/or discoloration of anthocyanins can be caused by many factors, e.g. pH of the environment, copigmentation, and presence of metallic ions or enzymes[13, 14]

Regarding the safety of fermented fish and egg tofu, since the responses of indicators were not sensitive enough to reflect the actual pH of the environment and/or the populations of microorganisms in the selected foods, the potential application of the developed indicators should be more towards notifying the consumers about the sensorial quality of the products[27].

Table 29: Pictures, and pH and color values of indicators; and APC of fermented fish as the fermentation progressed

Fermented fish	**Indicator**		**Day 0**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	6.0 and 7.0		6.62±0.30	4.0 and 5.0		4.55±0.27	3.0 and 4.0		3.68±0.30
Total plate count (log CFU/g)	4.69±0.14			7.37±0.14			8.83±0.23		
Indicator with red cabbage	7.0 6.0			5.0 4.0			4.0 3.0		
color values									
- L*			27.67±2.31			33.49±5.73			36.72±0.39
- a*			30.86±4.29			35.17±4.08			42.29±1.53
- b*			10.43±2.04			10.47±2.90			18.09±0.75
Indicator with Dendrobium orchid	7.0 6.0			5.0 4.0			4.0 3.0		
color values									
- L*			46.13±9.13			42.05±4.33			43.95±1.81
- a*			39.10±6.53			45.74±2.58			47.13±0.73
- b*			-8.20±1.11			8.09±3.47			21.22±1.25

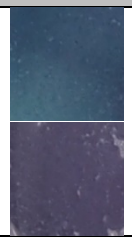
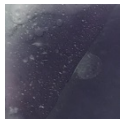
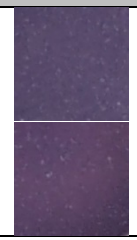
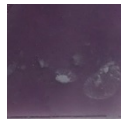
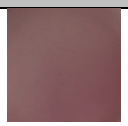
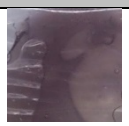
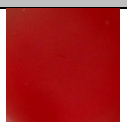
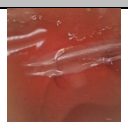
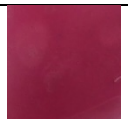
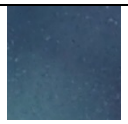
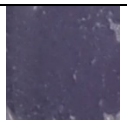
Fermented fish	**Indicator**		**Day 0**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	**6.0 and 7.0**		**6.62±0.30**	**4.0 and 5.0**		**4.55±0.27**	**3.0 and 4.0**		**3.68±0.30**
Total plate count (log CFU/g)	**4.69±0.14**			**7.37±0.14**			**8.83±0.23**		
Indicator with butterfly pea	7.0 6.0			5.0 4.0			4.0 3.0		
color values									
- L*			25.54±7.76			28.02±4.83			29.89±4.01
- a*			4.25±10.17			9.15±2.44			13.95±1.38
- b*			-12.00±1.01			-14.05±2.03			-9.79±0.58

Table 30: Pictures, and pH and color values of indicators; and APC of egg tofu during storage

Egg tofu	Indicator	Day 1	Indicator	Day 7
pH	7.0	7.49±0.22	6.0	6.19±0.46
Total plate count (log CFU/g)	< 1.00		3.83±0.12	
Indicator with red cabbage				
color values				
- L*		41.14±10.59		43.41±4.09
- a*		9.68±1.48		35.94±3.89
- b*		-0.60±3.43		24.30±2.48
Indicator with Dendrobium orchid				
color values				
- L*		26.20±6.05		30.45±2.74
- a*		33.07±0.46		44.07±1.14
- b*		-19.92±0.65		9.45±0.33
Indicator with butterfly pea				
color values				
- L*		31.04±3.37		27.56±10.33
- a*		0.61±2.58		5.34±0.61
- b*		-2.87±4.69		-2.49±2.20

7.2. Performance testing on fermented mushroom

Figure 16 shows placement of indicators on fermented mushroom; and Table 31 shows obtained pictures, and pH and color values of indicators; and APC of food sample as the fermentation progressed. The initial pH of homemade fermented mushroom was 7.11±0.14, and decreased to 4.44±0.08 and 4.24±0.10, at day 3 and 9 of fermentation process, respectively (Table 31). According to the TCPS, fermented mushroom must have pH equal to or less than 4.5[64]. Similar to fermented fish product, in small scale productions, product is stored at room temperature to initiate and accelerate fermentation, and then stored at 4ºC after 3-5 days of fermentation (at which point the product's pH values fall to around 4.0-4.5), depending on the type of mushroom used, to slow down activity of lactic acid bacteria. The shelf-life of this product is usually no longer than 10-14 days (since the day of production) [65].

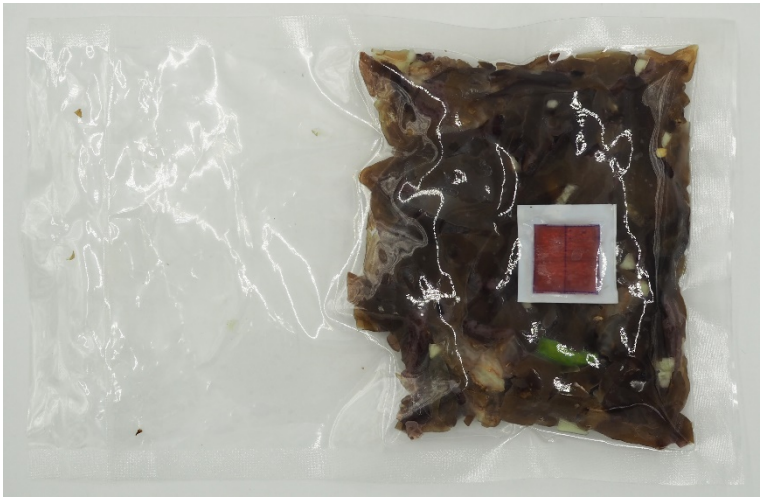
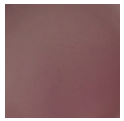
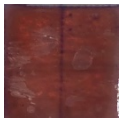
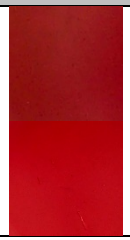
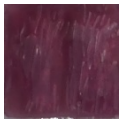
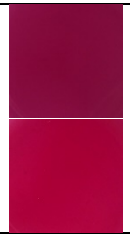
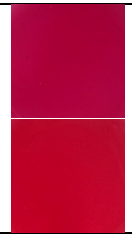
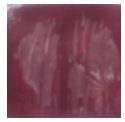


Figure 16. Placement of pH indicator with red cabbage extract on fermented mushroom

Similar to fermented fish, pH range associated with fermented mushroom was where color values at adjacent pH of indicators with red cabbage and butterfly pea were hard to distinguish (Table 25, 27, and 28), but color change of indicators with Dendrobium extract were more noticeable when the food's pH value decreased from ~7 to ~4.5 (pH of fermented mushroom at the 3rd day of fermentation). Since the food producers often recommend that the product be stored in refrigerator after 3-4 days of storage, visible change of indicator's color can help to ensure that. The pH of 4.5 and lower is also required by TCPS in consumption of fermented mushrooms[(64)].

Table 31: Pictures, and pH and color values of indicators; and APC of fermented mushroom as the fermentation progressed

Fermented fish	**Indicator**	**Day 0**	**Indicator**	**Day 3**	**Indicator**	**Day 9**
pH	7.0	7.11±0.14	4.0 and 5.0	4.44±0.08	3.0 and 4.0	4.24±0.10
Total plate count (log CFU/g)	4.63±0.23		7.51±0.10		10.70±0.07	
Indicator with red cabbage			5.0 4.0 		4.0 3.0 	
color values						
- L*		29.59±0.70		29.20±5.83		34.67±4.95
- a*		25.80±1.46		32.81±18.39		52.91±22.60
- b*		15.12±1.97		22.36±14.07		37.79±24.72
			5.0 4.0 		4.0 3.0 	
color values						
- L*		24.71±4.42		33.00±9.89		31.51±4.73
- a*		23.61±0.99		38.38±6.69		38.65±18.89
- b*		-1.79±2.30		0.01±3.61		9.32±10.72

Fermented fish	**Indicator**	**Day 0**	**Indicator**	**Day 3**	**Indicator**	**Day 9**
pH	7.0	7.11±0.14	4.0 and 5.0	4.44±0.08	3.0 and 4.0	4.24±0.10
Total plate count (log CFU/g)	4.63±0.23		7.51±0.10		10.70±0.07	
Indicator with butterfly pea			5.0 4.0			4.0 3.0		
color values								
- L*		36.49±4.87			32.63±7.47			37.32±6.00
- a*		-0.54±2.37			7.08±046			9.20±1.20
- b*		-4.90±2.58			-6.88±0.75			-7.66±2.26

7.3. Performance testing on fruit and fresh-cut fruit

Figure 17 shows placement of indicators on fruits and fresh-cut fruits; and Table 32-36 show obtained pictures, and pH and color values of indicators; and APC of food samples during storage. All indicators changed color according to pH and appeared to have colors closed to those of indicators immersed in pH buffers. However, in some products, e.g. coconut fruit, the indicator's colors visibly faded during storage. This could be due to prolonged immersion in coconut juice, causing the pigments to leak out or degrade. Anthocyanins dissolve in water and break down faster at neutral pH[15, 18]. Similar problem, to a lesser extent, also observed during the storage of sweet orange and watermelon.

Figure 17. From left to right, tom to bottom; placement of pH indicators with extracts from butterfly pea, butterfly pea, butterfly pea, Dendrobium orchid, red cabbage, and Dendrobium orchid on lemon, whole coconut fruit, sweet orange, yellow kiwi, pineapple, and watermelon, respectively

Table 32: Pictures, and pH and color values of indicators after 1 hour of placement, as compared to those of indicators immersed in pH buffers of similar (or closet to) the same pH values

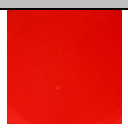
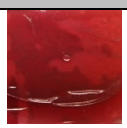
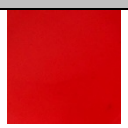
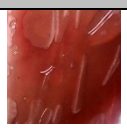
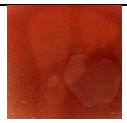
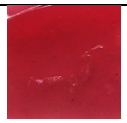
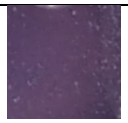
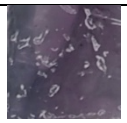
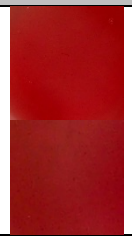
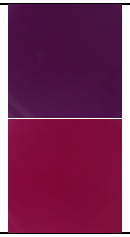
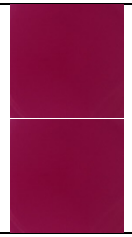
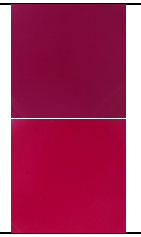
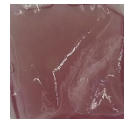
Food	**Indicator**	**Lemon**	**Indicator**	**Yellow kiwi**
pH	2.0	2.34±0.17	3.0	3.23±0.52
Indicator with red cabbage				
color values				
- L*		37.34±8.48		35.33±12.70
- a*		52.81±7.93		40.22±3.14
- b*		33.13±6.09		31.21±2.18
Indicator with Dendrobium orchid				
color values				
- L*		38.41±1.56		38.10±1.45
- a*		49.76±0.86		57.52±2.50
- b*		45.38±0.32		33.81±3.81
Indicator with butterfly pea				
color values				
- L*		39.69±12.44		34.89±2.79
- a*		19.10±2.89		6.67±3.12
- b*		1.84±1.52		-9.41±0.35

Table 33: Pictures, and pH and color values of indicators placed on whole coconut fruit, as compared to those of indicators immersed in pH buffers of similar (or closet to) the same pH values

Fermented fish	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	6.0 and 7.0		6.34±0.27	5.0 and 6.0		5.78±0.20	4.0 and 5.0		4.71±0.13
Total plate count (log CFU/g)	1.65±0.60			3.25±0.94			6.34±0.90		
Indicator with red cabbage	7.0 6.0			6.0 5.0			5.0 4.0		
color values									
- L*			38.23±7.03			43.94±5.39			50.45±4.06
- a*			19.23±0.59			29.62±2.64			8.69±3.94
- b*			2.91±0.58			11.74±1.58			9.24±3.77
Indicator with Dendrobium orchid	7.0 6.0			6.0 5.0			5.0 4.0		
color values									
- L*			32.79±6.43			44.04±3.49			42.63±6.47
- a*			29.47±5.55			23.90±3.83			21.17±4.01
- b*			7.04±0.47			8.62±0.45			6.62±1.13

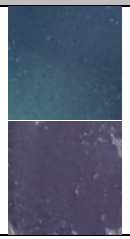
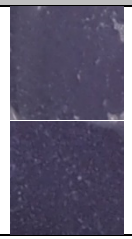
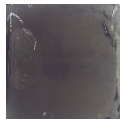
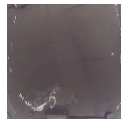
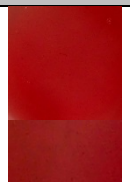
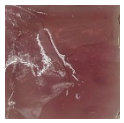
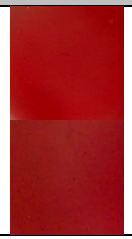
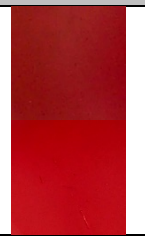
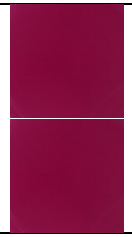
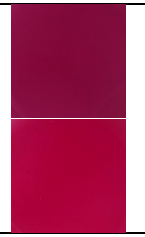
Fermented fish	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	6.0 and 7.0		6.34±0.27	5.0 and 6.0		5.78±0.20	4.0 and 5.0		4.71±0.13
Indicator with butterfly pea	7.0 6.0			6.0 5.0			5.0 4.0		
color values									
- L*			26.13±3.51			34.66±6.60			36.76±3.72
- a*			2.51±2.70			2.73±1.49			5.27±0.46
- b*			-4.60±0.77			0.98±2.68			0.42±1.95

Table 34: Pictures, and pH and color values of indicators placed on sweet orange, as compared to those of indicators immersed in pH buffers of similar (or closet to) the same pH values

Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	5.0 and 6.0		5.47±0.40	5.0 and 6.0		5.40±0.32	4.0 and 5.0		4.70±0.16
Total plate count (log CFU/g)	4.75±0.05			6.28±0.03			7.34±0.33		
Indicator with red cabbage	6.0 5.0			6.0 5.0			5.0 4.0		
color values									
- L*			37.97±0.41			33.60±3.53			38.01±6.08
- a*			23.32±0.66			16.00±5.21			15.35±3.95
- b*			10.94±1.87			10.55±2.78			10.72±1.02
Indicator with Dendrobium orchid	6.0 5.0			6.0 5.0			5.0 4.0		
color values									
- L*			25.57±5.26			18.99±3.36			20.32±2.15
- a*			26.62±4.52			25.74±1.22			22.58±3.80
- b*			9.39±1.32			5.79±0.83			9.27±1.54

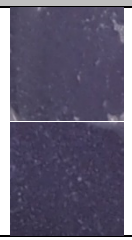
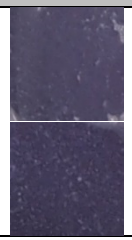
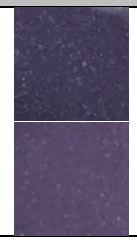
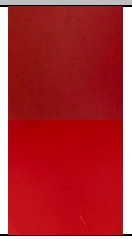
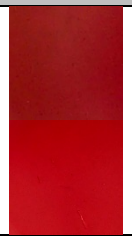
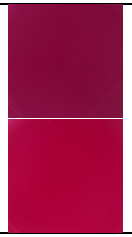
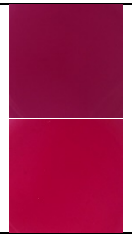
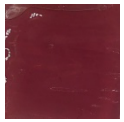
Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	5.0 and 6.0		5.47±0.40	5.0 and 6.0		5.40±0.32	4.0 and 5.0		4.70±0.16
Indicator with butterfly pea	6.0 5.0			6.0 5.0			5.0 4.0		
color values									
- L*			33.77±2.29			27.21±4.30			34.57±4.28
- a*			3.81±0.79			2.10±0.89			2.87±0.36
- b*			--4.38±1.21			-0.23±0.72			2.01±1.73

Table 35: Pictures, and pH and color values of indicators placed on pineapple, as compared to those of indicators immersed in pH buffers of similar (or closet to) the same pH values

Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**	**Day 5**
pH	4.0 and 5.0		4.42±0.35	4.0 and 5.0		4.35±0.32	4.0	4.18±0.38
Total plate count (log CFU/g)	3.80±0.44			5.36±0.12			7.01±0.35	
Indicator with red cabbage	5.0 4.0			5.0 4.0				
color values - L* - a* - b*			32.34±2.49 30.59±0.57 17.93±1.67			33.74±6.55 25.86±5.56 16.67±3.06		29.67±5.66 24.28±4.53 15.93±1.56
Indicator with Dendrobium orchid	5.0 4.0			5.0 4.0				
color values - L* - a* - b*			25.32±3.46 29.49±2.12 8.85±0.99			25.36±0.29 33.63±1.42 11.40±0.77		26.54±1.21 33.47±0.37 12.68±1.33

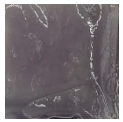
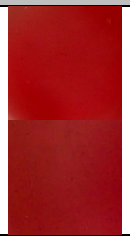
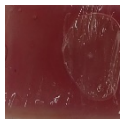
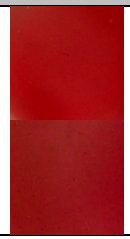
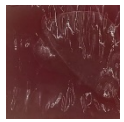
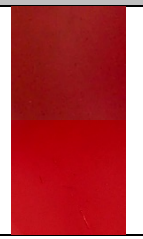
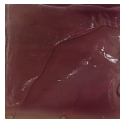
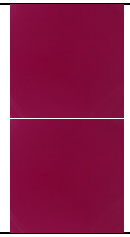
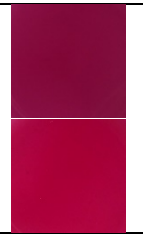
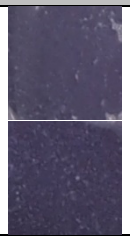
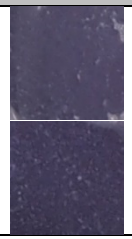
Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**	**Day 5**
pH	4.0 and 5.0		4.42±0.35	4.0 and 5.0		4.35±0.32	4.0	4.18±0.38
Total plate count (log CFU/g)	3.80±0.44			5.36±0.12			7.01±0.35	
Indicator with butterfly pea	5.0 4.0			5.0 4.0				
color values								
- L*			37.20±2.81			33.30±2.42		39.57±7.26
- a*			4.37±1.06			6.15±1.01		6.66±2.34
- b*			-2.62±0.50			3.00±1.41		-0.78±2.60

Table 36: Pictures, and pH and color values of indicators placed on watermelon, as compared to those of indicators immersed in pH buffers of similar (or closet to) the same pH values

Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	5.0 and 6.0		5.45±0.26	5.0 and 6.0		5.42±0.25	4.0 and 5.0		4.64±0.10
Total plate count (log CFU/g)	4.46±0.07			6.12±0.19			8.21±0.05		
Indicator with red cabbage	6.0 5.0			6.0 5.0			5.0 4.0		
color values - L* - a* - b*			26.97±1.69 32.00±0.91 16.61±1.09			27.26±7.25 24.06±2.68 10.39±2.92			26.81±3.66 24.35±4.33 17.69±1.69
Indicator with Dendrobium orchid	6.0 5.0			6.0 5.0			5.0 4.0		
color values - L* - a* - b*			27.14±1.20 22.55±0.96 7.82±2.40			25.99±4.57 21.77±2.51 9.73±2.10			33.12±4.53 28.20±5.47 16.22±1.59

Sweet orange	**Indicator**		**Day 1**	**Indicator**		**Day 3**	**Indicator**		**Day 5**
pH	5.0 and 6.0		5.45±0.26	5.0 and 6.0		5.42±0.25	4.0 and 5.0		4.64±0.10
Indicator with butterfly pea	6.0 5.0			6.0 5.0			5.0 4.0		
color values									
- L*			33.94±3.60			28.02±7.02			35.01±5.28
- a*			3.58±1.16			2.51±0.56			2.74±0.34
- b*			-5.05±0.86			-1.17±2.65			3.28±3.20

Another limitation of pH indicator incorporated into the system to monitor the product's quality was that direct contact between the food's surface and the indicator is crucial[27]; in this case, the indicator must be at least partially submersed into the foods. It was observed during the study that there were possible interferences from the foods' colors (Figure 18). In some case, even though the indicator prototypes had white backing, it was difficult to notice/measure current color of the indicators without removing them from the products and cleaning/wiping out the colored fluids from the foods.

a b

Figure 18. Placement of pH indicator with Dendrobium orchid on grass jelly (a); and pH indicator with red cabbage extract on purple dragon fruit (b)

8. CO_2 sensitivity of colorimetric films

Figure 19 shows colorimetric layers at various CO_2 concentrations; and Table 37-39 show ΔE and ΔH of colorimetric layers with red cabbage, Dendrobium orchid, and butterfly pea, respectively, at different CO_2 concentrations.

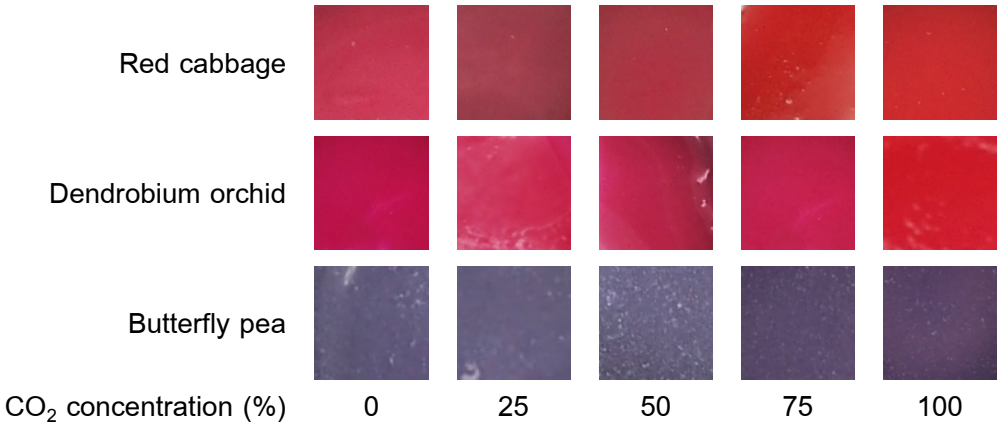


Figure 19. Colorimetric layers with different plant extracts at CO_2 level from 0 to 100%

Table 40 shows results of 'Two out of five' difference test of colorimetric layers with red cabbage and butterfly pea. The results indicated the developed colorimetric films' limitations, i.e. majority of consumers might have problem distinguish indicators, with red cabbage, Dendrobium orchid, and butterfly pea, exposed to CO_2 at concentration of less than 75, 100, or 75, respectively, from each other. Similarly, there were no difference in color characteristics of indicators with red cabbage or butterfly pea between samples exposed to CO_2 at concentrations of 75-100%. On the other hand, there were significant color differences between indicators that were exposed to <75% CO_2 and ≥75% CO_2.

Table 37: Color change in response to CO_2 concentration of colorimetric films with red cabbage

[CO2] (%)	L*	a*	b*	[CO2] (%)	ΔE	ΔH	Further test
0	44.86±0.61[a]	48.09±0.41[c]	34.78±0.63[c]	25	2.25	1.28	-
				50	7.35	1.60	Difference test
				75	13.08	0.75	-
				100	13.22	0.06	-
25	43.36±1.29[b]	49.72±1.65[c]	34.36±2.32[b,c]	50	6.05	0.26	Difference test
				75	11.83	0.65	Difference test
				100	12.12	1.48	-
50	43.89±0.47[b]	54.83±1.18[b]	37.56±1.94[b]	75	5.97	0.97	Difference test
				100	6.32	1.84	Difference test
75	42.74±0.65[a,b]	59.02±1.41[a]	41.65±0.48[a]	100	1.04	0.90	-
100	43.21±1.48[b]	58.68±1.75[a,b]	42.52±0.94[a]	-	-	-	-

Table 38: Color change in response to CO_2 concentration of colorimetric films with Dendrobium orchid

[CO2] (%)	L*	a*	b*	[CO2] (%)	ΔE	ΔH	Further test
0	45.08±0.69[b]	63.77±0.50[a]	19.97±0.69[b]	25	2.92	1.53	-
				50	2.10	1.06	-
				75	1.77	0.91	-
				100	21.17	19.57	-
25	46.51±1.61[a,b]	63.82±1.57[a]	21.59±1.94[b]	50	2.04	0.45	-
				75	3.12	0.60	-
				100	19.26	18.06	-
50	46.48±0.79[a,b]	62.35±1.23[a]	20.62±1.77[b]	75	1.30	0.15	-
				100	20.23	18.30	
75	45.24±1.32[a,b]	62.06±1.23[a]	20.37±0.93[b]	100	20.65	18.41	-
100	48.91±2.04[a]	61.72±0.11[b]	40.69±1.45[a]	-	-	-	-

Table 39: Color change in response to CO_2 concentration of colorimetric films with butterfly pea

[CO2] (%)	L*	a*	b*	[CO2] (%)	ΔE	ΔH	Further test
0	50.29±0.86[a]	11.51±0.58[c]	-14.49±2.07[c]	25	2.71	0.50	-
				50	6.59	1.90	Difference test
				75	13.31	2.49	-
				100	12.87	2.37	-
25	49.12±1.25[b]	13.41±2.54[c]	-16.03±2.08[b,c]	50	4.28	1.47	Difference test
				75	10.78	2.03	Difference test
				100	10.42	1.91	Difference test
50	45.46±2.68[b]	15.62±1.39[b]	-16.29±1.49[b]	75	6.84	0.41	Difference test
				100	6.36	0.32	Difference test
75	41.10±0.93[a,b]	19.58±0.64[a]	-19.76±0.44[a]	100	0.96	0.10	-
100	40.81±1.88[b]	18.87±2.30[a,b]	-19.18±1.45[a]	-	-	-	-

Table 40: Results of 'Two out of five' difference test

Plant type	[CO_2] pair	Corrected answer (panelist)	Total (panelist)	Probability of a correct response (%)
Red cabbage	0,50	26	50	52%
	25,50	14		28%
	25,75	40		80%
	50,75	43		86%
	50,100	41		82%
Butterfly pea	0,50	13		26%
	25,50	11		22%
	25,75	39		78%
	25,100	47		94%
	50,75	49		98%
	50,100	46		92%

9. Color-changing mechanism of colorimetric films

Figure 20 and 21 show IR spectrum of colorimetric layers with red cabbage and Dendrobium orchid extract, respectively, at pH values of 2, 3, 5, and 7.

Both red cabbage and Dendrobium orchid consist mainly of cyanidin type anthocyanin pigment[16, 17, 30]. In environment with pH ~2, cyanidin is in its flavylium cation form which gives off the reddish color; the structure changes to hemiketal cyanidin at pH value of ~5, and then to quinoidal base cyanidin, at pH of ~6 or more. This form turns the color bluish[66]. Thus, in the range of pH 2-7, the colors shifted from reddish toward purple at neutral pH.

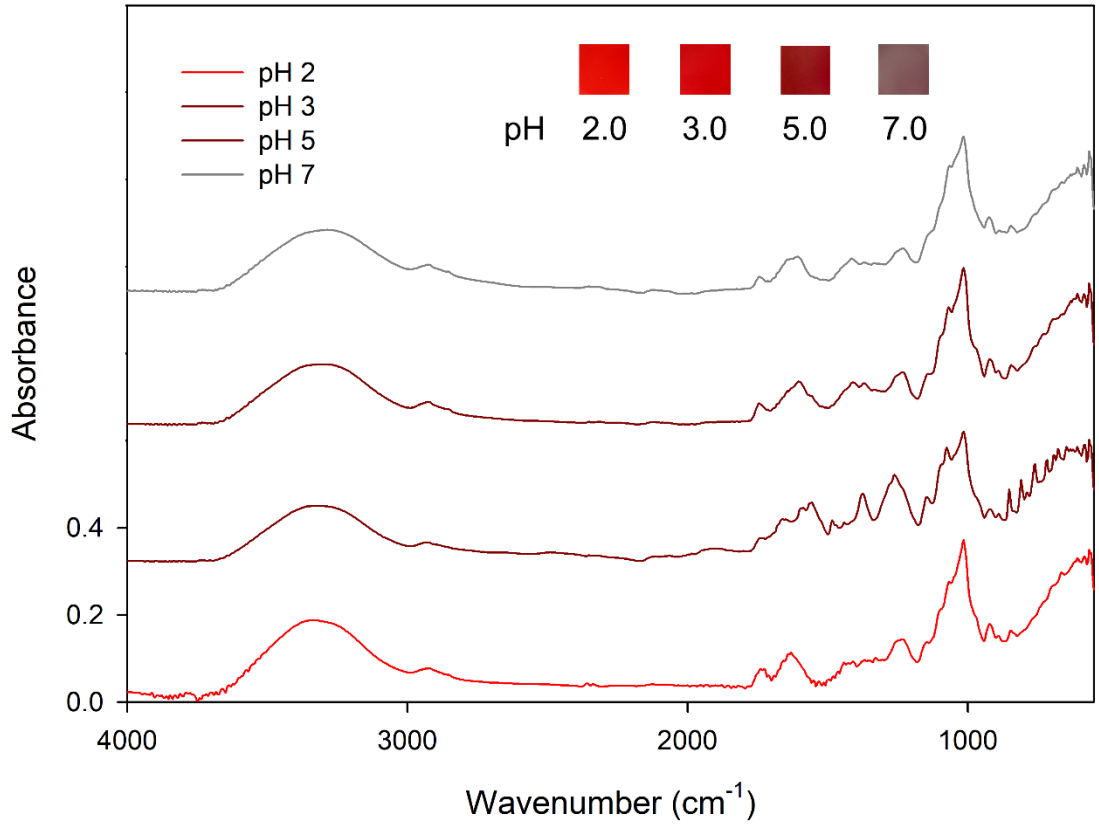


Figure 20: IR spectrum of colorimetric layer with red cabbage extract

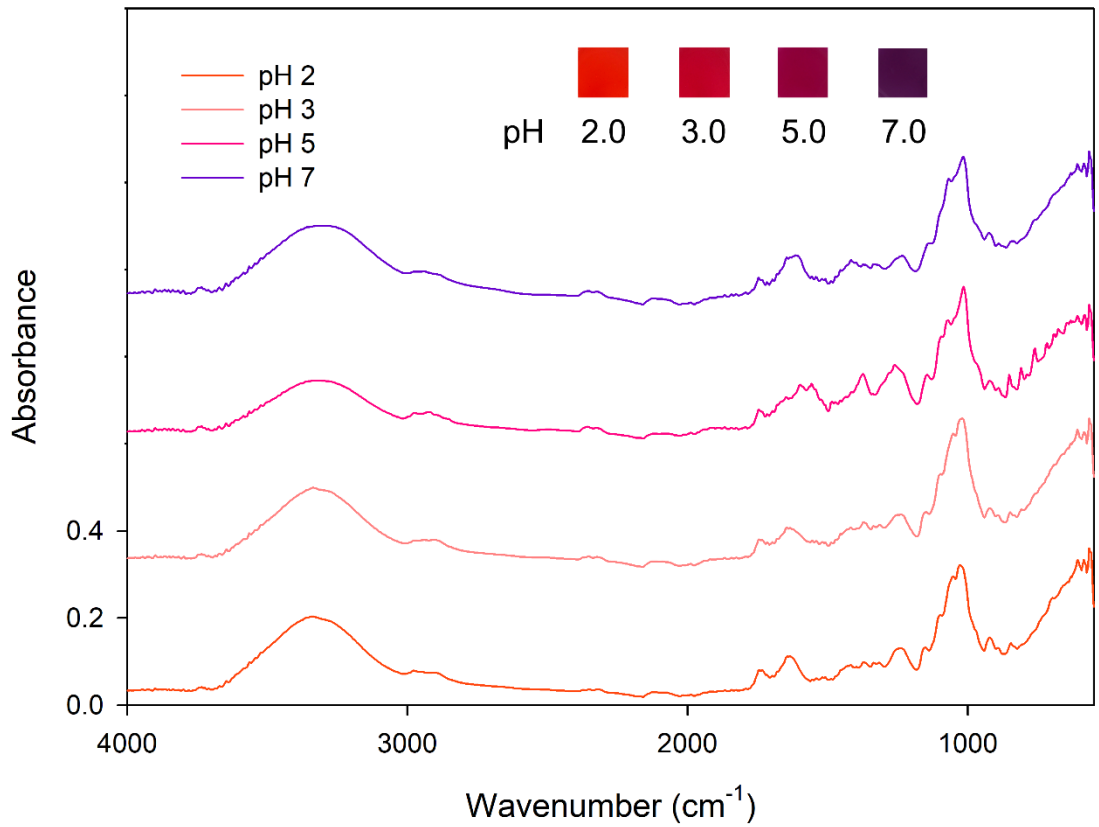


Figure 21: IR spectrum of colorimetric layer with Dendrobium orchid extract

IR spectrum of both indicators show changes in 3 main areas indicating changes in cyanidin structure, resulting in noticeable color changes. The 3,600–3,200 cm^{-1} area represents intermolecular bonded hydroxyl (-OH) bond; 1,685-1,660 cm^{-1} represents carbonyl (-C=O) group in conjugated ketone; 1,620-1,610 cm^{-1} represents alkene (-C=C-) bond in structure of α,β-unsaturated ketone; and 1,130-1,070 cm^{-1} area represents -C-O- bond in secondary alcohol[67, 68]. The 3,600-3,200 cm^{-1} peaks of indicator with red cabbage extract at pH of 2 and indicator with Dendrobium orchid extract at pH of 2 and 3 were large and shifted toward higher wavenumber, this indicated that, in such environment, the bond length of hydroxyl group decreased which could be due to the higher electronegativity of O+ in flavylium cation form[68, 69]. The increasing prominence of peaks in 1,130-1,070 cm^{-1} area in indicator with red cabbage and Dendrobium extracts at pH of 3 and 5, and 5, respectively indicated the presence of -C-O- bond in secondary alcohol, which referred to cyanidin in its hemiketal form. Finally, the increase in peak high at 1,609.33 and 1 ,612.83 cm^{-1} in indicator with red cabbage and Dendrobium orchid extract at pH 7, respectively, represented the presence of α,β-unsaturated ketone in quinoidal base form. The predicting color-changing mechanism of anthocyanin (cyanidin type) in colorimetric films with red cabbage and Dendrobium orchid extract was as follows:

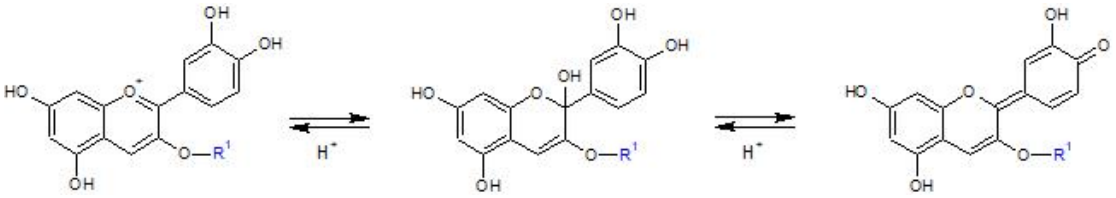


Figure 22: Purposed color-changing mechanism of colorimetric film with red cabbage or Dendrobium extract in the form of cyanidin in different pH environment

Figure 23 shows IR spectrum of colorimetric layers with butterfly pea extract, at pH values of 2, 3, 5, and 7.

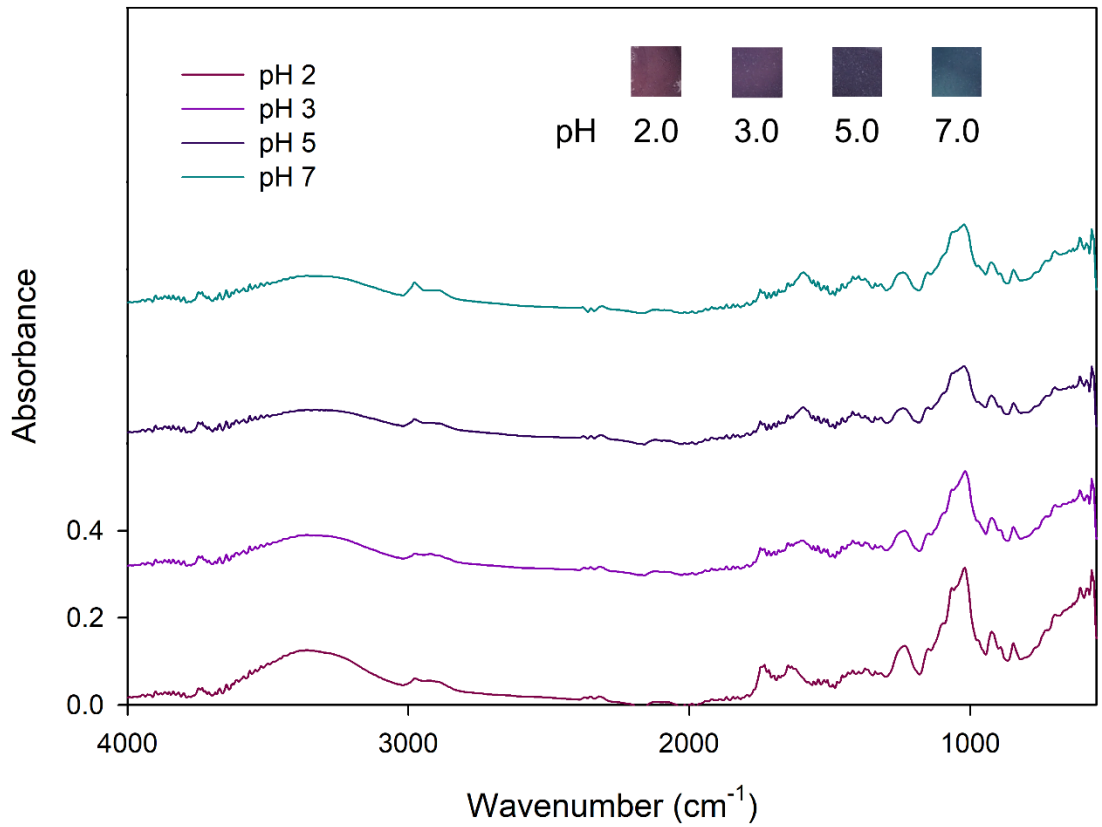


Figure 23: IR spectrum of colorimetric layer with butterfly pea extract

Unlike red cabbage and Dendrobium orchid, butterfly pea consists mainly of delphinidin type anthocyanin pigment[12, 42]. In environment with pH ~2, delphinidin is in its flavylium cation form which gives off the reddish color; the structure changes to anhydrobase and, later, anhydrobase anion as the pH of the environment increased, turning the color to purple and blue, respectively[12]. Thus, in the range of pH 2-7, the colors shifted from reddish toward purple to blue at neutral pH.

IR spectrum shows changes in 3 main areas indicating changes in delphinidin structure. The 3,600–3,200 cm^{-1} indicates the presence of intermolecular bonded hydroxyl bond; 1,685-1,660 cm^{-1} represents carbonyl group in conjugated ketone; and 1,650-1,600 cm^{-1} represents alkene group in conjugated alkene[5, 6, 67, 68]. The 3,600-3,200 cm^{-1} peaks of indicator with butterfly pea extract at pH of 2 were significantly larger than those of indicator at other pH values, indicating that the structure of delphinidin, at this pH, had higher amount of hydroxyl group, confirming the structure of flavylium cation[68]. The presence of ~1,660 cm^{-1} peak of indicator at pH 3, 5, and 7 meant that, at these pH, (at least) some of delphinidin were in the form of either anhydrobase or anhydrobase anion; and the peaks at 1,620-1,610 cm^{-1} in indicator at pH 3, 5, and 7 indicated the presence of conjugated alkene in the structure, also confirming either anhydrobase or anhydrobase anion of delphinidin. It was not possible, based on observation of these IR spectrum,

to distinguish between anhydrobase and anhydrobase anion. However, based on the blueish color of the indicator at pH 7, it was reasonable to concluded that (at least some of) delphinidin were in anhydrobase anion. The predicting color-changing mechanism of anthocyanin (delphinidin type) in colorimetric films with butterfly pea extract was as follows:

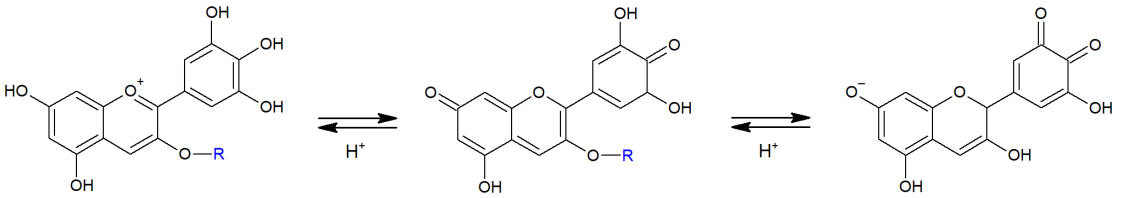


Figure 24: Purposed color-changing mechanism of colorimetric film with butterfly pea extract in the form of delphinidin in different pH environment

10. Shelf-life of developed pH indicator

Table 42-44 show color values and ΔE of pH indicators with red cabbage, Dendrobium orchid, and butterfly pea extract measured during storage, respectively. From the table, it was found that, at different pH, the colors of pH indicators during storage did not become less distinguishable (ΔE_{pH} values were either equal or higher as compared between different storage time), but the colors of indicators at particular pH point (3, 5, or 7) changed ($\Delta E_{storage}$ values) during storage. At the end of week 3, 4, and 6 of storage, in sealed vacuum package, at 25ºC, $\Delta E_{storage}$ values of pH indicators with red cabbage, Dendrobium orchid, and butterfly pea extract, respectively, exceeded 4.0[4], indicating noticeable discoloration of pH indicators immersed in pH buffer pH 3, 5, or 7[68]. The most changed parameter was L* value, indicating that the film color became darker. This could be due to decolorization and browning of destructed anthocyanin pigments[21, 68]. Similar trend was observed by Luna-Vital *et al*. (2018), i.e. significant degradation of copigmented anthocyanins from purple corn in beverage model could be observed through the changes in their color values. The study found that L*, a*, and b* values of the samples significantly decreased[21].

Table 41: Color values and ΔE of pH indicator with red cabbage extract during storage at 25ºC

Week	Color value of pH indicator during storage period at 25ºC									ΔE_{pH}			$\Delta E_{storage}$			
	3			5			7			3,5	3,7	5.7	Week	3	5	7
	L*	a*	b*	L*	a*	b*	L*	a*	b*							
0	35.47 ±2.54	52.57 ±1.37	20.80 ±0.80	35.21 ±3.07	35.64 ±1.52	-0.24 ±2.21	37.05 ±2.22	25.61 ±2.02	-3.74 ±0.88	27.01	36.50	10.78	-	-	-	-
2	32.39 ±1.24	54.14 ±2.20	20.64 ±0.77	33.03 ±2.09	36.92 ±1.78	-0.17 ±1.53	36.34 ±2.31	26.11 ±2.03	-3.34 ±1.13	27.02	37.10	11.74	0,2	3.45	2.53	0.96
3	30.33 ±1.62	53.90 ±1.40	22.13 ±1.34	29.03 ±2.33	36.82 ±2.65	-0.24 ±1.60	33.99 ±2.04	26.52 ±2.38	-3.44 ±1.25	28.18	37.64	11.87	0,3	5.47	6.29	3.21
4	25.67 ±1.85	50.18 ±1.30	24.82 ±2.31	25.46 ±2.43	35.99 ±1.71	-0.31 ±1.44	30.83 ±1.86	27.06 ±2.28	-4.02 ±0.87	28.86	37.31	11.05	0,4	10.86	9.75	6.40

Table 42: Color values and ΔE of pH indicator with Dendrobium orchid extract during storage at 25ºC

Week	Color value of pH indicator during storage period at 25ºC									ΔE_{pH}			$\Delta E_{storage}$			
	3			5			7			3,5	3,7	5.7	Week	3	5	7
	L*	a*	b*	L*	a*	b*	L*	a*	b*							
0	42.15 ±2.46	62.85 ±2.69	31.73 ±1.63	31.50 ±2.74	51.06 ±4.71	18.35 ±6.45	19.82 ±0.76	33.77 ±1.80	-7.36 ±1.47	20.77	53.59	33.11	-	-	-	-
2	41.58 ±3.10	62.76 ±2.36	30.99 ±2.10	30.36 ±1.66	52.23 ±2.24	18.52 ±3.14	17.30 ±2.57	33.37 ±2.94	-7.11 ±2.99	19.81	53.90	34.40	0,2	0.93	1.64	2.56
4	39.48 ±3.37	63.27 ±2.00	30.43 ±2.15	30.07 ±4.54	53.12 ±4.47	17.35 ±5.58	15.23 ±3.01	32.28 ±3.67	-7.65 ±3.19	19.05	54.76	35.77	0,4	2.99	2.70	4.84
5	36.80 ±2.29	62.24 ±2.56	30.27 ±2.25	28.10 ±4.74	52.81 ±4.91	16.76 ±2.81	14.06 ±3.69	29.00 ±2.27	-7.16 ±1.87	18.63	54.98	36.55	0,5	5.58	4.14	7.48

Table 43: Color values and ΔE of pH indicator with butterfly pea extract during storage at 25ºC

Week	Color value of pH indicator during storage period at 25ºC									ΔE_{pH}			$\Delta E_{storage}$			
	3			5			7			3,5	3,7	5.7	Week	3	5	7
	L*	a*	b*	L*	a*	b*	L*	a*	b*							
0	33.41 ±2.49	15.58 ±1.07	-14.92 ±1.15	27.10 ±2.06	8.18 ±0.73	-15.51 ±1.22	35.34 ±3.46	-4.91 ±1.63	-16.81 ±1.09	9.75	20.67	15.52	-	-	-	-
2	33.14 ±2.29	15.85 ±1.24	-15.63 ±1.96	26.29 ±1.27	7.84 ±0.92	-15.03 ±1.20	36.26 ±1.67	-4.45 ±1.33	-16.30 ±1.37	10.55	20.59	15.91	0,2	0.81	1.00	1.13
4	31.64 ±1.91	15.94 ±1.14	-15.75 ±1.09	24.89 ±2.54	7.82 ±1.06	-15.05 ±1.10	37.91 ±2.10	-4.37 ±1.25	-15.95 ±0.96	10.59	21.26	17.86	0,4	1.99	2.29	2.76
6	26.49 ±2.68	17.73 ±1.72	-16.72 ±1.24	25.78 ±2.29	9.07 ±1.06	-15.40 ±1.53	34.86 ±2.77	-4.12 ±1.94	-15.46 ±1.42	8.79	23.43	16.01	0,6	7.48	1.60	1.65
7	22.94 ±2.55	18.21 ±1.03	-18.03 ±1.23	24.75 ±1.91	10.41 ±0.95	-15.51 ±1.22	31.75 ±1.12	-3.49 ±0.98	-15.43 ±1.78	8.39	23.57	15.57	0,7	11.24	3.25	4.11

Conclusion and Future Work

(สรุป และข้อเสนอแนะสำหรับงานวิจัยในอนาคต)

Colorimetric indicators for pH and CO_2 to be used primarily in packaging system for short shelf-life foods were developed from plant extracts. The plants, i.e. fresh red cabbage (*Brassica oleraces* var. *capitate* f. *rubra*), Dendrobium orchid (*Dendrobium* Sonia 'Earsakul'), and butterfly pea (*Clitoria ternatea* L.), were selected based on their abilities to visibly change colors when exposed to different pH environments. Visible spectrum (400 - 700 nm) and wavelengths with maximum absorbance (λ_{max}) of each plant extract prepared using hot water extraction were obtained. The λ_{max} of red cabbage, Dendrobium orchid, and butterfly pea extracts were 553 nm, 543 and 583 nm, and 574 and 618 nm, respectively.

Optimization of microwave assisted hot water extraction (mHWE) for each selected plant were achieved using response surface methodology (RSM) – Box-Behnken design and desirability analysis. The parameters included in the study were sample to water ratio (g/mL), extraction power (W), and extraction time (s); the response was absorbance at λ_{max}. Total monomeric anthocyanin pigment content of all extracts were also determined. Positive correlations between total monomeric anthocyanin contents and absorbance at λ_{max} were observed in all plant extract samples. According to the constructed mathematical models, 1) sample to water ratio and 2) extraction time were the parameters that prominently influenced the extraction yields. The conditions for mHWE for all selected plants were identified, i.e. sample to water ratio of 1:30 g/mL, power of 800 W, and extraction time of 180 s for butterfly pea; and 1:3 g/mL, 800 W, and 480 s for red cabbage and Dendrobium orchid. These selected conditions gave maximum absorbance at λ_{max} and total monomeric anthocyanin content, while maintaining the quality of the extracts and did not cause violent boiling.

RSM and deniability analysis were used to determine optimal formula of colorimetric layer, with color change in response to pH and solubility of the prepared films as response. Suitable amount of each plant extract for the colorimetric layer was identified through difference test (Two out of five) by 50 untrained panelists. The final formula for colorimetric layers were 3% (w/w) of carrageenan, 2% (w/w) of pectin, 1% (w/w) of cellulose powder, and 40 % (v/w) of extract for colorimetric layer with red cabbage or Dendrobium orchid extract; and 1.5% (w/w) of CMC, 1.5% (w/w) of carrageenan, 1.5% (w/w) of pectin, 1% (w/w) of cellulose powder, and 6 % (v/w) of extract for colorimetric layer with butterfly pea. These formula gave films with high color-changing capacity (ΔE and/or ΔH) when exposed to different pH values, and low films' solubility. The colorimetric films prepared based on final formula were determined their physical-, optical-,

morphological, mechanical-, and thermal properties. Apart from their color characteristics, there was no significant difference in properties among films with different plant extracts.

The films were also studied their color-changing mechanism by observing their IR spectrum and color appearances at pH value of 2, 3, 5, and 7. In indicator with red cabbage and Dendrobium orchid extract, which the main anthocyanin was reported to be cyanidin[30], the pigment changed its form from flavylium cation to hemiketal to quinoidal base, at pH of 2-3, 4-5, and 6-7, respectively, changing the film's color from red to purple[11]. On the other hand, delphinidin was reported to be the main anthocyanin found in butterfly pea[42]. Comparison of IR spectrum and the film's colors at different pH values indicated that the pigment changed from flavylium cation to anhydrobase to anhydrobase antion, at pH 2, 3-5, and 7, respectively, resulting in the film's color from reddish purple to purple to blue.

developed indicator films were assessed their sensitivity to the changes of pH and CO_2 concentration. For sensitivity to pH of the environment, ΔE values of colorimetric layers that were immersed in different pH buffer (pH of 2-7) were determined, and, for sensitivity to CO_2, the films were exposed to gas mixture with various CO_2 concentration (0-100% v/v). The pairs of indicators treated with different pH buffer or CO_2 concentration that had low ΔE were then subjected to difference test by 50 untrained panelists to further assess if the color difference can be distinguish by the naked eyes. It was found that colorimetric films with red cabbage, Dendrobium orchid, and butterfly pea extracts responded well to changes in pH of the environments. However, similarities of the films' colors at some pH values might make it difficult for observers to identify pH of the environment, in some cases, especially, environments with pH 4-6, for films with red cabbage or butterfly pea extract. The ΔE values obtained from exposing colorimetric layers to CO_2 at different concentrations showed that visual changes of the films' colors were low and occurred mostly at CO_2 level of 75 and 100% CO_2. Based on this finding, the performance of developed films as CO_2 indicator was not studied.

Colorimetric films were made into pH indicator prototype. The final designs consisted of outer layers of cellophane film and PLA sheet, and white plastic backing film. The indicators changed color when exposed to food products with different pH, but the sensitivity was not high enough to be used as safety measure for short shelf-life food products. However, all indicators, especially indicator with Dendrobium extract, might have potential use in informing the consumers about the foods' qualities, especially for fermented foods, such as fermented fish and mushroom, since the change in pH helps the consumers in decision making regarding taste acceptability, and partly indicates the safety of the foods. The use of these indicators in an acidic environment can also maintain quality of anthocyanin longer than using them at higher pH values[11]. During

the performance study, it was found that, in some cases, the indicators' colors were visibly fading after 2-3 days of attachment to the food samples, especially in foods that had 1) low acidic to neutral pH, or 2) large amount of fluid, which could be due to low stability of anthocyanins in high pH value[70] and/or the leaching of color compounds from colorimetric layer into the foods[27]. Other limitations of the developed indicators included the changed colors were not similar to the colors they produced after immersing in pH buffer solutions; and the color of the food could obscure visual observation of the indicators during use.

With appropriate packaging and storage condition, the developed indicators, with red cabbage, Dendrobium orchid, and butterfly pea, had shelf-life of 2, 4, 4 weeks, respectively. At the end of shelf-life, ΔE values of indicators with different pH were equal to or higher than 4.0[4].

The devices included in any intelligent packaging system should have high stability and sensitivity; should be non-toxic, economical, and easy to introduce into the package[71]. Thus, to further develop the pH indicator with plant extract, especially one that is rich in anthocyanins, it is important to improve the pigments' stability within the colorimetric layer. There are several potential approaches, for example, copigmentation with other organic compounds or natural pigments to form a more stable complex, such as phenolic acids, amino acids, or among anthocyanins themselves[18, 19], or with metal ions, e.g. ferric ion (Fe^{+3}) or Zine ion ($Zinc^{+2}$)[12, 20]. This can result in a more stable complex, and might increase the indicator's shelf-life as well. On the other hand, the sensitivity to pH of the environment of the formed complex will need to be reassessed since copigmentation can significantly change anthocyanins' color characteristics[19, 21].

Changing film-forming chemicals for colorimetric layer can increase anthocyanins' stability. The uses of polymeric compounds, such as pectin and whey protein could improve the pigment's stability[22]. The use of whey protein as film-forming compound also decrease solubility of the film[23]. However, the study on influences of protein denaturation on color-changing capacity of the pigment/film should also be assessed. There are several other film-forming chemicals that can be used to develop pH indicator as well, for example chitosan, zein protein, or combination of film-forming compounds[3, 23, 72].

Reference (เอกสารอ้างอิง)

[1]Kham-ngam, C., Netramai, S., and Kijchavengkul, T. *The Development of pH-indicating Film from Red Cabbage (Brassica oleracea var. capitata f. rubra) and Butterfly Pea (Clitoria ternatea L.)* in *Science & Management Project Exhibition 2015*. 2015. Kanchanaburi, Thailand: Mahidol University.

[2]Netramai, S., Kijchavengkul, T., Akanimanee, S., and Vongsaravit, I. *The Development of pH-indicating Labels From Plant Extracts*. in *IFT14 - IFT Annual Meeting & Food Expo*. 2014. New Orleans Morial Convention Center, New Orleans, LA: Institute of Food Technologists.

[3]Hosseini, M. H., Razavi, S. H., and Mousavi, M. A. *Antimicrobial, Physical and Mechanical Properties of Chitosan-based Films Incorporated with Thyme, Clove and Cinnamon Essential Oils* Journal of Food Processing and Preservation, 2009. **33**(6): p. 727-743.

[4]Witzel, R. F., Burnham, R. W., and Onley, J. W. *Threshold and Suprathreshold Perceptual Color Differences.* Journal of the Optical Society of America, 1973. **63**(5): p. 615-625.

[5]Ahmed, J. K., Amer, Z. J. A., and Al-Bahate, M. J. M. *Effect of Chlorophyll and Anthocyanin on the Secondary Bonds of Poly Vinyl Chloride (PVC)* International Journal of Materials Science and Applications, 2015. **4**(2-1): p. 21-29.

[6]Espinosa-Morales, Y., Reyes, J., Hermosín, B., and Azamar-Barrios, J. A. *Characterization of a Natural Dye by Spectroscopic and Chromatographic Techniques* Materials Research Society, 2012. **1374**.

[7]Bingol, E. B. and Ergun, O. *Effects of Modified Atmosphere Packaging (MAP) on the Microbiological Quality and Shelf Life of Ostrich Meat.* Meat Science, 2011. **88**: p. 774-785.

[8]Coffin, D. R. and Fishman, M. L. *Physical and Mechanical Properties of Highly Plasticized Pectin/Starch Films.* Journal of Applied Polymer Science, 1994. **54**(9): p. 1311-1320.

[9]Pitak, N. and Rakshit, S. K. *Physical and Antimicrobial Properties of Banana Flour/Chitosan Biodegradable and Self Sealing Films Used for Preserving Fresh-cut Vegetables.* LWT - Food Science and Technology, 2011. **44**(10): p. 2310-2315.

[10]Hu, X., Su, T., Pan, W., Li, P., and Wang, Z. *Difference in Solid-state Properties and Enzymatic Degradation of Three Kinds of Poly(butylene Succinate)/Cellulose Blends.* The Royal Society of Chemistry 2017. **7**: p. 35496-36603.

[11]Rakić, V., Rinnan, A., Polak, T., Skrt, M., Miljković, M., and Ulrih., N. P. *pH-induced Structural Forms of Cyanidin and Cyanidin 3-O-β-glucopyranoside.* Dyes and Pigments, 2019. **165**: p. 71-80.

[12]Takeda, K. *Blue Metal Complex Pigments Involved in Blue Flower Color.* Proceedings of the Japan Academy, Series B - Physical and Biological Sciences, 2006. **82**(4): p. 142-154.

[13]Tanaka, Y., Sasaki, N., and Ohmiya, A. *Biosynthesis of Plant Pigments: Anthocyanins, Betalains and Carotenoids.* The Plant Journal, 2008. **54**(4): p. 733-749.

[14]Huang, H. T. *Decolorization of Anthocyanins by Fungal Enzymes.* Fruit Color Destruction, 1955. **3**(2): p. 141-146.

[15]Kungsuwan, K., Singh, K., Phetkao, S., and Utama-ang, N. *Effects of pH and Anthocyanin Concentration on Color and Antioxidant Activity of Clitoria ternatea Extract.* Food and Applied Bioscience Journal, 2014. **2**(1): p. 31-46.

[16]Prietto, L., Mirapalhete, T. C., Pinto, V. Z., Hoffmann, J. F., Vanier, N. L., Lim, L.-T., Dias, A. R. G., and da Rosa Zavareze, E. *pH-sensitive Films Containing Anthocyanins Extracted from Black Bean Seed Coat and Red Cabbage.* LWT - Food Science and Technology, 2017. **80**: p. 492-500.

[17]Kuehnle, A. R., Lewis, D. H., Markham, K. R., Mitchell, K. A., Davies, K. M., and Jordan, B. R. *Floral Flavonoids and pH in Dendrobium orchid Species and Hybrids.* Euphytica, 1997. **95**: p. 187-194.

[18]Cortez, R., Luna-Vital, D. A., Margulis, D., and de Mejia, E. G. *Natural Pigments: Stabilization Methods of Anthocyanins for Food Applications.* Comprehensive Reviews in Food Science and Food Safety, 2017. **16**: p. 180-198.

[19]Bimpilas, A., Panagopoulou, M., Tsimogiannis, D., and Oreopoulou, V. *Anthocyanin Copigmentation and Color of Wine: The Effect of Naturally Obtained Hydroxycinnamic Acids as Cofactors.* Food Chemistry, 2016. **197**: p. 39-46.

[20]Tachibana, N., Kimura, Y., and Ohno, T. *Examination of Molecular Mechanism for the Enhanced Thermal Stability of Anthocyanins by Metal Cations and Polysaccharides.* Food Chemistry, 2014. **143**: p. 452-458.

[21]Luna-Vital, D., Cortez, R., Ongkowijoyo, P., and de Mejia, E. G. *Protection of Color and Chemical Degradation of Anthocyanin from Purple Corn (Zea mays L.) by Zinc Ions and Alginate through Chemical Interaction in a Beverage Model.* Food Research International, 2018. **105**: p. 169-177.

[22]Chung, C., Rojanasasithara, T., Mutilangi, W., and McClements, D. J. *Enhanced Stability of Anthocyanin-based Color in Model Beverage Systems through Whey Protein Isolate Complexation.* Food Research International, 2015. **76**(3): p. 761-768.

[23]Bourtoom, T. *Edible Films and Coatings: Characteristics and Properties.* International Food Research Journal, 2008. **15**(3): p. 237-248.

[24]Lee, D. S., Yam, K. L., and Piergiovanni, L. *Food Packaging Science and Technology*. 2008, Boca Raton, FL: CRC Press.

[25]Robertson, G. L. *Food Packaging and Shelf Life*, in *Food Packaging and Shelf Life: A Practical Guide*, Robertson, G. L., Editor. 2010, CRC Press, LLC: New York. p. 1-16.

[26]Puligundla, P., Jung, J., and Ko, S. *Carbon Dioxide Sensors for Intelligent Food Packaging Applications.* Food Control, 2012. **25**(1): p. 328-333.

[27]Dainelli, D., Gontard, N., Spyropoulos, D., Beuken, E. Z.-v. d., and Tobback, P. *Active and Intelligent Food Packaging: Legal Aspects and Safety Concerns.* Trends in Food Science & Technology, 2008. **19**(1): p. S103-S112.

[28]Yam, K. L. and Lee, D. S. *Emerging Food Packaging Technologies: Principles and Practice* 1st ed. 2012, Oxford, UK: Woodhead Publishing.

[29]The World Bank. *What a Waste: A Global Review of Solid Waste Management*. 2012. Available: https://siteresources.worldbank.org/INTURBANDEVELOPMENT/Resources/336387-1334852610766/What_a_Waste2012_Final.pdf [Accessed: April 18, 2019]

[30]Chandrasekhar, J., Madhusudhan, M. C., and Raghavarao, K. S. M. S. *Extraction of Anthocyanins from Red Cabbage and Purification Using Adsorption.* Food and Bioproducts Processing, 2012. **90**(4): p. 615-623.

[31]Gupta, P., Jain, P., and Jain, P. K. *Flower Sap: A Natural Resource as Indicator in Acidimetry and Alkalimetry.* International Journal of ChemTech Research, 2012. **4**(4): p. 1619-1622.

[32]Ogata, J., Kanno, Y., Itoh, Y., Tsugawa, H., and Suzuki, M. *Plant Biochemistry: Anthocyanin Biosynthesis in Roses.* Nature, 2005. **435**(7043): p. 757-758.

[33]Junka, N., Kanlayanarat, S., Buanong, M., and Wongs-Aree, C. *Characterisation of Floral Anthocyanins and Their Antioxidant Activity in Vanda hybrid (V. teres x V. hookeriana).* Journal of Food, Agriculture & Environment, 2012. **10**(2): p. 221-226.

[34]Terahara, N., Toki, K., Saito, N., Honda, T., Matsui, T., and Osajima, Y. *Eight New Anthocyanins, Ternatins C1-C5 and D3 and Preternatins A3 and C4 from Young Clitoria ternatea Flowers.* Journal of Natural Products, 1998. **61**(11): p. 1361-1367.

[35]นิษฐกานต์ ประดิษฐศรีกุล, เสน่ห์ บัวสนิท, ทศพร นามโฮง, ศิรัญญา โพธิคำ, and สุจิตรา แจ้งมงคล. การพัฒนาผลิตภัณฑ์แหนมเห็ดนางฟ้า. in การประชุมวิชาการระดับชาติมหาวิทยาลัย

เทคโนโลยีราชมงคลสุวรรณภูมิ ครั้งที่ *1*. 2559. มหาวิทยาลัยเทคโนโลยีราชมงคลสุวรรณภูมิ ศูนย์พระนครศรีอยุธยา หันตรา, พระนครศรีอยุธยา.

[36]อังคณา ชมภูมิ่ง, ตะวัน ฉัตรสูงเนิน, and ธวัชชัย ชัยธวัชวิถี. การปรับปรุงคุณภาพผลิตภัณฑ์ปลาส้มด้วยกระบวนการทางเทคโนโลยีชีวภาพ : กรณีศึกษาพื้นที่จังหวัดแพร่ และจังหวัดพะเยา. 2553. Available: http://librae.mju.ac.th/goverment/20111119104834_librae/15336.pdf [Accessed: April 18, 2019]

[37]Lee, J. *Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study.* Journal of AOAC International, 2005. **88**(5): p. 1269-1278.

[38]Dasamut, B. *Development of Edible Coating from Cultivated Banana (Musa ABB cv. Kluai 'Namwa') for Tropical Fruits* [B.Sc. Thesis], 2015, Kanchanaburi: Mahidol University Kanchanaburi Campus.

[39]Dubois, A., Remay, A., Raymond, O., Balzergue, S., Chauvet, A., Maene, M., Pe´crix, Y., Yang, S.-H., Jeauffre, J., Touroude, T., Boltz, V., Martin-Magniette, M.-L., Janczarski, S., Legeai, F., Renou, J.-P., Vergne, P., Le Bris, M., Foucher, F., and Bendahmane, M. *Genomic Approach to Study Floral Development Genes in Rosa sp.* PLoS ONE, 2011. **6**(12): p. 1-14.

[40]Pitakdantham, W., Sutabutra, T., Chiemsombat, P., and Pitaksutheepong, C. *Isolation and Characterization of Dihydroflavonol 4-reductase Gene in Dendrobium Flowers.* Journal of Plant Sciences, 2011. **6**: p. 88-94.

[41]Netramai, S., Kijchavengkul, T., Tangsirimongkhon, P., and Lohachitpitak, R. *The Effects of Citric Acid and Storage Condition on Color Stability of Butterfly Pea (Clitoria ternatea L.) Extract*. in *IFT15 - IFT Annual Meeting & Food Expo*. 2015. McCormick Place South, Chicago, IL: Institute of Food Technologists.

[42]Sinha, K., Saha, D. P., Ramya, V., and Datta, S. *Improved Extraction of Natural Blue Dye from Butterfly Pea Using Microwave Assisted Methodology to Reduce the Effect of Synthetic Blue Dye.* International Journal of Chemistry, 2012. **4**(2): p. 57-65.

[43]Chavda, H. V. and Patel, C. N. *Effect of Crosslinker Concentration on Characteristics of Superporous Hydrogel* International Journal of Pharmaceutical Investigation, 2011. **1**(1): p. 17-21.

[44]AOAC. *Official Methods of Analysis of AOAC international.* 17th ed. 2000, AOAC International: Gaithersburg, MD.

[45]Xu, J. B., Bartley, J. P., and Johnson, R. A. *Preparation and Characterization of Alginate-carrageenan Hydrogel Films Crosslinked Using a Water Soluble Carbodiimide (WSC).* Journal of Membrane Science, 2003. **218**(1-2): p. 131-146.

[46]ASTM. *ASTM D882-02 Standard Test Method for Tensile Properties of Thin Plastic Sheeting*. 2002, ASTM International: West Conshohocken, PA.

[47]ASTM. *ASTM D3418-03 Standard Test Method for Transition Temperatures and Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry*. 2003, ASTM International: West Conshohocken, PA.

[48]Ploumidis, D. *Color Perception - Part III of an ongoing Color Management Series*. 2008. Available: https://www.flexoglobal.com/flexomag/08-September/flexomag-ploumidis.htm [Accessed: April 18, 2019]

[49]ASTM. *ASTM E1871-97, Standard Practice for Serving Protocol for Sensory Evaluation of Foods and Beverages*. 1997, ASTM International: West Conshohocken, PA.

[50]Ennis, J. M., Rousseau, B., and Ennis, D. M. *Proper Task Instructions and the Two-out-of-Five Test*. 2012. Available: http://ifpress.com/wp-content/uploads/2012/11/15-4-Proper-Task-Instructions-and-the-Two-out-of-Five-Test.pdf [Accessed: April 18, 2019]

[51]Smith, G. L. *Statistical Properties of Simple Sensory Difference Tests: Confidence Limits and Significance Test.* Journal of the Science of Food and Agriculture, 1981. **32**(5): p. 513-520.

[52]Arditi, A. and Knoblauch, K. *Effective Color Contrast and Low Vision*, in *Functional Assessment of Low Vision*, Rosenthal, B. and Cole, R., Editors. 1996, Mosby: St. Louis, MO. p. 129-135.

[53]Abdullah, R., Lee, P. M., and Hung, L. K. *Multiple Color and pH Stability of Floral Anthocyanin Extract: elitaria Ternatea*. in *2010 International Conference on Science and Social Research*. 2010. Kuala Lumpur, Malaysia.

[54]Katsumoto, Y., Fukuchi-mizutani, M., Fukui, Y., Holton, T. A., Karan, M., Nakamura, N., Yonekura-sakakibara, K., Togami, J., Pigeaire, A., Tao, G.-q., Nehra, N. S., Lu, C.-y., Dyson, B. K., Tsuda, S., Ashikari, T., Kusumi, T., Mason, J. G., and Tanaka, Y. *Engineering of the Rose Flavonoid Biosynthetic Pathway Successfully Generated Blue-hued Flowers Accumulating Delphinidin.* Plant and Cell Physiology, 2007. **48**(11): p. 1589-1600.

[55]Limsitthichaikoon, S., Saodaeng, K., Priprem, A., and Damrongrungruang, T. *Anthocyanin complex: Characterization and cytotoxicity studies.* International Journal of Chemical and Molecular Engineering, 2015. **9**(2): p. 147-153.

[56]Routray, W. and Orsat, V. *Microwave-Assisted Extraction of Flavonoids: A Review.* Food and Bioprocess Technology, 2012. **5**: p. 409-424.

[57]Witzel, C. and Gegenfurtner, K. R. *Categorical Sensitivity to Color Differences.* Journal of Vision, 2013. **13**(7): p. 1-33.

[58]Shojaee-Aliabadi, S., Hosseini, H., Mohammadifar, M. A., Mohammadi, A., Ghasemlou, M., Hosseini, S. M., and Khaksar, R. *Characterization of κ-carrageenan films incorporated plant essential oils with improved antimicrobial activity.* Carbohydrate Polymers, 2013. **101**: p. 582-591.

[59]Veiga-Santos, P., Ditchfield, C., and Tadini, C. C. *Development and Evaluation of a Novel pH Indicator Biodegradable Film Based on Cassava Starch.* Journal of Applied Polymer Science, 2011. **120**(2): p. 1069-1079.

[60]Bento, L. M., Silva, M. C. P. D., Chaves, K. D. S., and Stefani, R. *Development and Evaluation of a Smart Packaging for the Monitoring of Ricotta Cheese Spoilage.* MOJ Food Processing & Technology, 2015. **1**(1): p. 00004-00006.

[61]สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม. มผช.*26/2546* มาตรฐานผลิตภัณฑ์ชุมชน ปลาส้ม. 2546, สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม: กรุงเทพฯ.

[62]สานักหอสมุดและศูนย์สารสนเทศวิทยาศาสตร์และเทคโนโลยี. ประมวลสารสนเทศพร้อมใช้ เรื่อง การพัฒนาผลิตภัณฑ์ปลาส้ม. 2558. Available: http://siweb.dss.go.th/repack/fulltext/IR%2034.pdf [Accessed: April 18, 2019]

[63]สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม. มผช.*462/2546* มาตรฐานผลิตภัณฑ์ชุมชน เต้าหู้ไข่. 2546, สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม: กรุงเทพฯ.

[64]สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม. มผช.*472/2547* มาตรฐานผลิตภัณฑ์ชุมชน แหนมเห็ด. 2547, สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม: กรุงเทพฯ.

[65]นิษฐกานต์ ประดิษฐศรีกุล, เสน่ห์ บัวสนิท, ทศพร นามโฮง, ศิรัญญา โพธิคำ, and สุจิตรา แจ้งมงคล. การพัฒนาผลิตภัณฑ์แหนมเห็ดนางฟ้า. in การประชุมวิชาการระดับชาติมหาวิทยาลัยเทคโนโลยีราชมงคลสุวรรณภูมิ ครั้งที่ *1*. 2559. พระนครศรีอยุธยา: สถาบันวิจัยและพัฒนามหาวิทยาลัยเทคโนโลยีราชมงคลสุวรรณภูมิ.

[66]Rakić, V., Rinnan, A., Polak, T., Skrt, M., Miljković, M., and Ulrih, N. P. *pH-induced Structural Forms of Cyanidin and Cyanidin 3-O-β-glucopyranoside.* Dyes and Pigments, 2019. **165**: p. 71-80.

[67]Sigma-Aldrich. *IR Spectrum Table & Chart*. n.a. Merck KGaA: https://www.sigmaaldrich.com. Available: https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html [Accessed: April 18, 2019]

[68]Wahyuningsih, S., Wulandari, L., Wartono, M. W., Munawaroh, H., and Ramelan, A. H. *The Effect of pH and Color Stability of Anthocyanin on Food Colorant.* IOP Conference Series: Materials Science and Engineering, 2017. **193**: p. 1-9.

[69]Nie, B., Stutzman, J., and Xie, A. *A Vibrational Spectral Maker for Probing the Hydrogen-Bonding Status of Protonated Asp and Glu Residues.* Biophysical Journal, 2005. **88**: p. 2833-2847.

[70]Khoo, H. E., Azlan, A., Tang, S. T., and Lim, S. M. *Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits.* Food & Nutrition Research, 2017. **61**(1).

[71]Mills, A. *Oxygen Indicators and Intelligent Inks for Packaging Food.* Chemical Society Reviews, 2005. **34**(12): p. 1003-1011.

[72]Jung, J., Puligundla, P., and Ko, S. *Proof-of-concept Study of Chitosan-based Carbon Dioxide Indicator for Food Packaging Applications.* Food Chemistry, 2012. **135**(4): p. 2170-2174.

Appendix (ภาคผนวก)

Appendix 1 – Color values of plant extracts at different pH

Table A1.1: Color values of plant extracts included in the study

Plant Type		pH 3.0			pH 5.0			pH 7.0		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
Red cabbage	1	72.96	38.67	-7.25	81.42	13.84	-8.73	80.79	3.26	-8.59
	2	64.21	50.55	-1.78	82.59	15.75	-9.06	76.34	2.41	-11.00
	3	67.46	42.04	-2.80	79.95	12.09	-8.30	73.22	3.50	-12.66
	Ave. ± S.D.	68.21±4.42	43.45±6.12	-3.94±2.91	81.32±1.32	13.89±1.83	-8.70±0.38	76.78±3.80	3.06±0.57	-10.75±2.05
Rose	1	60.47	62.88	52.17	55.81	66.34	60.28	54.34	60.39	55.73
	2	55.31	55.07	51.54	51.25	68.36	63.81	60.18	58.15	63.02
	3	58.10	63.10	52.64	59.00	48.02	50.09	58.46	50.45	60.69
	Ave. ± S.D.	57.96±2.58	60.35±4.57	52.12±0.55	55.35±3.90	60.91±11.21	58.06±7.12	57.66±3.00	56.33±5.21	59.81±3.72
Dendrobium orchid	1	68.59	53.82	-28.22	82.11	24.95	-5.35	71.48	25.63	-28.56
	2	70.68	47.65	-27.74	80.52	26.48	-3.84	58.03	22.59	-26.8
	3	61.51	58.21	-31.00	80.62	28.02	-3.13	62.00	27.89	-33.61
	Ave. ± S.D.	66.93±4.81	53.23±5.30	-28.99±1.76	81.08±0.89	26.48±1.54	-4.11±1.13	63.84±6.91	25.37±2.66	-29.66±3.53
Butterfly pea	1	46.91	60.41	-34.73	42.45	34.26	-70.42	51.01	-13.15	-38.61
	2	44.72	54.39	-42.25	43.6	31.58	-72.16	50.15	-15.93	-28.58
	3	39.35	47.02	-30.86	52.05	22.23	-57.07	44.24	-13.8	-35.08
	Ave. ± S.D.	43.66±3.89	53.94±6.71	-35.95±5.79	46.03±5.24	29.36±6.32	-66.55±8.26	48.47±3.69	-14.29±1.45	-34.09±5.09

Table A1.2: pH values of plant extracts

Plant Type	Replication	Temperature of Extract	pH of plant extract	Average ± S.D.
Red cabbage	1	32.1	6.85	6.82±0.02
	2	32.4	6.81	
	3	32.1	6.83	
	4	32.1	6.81	
	5	32.5	6.81	
Dendrobium orchid	1	31.4	6.83	6.84±0.01
	2	31.4	6.85	
	3	32.5	6.83	
	4	31.5	6.84	
	5	31.5	6.83	
Butterfly pea	1	32.1	6.84	6.84±0.01
	2	32.1	6.85	
	3	32.3	6.83	
	4	32.4	6.84	
	5	32.1	6.84	

Appendix 2 – RSM and desirability analysis for microwave assisted hot water extraction of plant extracts

2.1. Optimization of microwave assisted hot water extraction of red cabbage (using absorbance at λ_{max} = 553 nm as response)

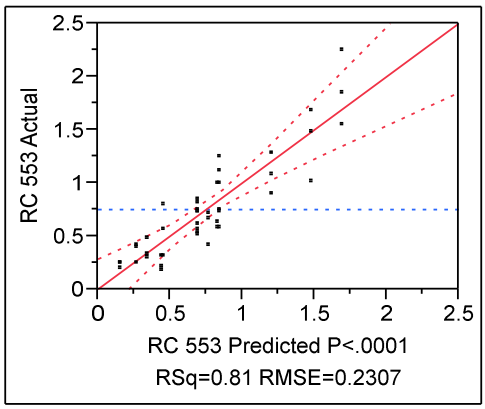
Table A2.1: Absorbance at λ_{max} = 553 nm and corresponding total monomeric anthocyanin content of red cabbage extracts obtained from microwave assisted hot water extraction

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance	TAC (cyd-3-glu equivalents, mg/L)
1	0 0 0	1:4	640	300	1	0.7295	118.63
					2	0.5378	127.45
					3	0.8210	114.49
2	0 - +	1:4	480	480	1	0.6310	122.97
					2	0.5890	136.58
					3	1.0063	138.38
3	- - 0	1:5	480	300	1	0.8073	122.54
					2	0.3180	63.22
					3	0.5668	75.95
4	0 0 0	1:4	640	300	1	0.7558	155.78
					2	0.5723	133.42
					3	0.6250	129.02
5	- 0 -	1:5	640	120	1	0.2518	46.39
					2	0.1958	35.92
					3	0.2428	31.54
6	0 0 0	1:4	640	300	1	0.7383	129.89
					2	0.5108	115.71
					3	0.8580	120.24
7	+ - 0	1:3	480	300	1	1.2563	160.58
					2	0.7505	136.33
					3	1.0030	114.10
8	+ + 0	1:3	800	300	1	1.0825	124.66
					2	0.9005	156.22

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance	TAC (cyd-3-glu equivalents, mg/L)
					3	1.2873	155.73
9	+ 0 -	1:3	640	120	1	0.4798	85.85
					2	0.2930	66.80
					3	0.3263	40.59
10	0 - -	1:4	480	120	1	0.2118	37.32
					2	0.1915	41.36
					3	0.3100	29.89
11	0 + -	1:4	800	120	1	0.3963	40.66
					2	0.2518	32.79
					3	0.4188	41.41
12	0 + +	1:4	800	480	1	2.2535	284.11
					2	1.5425	189.38
					3	1.8440	195.23
13	- + 0	1:5	800	300	1	0.6653	120.98
					2	0.4175	103.53
					3	0.7130	92.14
14	+ 0 +	1:3	640	480	1	1.4778	172.29
					2	1.0135	165.02
					3	1.6833	202.31
15	- 0 +	1:5	640	480	1	0.7253	130.06
					2	0.5798	126.73
					3	1.1158	161.49

*Trt. = Treatment; S:W = Sample to water ratio; A = Absorbance; and TAC = Total monomeric anthocyanin content

Response RC 553
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.806566
RSquare Adj	0.756826
Root Mean Square Error	0.23067
Mean of Response	0.7544
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	7.7652818	0.862809	16.2156
Error	35	1.8622974	0.053208	**Prob > F**
C. Total	44	9.6275792		<.0001*

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	0.5673497	0.189117	4.6733
Pure Error	32	1.2949477	0.040467	**Prob > F**
Total Error	35	1.8622974		0.0081*
				Max RSq
				0.8655

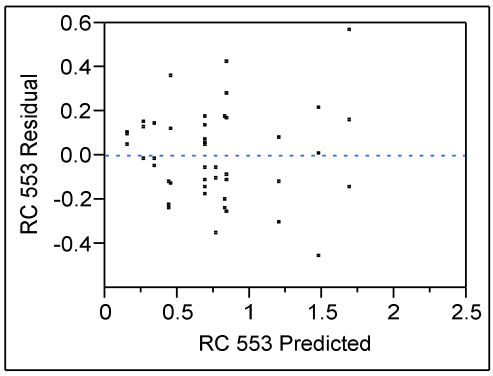
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.761812	0.218043	-3.49	0.0013*
Ratio	0.2064417	0.047085	4.38	0.0001*
Power	0.0010759	0.000294	3.66	0.0008*
Time	0.1512792	0.015695	9.64	<.0001*
Ratio*Ratio	0.0128542	0.069308	0.19	0.8539
Ratio*(Power-640)	8.1667e-5	0.000416	0.20	0.8456
(Power-640)*(Power-640)	4.6086e-6	2.707e-6	1.70	0.0976
Ratio*(Time-5)	0.0373611	0.022196	1.68	0.1012
(Power-640)*(Time-5)	0.0005313	0.000139	3.83	0.0005*
(Time-5)*(Time-5)	0.0003032	0.007701	0.04	0.9688

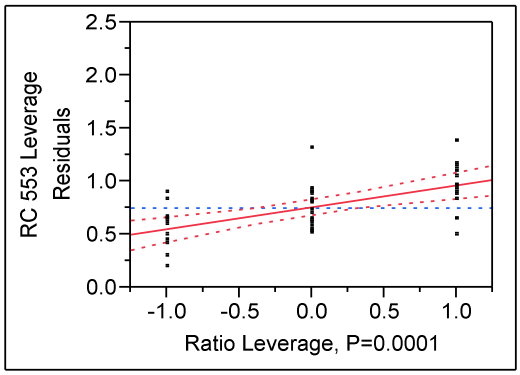
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Ratio	1	1	1.0228359	19.2232	0.0001*
Power	1	1	0.7112205	13.3667	0.0008*
Time	1	1	4.9432434	92.9033	<.0001*
Ratio*Ratio	1	1	0.0018302	0.0344	0.8539
Ratio*Power	1	1	0.0020489	0.0385	0.8456
Power*Power	1	1	0.1541806	2.8977	0.0976
Ratio*Time	1	1	0.1507521	2.8332	0.1012
Power*Time	1	1	0.7803510	14.6659	0.0005*
Time*Time	1	1	0.0000825	0.0016	0.9688

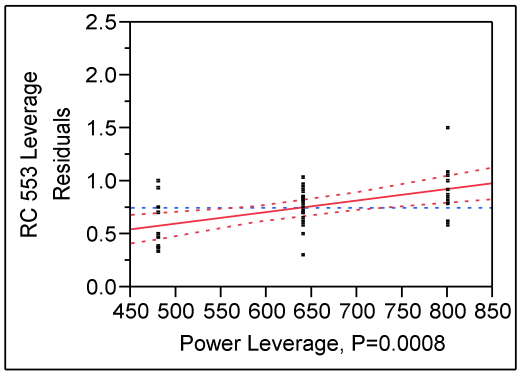
Residual by Predicted Plot



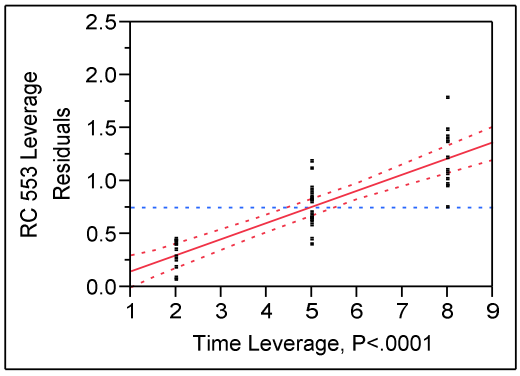
Ratio Leverage Plot



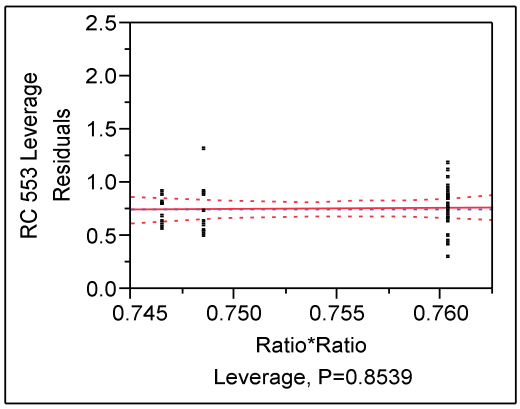
Power Leverage Plot



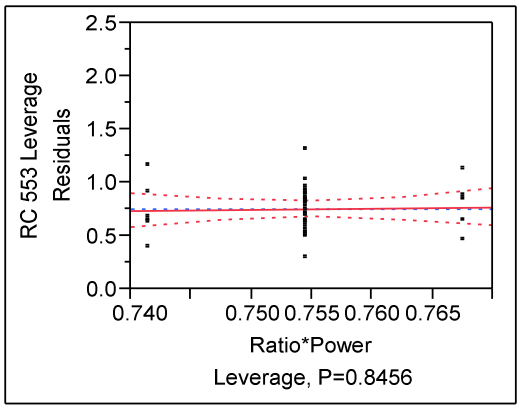
Time Leverage Plot



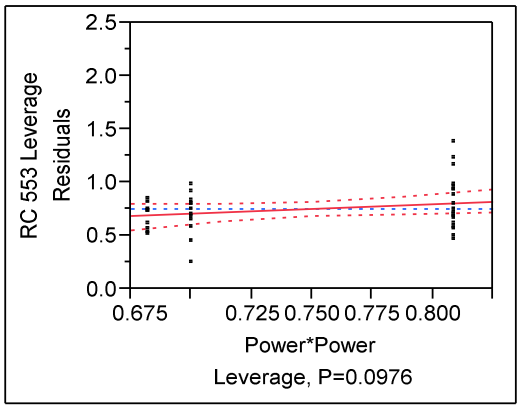
Ratio*Ratio Leverage Plot



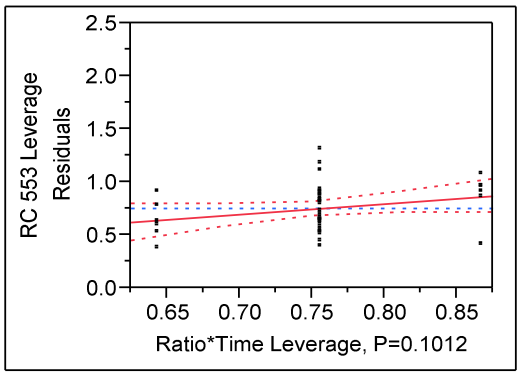
Ratio*Power Leverage Plot



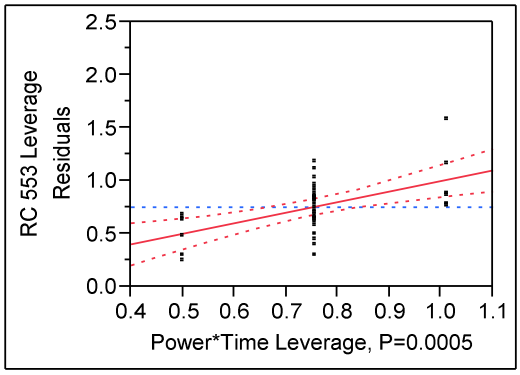
Power*Power Leverage Plot



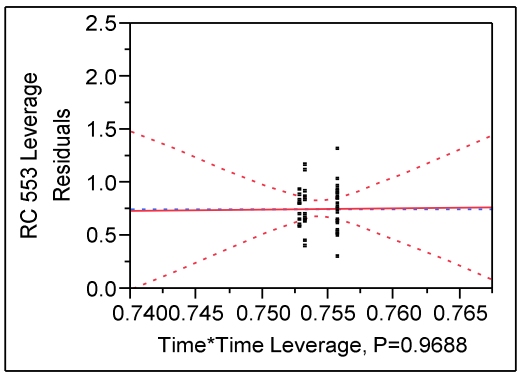
Ratio*Time Leverage Plot



Power*Time Leverage Plot



Time*Time Leverage Plot



Response Surface

Coef	Ratio	Power	Time	RC 553
Ratio	0.0128542	8.1667e-5	0.0373611	0.2064417
Power	.	4.6086e-6	0.0005313	0.0010759
Time	.	.	0.0003032	0.1512792

Solution

Variable	Critical Value
Ratio	-4.859102
Power	699.6021
Time	2.6877092

Solution is a SaddlePoint

Critical values outside data range

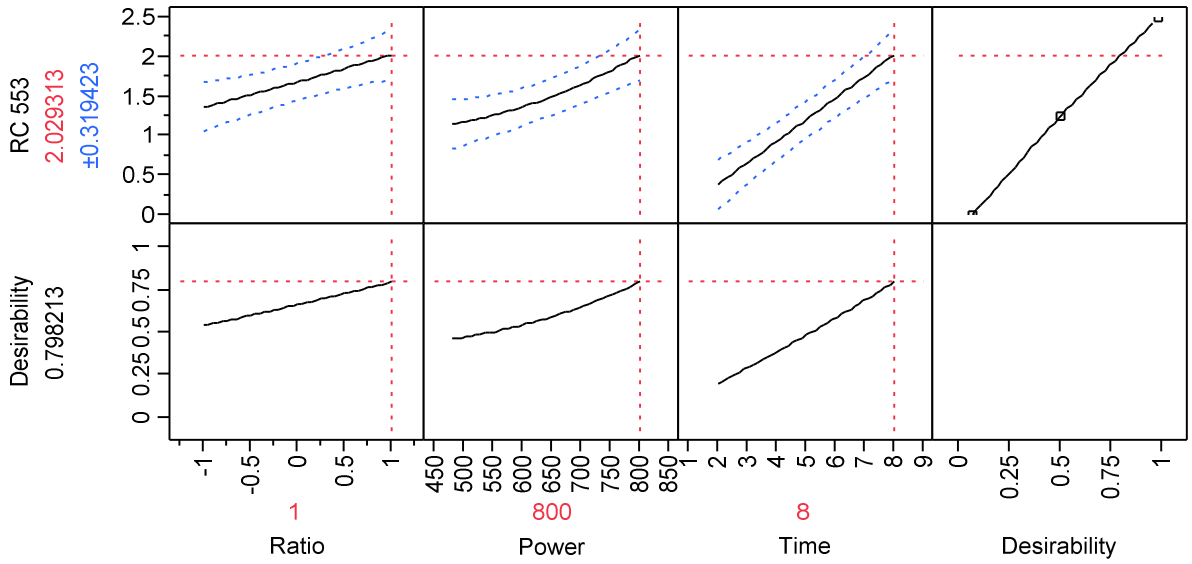
Predicted Value at Solution
0.0387687

Canonical Curvature

Eigenvalues and Eigenvectors

Eigenvalue	0.0263	0.0000	-0.0131
Ratio	0.81188	-0.01434	-0.58364
Power	0.00716	0.99987	-0.01460
Time	0.58377	0.00768	0.81188

Prediction Profiler



2.2. Optimization of microwave assisted hot water extraction of Dendrobium orchid (using absorbance at λ_{max} = 543 and 583 nm as responses)

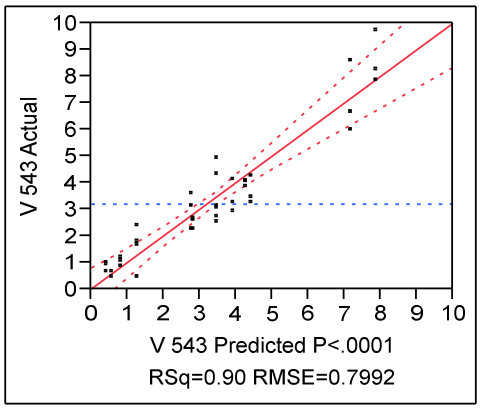
Table A2.2: Absorbance at λ_{max} = 543 and 583 nm and corresponding total monomeric anthocyanin content of Dendrobium orchid extracts obtained from microwave assisted hot water extraction

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance		TAC*
						λ_{543}	λ_{583}	
1	0 0 0	1:4	640	300	1	3.1475	3.0410	25.75
					2	3.1340	3.0135	26.45
					3	2.5338	2.4610	24.70
2	0 - +	1:4	480	480	1	4.0955	3.9633	25.58
					2	3.8928	3.7658	23.02
					3	4.0618	3.9150	20.66
3	- - 0	1:5	480	300	1	2.4283	2.3663	11.52
					2	1.6810	1.6335	11.42
					3	1.7695	1.7210	11.22
4	0 0 0	1:4	640	300	1	4.9243	4.7418	27.87
					2	3.0793	2.9783	22.44
					3	2.7595	2.6688	25.99
5	- 0 -	1:5	640	120	1	0.6601	0.6478	2.75
					2	0.4830	0.4566	2.91
					3	0.6470	0.6328	2.91
6	0 0 0	1:4	640	300	1	4.2793	4.1243	28.14
					2	3.0805	2.9735	27.53
					3	4.3108	4.1625	27.62
7	+ - 0	1:3	480	300	1	3.6305	3.5233	24.70
					2	2.2680	2.2158	20.68
					3	3.1553	3.0338	11.87
8	+ + 0	1:3	800	300	1	3.4403	3.3140	21.64
					2	4.2848	4.1108	20.47
					3	3.2815	3.1695	19.67
9	+ 0 -	1:3	640	120	1	0.6788	0.6763	5.04

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance		TAC*
						λ_{543}	λ_{583}	
					2	0.9670	0.9365	4.87
					3	0.9298	0.9005	3.82
10	0 - -	1:4	480	120	1	0.4840	0.4815	2.92
					2	0.4820	0.4396	3.10
					3	0.4933	0.4815	3.22
11	0 + -	1:4	800	120	1	1.1853	1.1730	7.92
					2	1.0565	1.0335	4.49
					3	0.8935	0.8753	8.17
12	0 + +	1:4	800	480	1	9.7250	9.4195	46.52
					2	7.8365	7.5970	48.26
					3	8.2530	7.9775	39.38
13	- + 0	1:5	800	300	1	2.6945	2.6290	19.85
					2	2.5955	2.5170	14.26
					3	2.2548	2.1960	16.05
14	+ 0 +	1:3	640	480	1	5.9925	5.7578	37.84
					2	6.6920	6.3613	36.37
					3	8.6035	8.2715	38.09
15	- 0 +	1:5	640	480	1	3.2455	3.0653	14.29
					2	4.1003	3.9895	25.00
					3	2.9100	2.8215	26.90

*Trt. = Treatment; S:W = Sample to water ratio; A = Absorbance; TAC = Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L)

Least Squares Fit
Response V 543
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.902554
RSquare Adj	0.877496
Root Mean Square Error	0.799211
Mean of Response	3.180038
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	207.06101	23.0068	36.0191
Error	35	22.35584	0.6387	**Prob > F**
C. Total	44	229.41686		<.0001*

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	8.487633	2.82921	6.5282
Pure Error	32	13.868211	0.43338	**Prob > F**
Total Error	35	22.355844		0.0014*
				Max RSq
				0.9396

Parameter Estimates

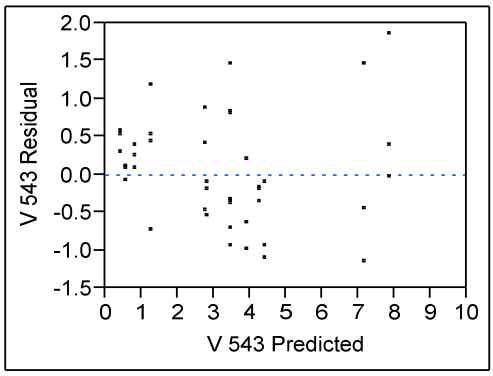
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.902207	0.755463	-5.17	<.0001*
Ratio	0.7689375	0.163138	4.71	<.0001*
Power	0.0049633	0.00102	4.87	<.0001*
Time	0.8395569	0.054379	15.44	<.0001*
Ratio*Ratio	-0.613793	0.240133	-2.56	0.0151*
Ratio*(Power-640)	0.0001494	0.001442	0.10	0.9181
(Power-640)*(Power-640)	-2.656e-6	9.38e-6	-0.28	0.7788
Ratio*(Time-5)	0.2846306	0.076904	3.70	0.0007*
(Power-640)*(Time-5)	0.0020987	0.000481	4.37	0.0001*
(Time-5)*(Time-5)	0.0149045	0.026681	0.56	0.5800

Effect Tests

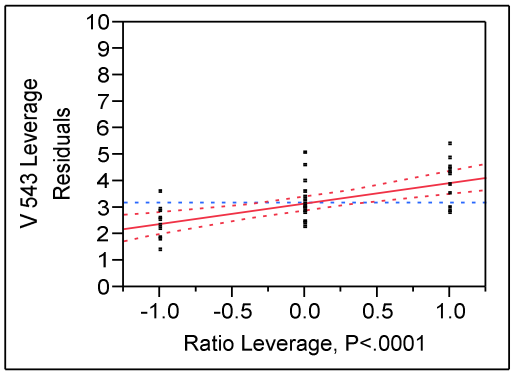
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Ratio	1	1	14.19036	22.2162	<.0001*
Power	1	1	15.13555	23.6960	<.0001*
Time	1	1	152.24887	238.3587	<.0001*
Ratio*Ratio	1	1	4.17314	6.5334	0.0151*
Ratio*Power	1	1	0.00685	0.0107	0.9181
Power*Power	1	1	0.05120	0.0802	0.7788
Ratio*Time	1	1	8.74957	13.6982	0.0007*
Power*Time	1	1	12.17745	19.0648	0.0001*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time*Time	1	1	0.19931	0.3120	0.5800

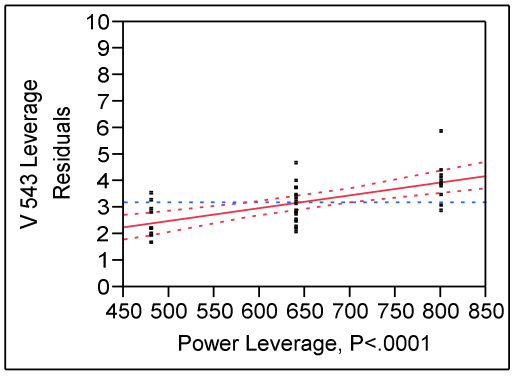
Residual by Predicted Plot



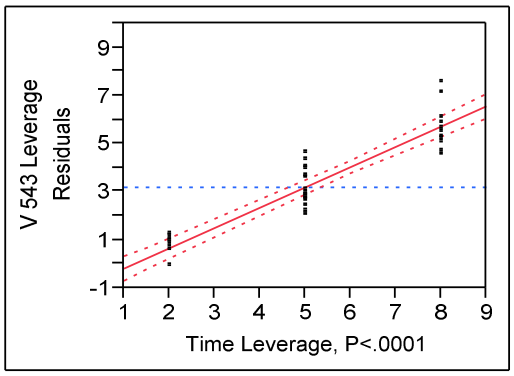
Ratio Leverage Plot



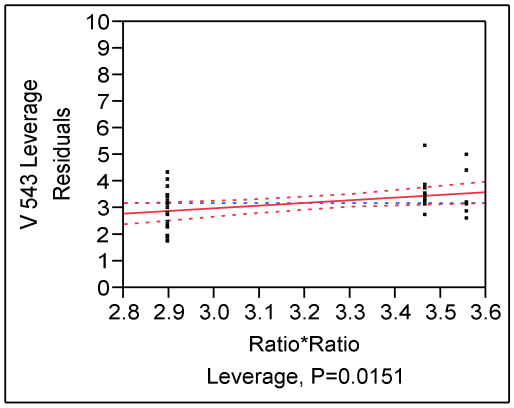
Power Leverage Plot



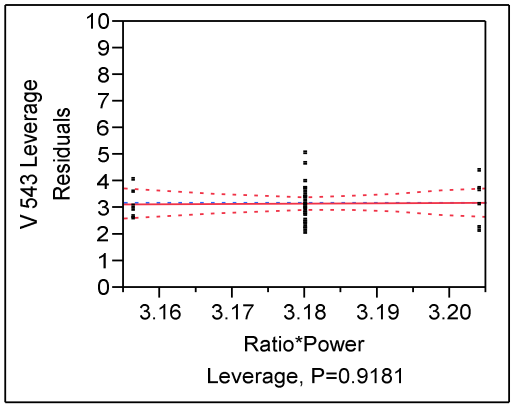
Time Leverage Plot



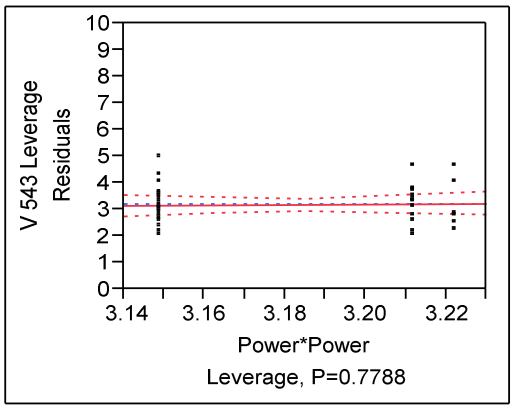
Ratio*Ratio Leverage Plot



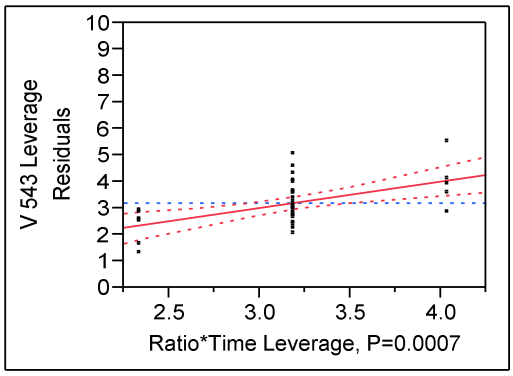
Ratio*Power Leverage Plot



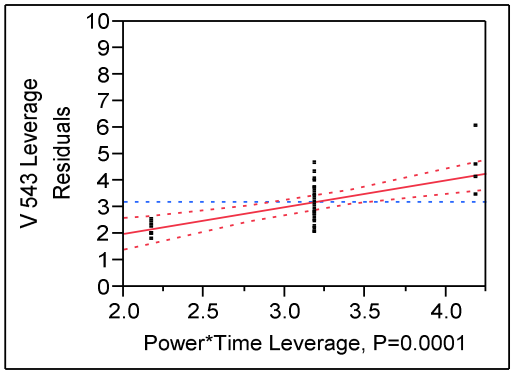
Power*Power Leverage Plot



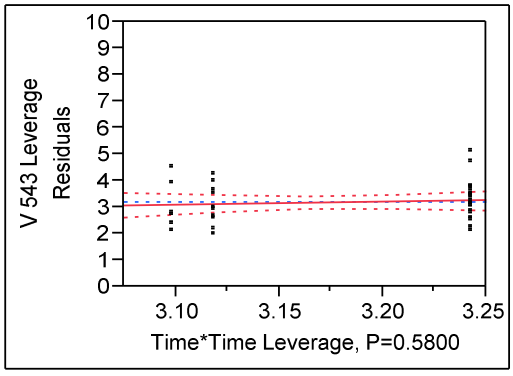
Ratio*Time Leverage Plot



Power*Time Leverage Plot



Time*Time Leverage Plot



Response Surface

Coef	Ratio	Power	Time	V 543
Ratio	-0.613793	0.0001494	0.2846306	0.7689375
Power	.	-2.656e-6	0.0020987	0.0049633
Time	.	.	0.0149045	0.8395569

Solution

Variable	Critical Value
Ratio	-0.155677
Power	306.49538
Time	1.8020744

Solution is a
SaddlePoint

Critical values outside data range

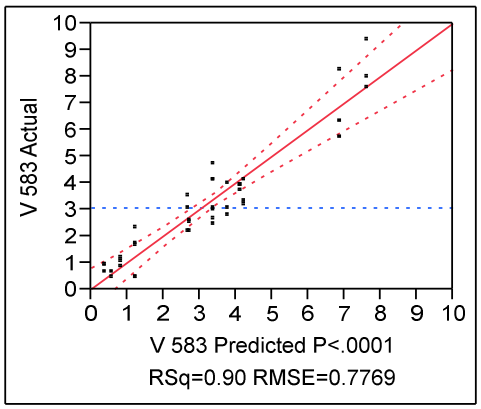
Predicted Value at Solution
1.2421905

Canonical Curvature

Eigenvalues and Eigenvectors

Eigenvalue	0.0456	-0.0000	-0.6445
Ratio	0.21090	-0.00504	0.97749
Power	0.02281	0.99974	0.00023
Time	0.97724	-0.02225	-0.21096

Response V 583
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.900717
RSquare Adj	0.875187
Root Mean Square Error	0.776946
Mean of Response	3.07188
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	191.67460	21.2972	35.2809
Error	35	21.12759	0.6036	**Prob > F**
C. Total	44	212.80219		<.0001*

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	8.198901	2.73297	6.7644
Pure Error	32	12.928689	0.40402	**Prob > F**
Total Error	35	21.127591		0.0012*
				Max RSq
				0.9392

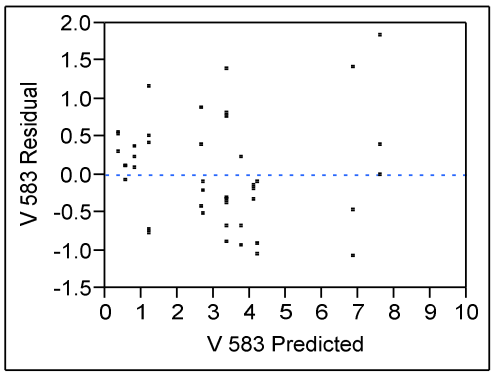
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.766574	0.734417	-5.13	<.0001*
Ratio	0.7331167	0.158593	4.62	<.0001*
Power	0.0048103	0.000991	4.85	<.0001*
Time	0.8079181	0.052864	15.28	<.0001*
Ratio*Ratio	-0.599779	0.233443	-2.57	0.0146*
Ratio*(Power-640)	0.0001043	0.001402	0.07	0.9411
(Power-640)*(Power-640)	-1.928e-6	9.119e-6	-0.21	0.8338
Ratio*(Time-5)	0.2705056	0.074762	3.62	0.0009*
(Power-640)*(Time-5)	0.0020262	0.000467	4.34	0.0001*
(Time-5)*(Time-5)	0.013844	0.025938	0.53	0.5969

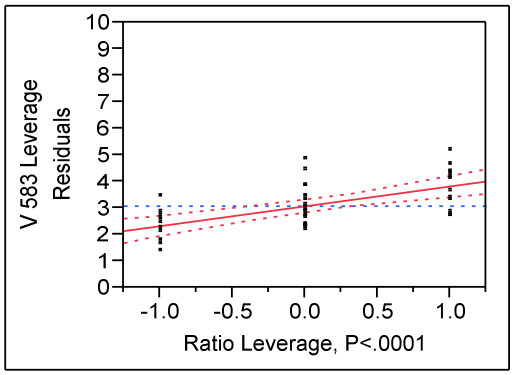
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Ratio	1	1	12.89904	21.3686	<.0001*
Power	1	1	14.21682	23.5516	<.0001*
Time	1	1	140.99002	233.5643	<.0001*
Ratio*Ratio	1	1	3.98476	6.6012	0.0146*
Ratio*Power	1	1	0.00334	0.0055	0.9411
Power*Power	1	1	0.02698	0.0447	0.8338
Ratio*Time	1	1	7.90271	13.0916	0.0009*
Power*Time	1	1	11.35044	18.8032	0.0001*
Time*Time	1	1	0.17196	0.2849	0.5969

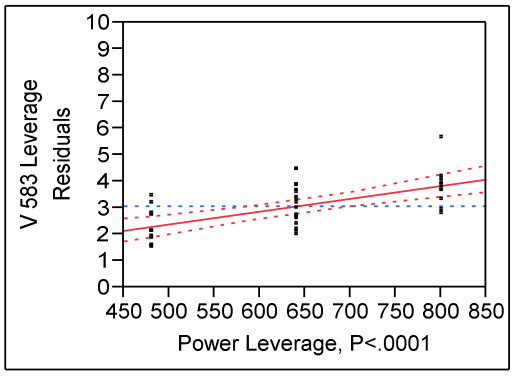
Residual by Predicted Plot



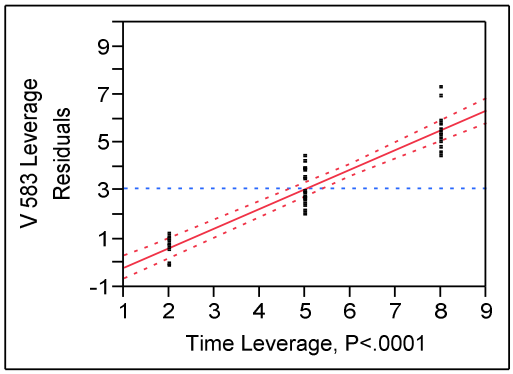
Ratio Leverage Plot



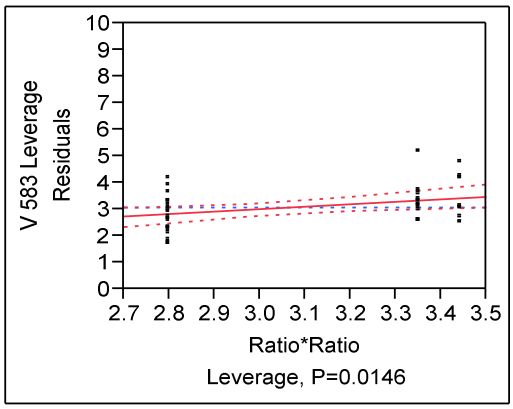
Power Leverage Plot



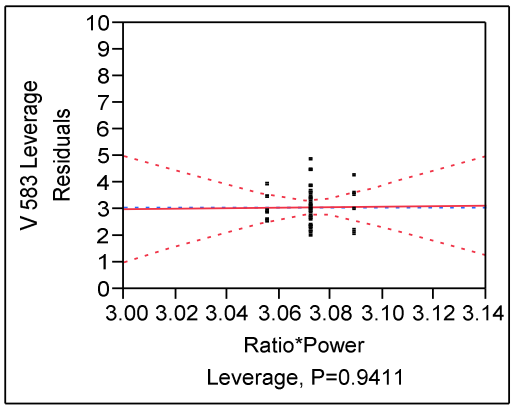
Time Leverage Plot



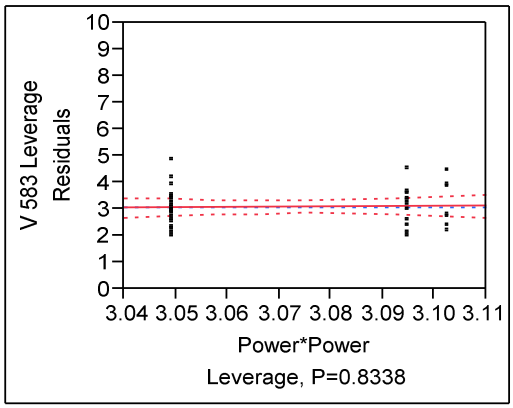
Ratio*Ratio Leverage Plot



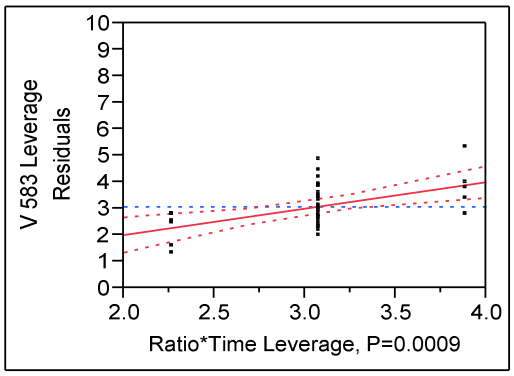
Ratio*Power Leverage Plot



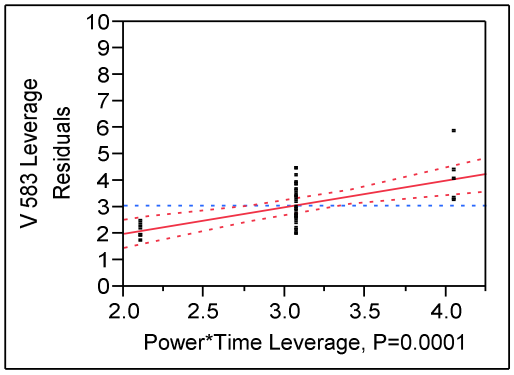
Power*Power Leverage Plot



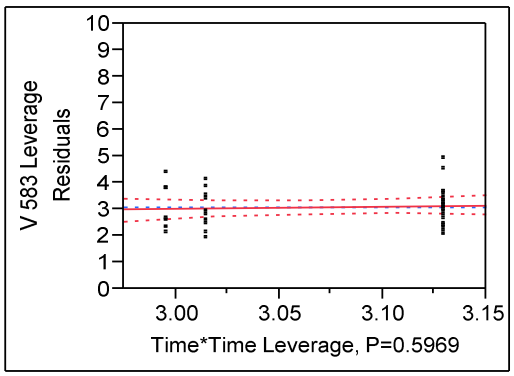
Ratio*Time Leverage Plot



Power*Time Leverage Plot



Time*Time Leverage Plot



Response Surface

Coef	Ratio	Power	Time	V 583
Ratio	-0.599779	0.0001043	0.2705056	0.7331167
Power	.	-1.928e-6	0.0020262	0.0048103
Time	.	.	0.013844	0.8079181

Solution

Variable	Critical Value
Ratio	-0.100573
Power	296.00202
Time	1.9764345

Solution is a SaddlePoint

Critical values outside data range

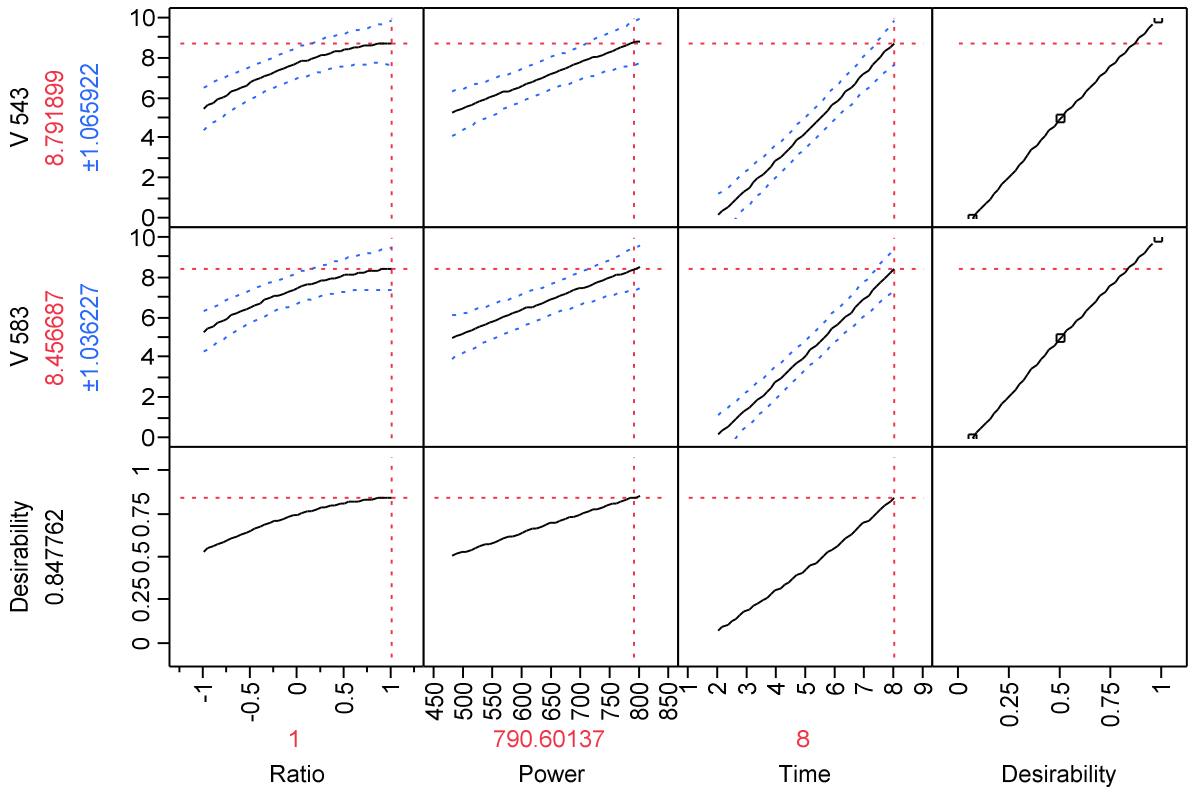
Predicted Value at Solution
1.2659974

Canonical Curvature

Eigenvalues and Eigenvectors

Eigenvalue	0.0424	-0.0000	-0.6283
Ratio	0.20605	-0.00512	0.97853
Power	0.02365	0.99972	0.00025
Time	0.97826	-0.02309	-0.20611

Prediction Profiler



2.3. Optimization of microwave assisted hot water extraction of butterfly pea (using absorbance at λ_{max} = 574 and 618 nm as responses)

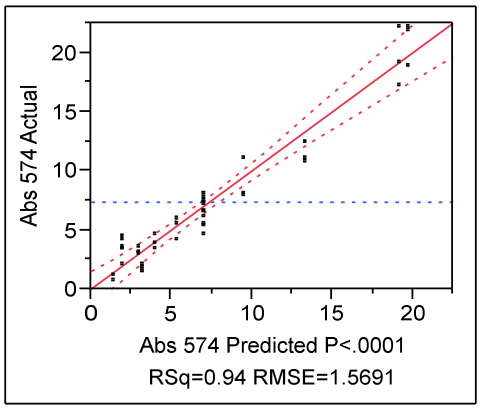
Table A2.3: Absorbance at λ_{max} = 574 and 618 nm and corresponding total monomeric anthocyanin content of butterfly pea extracts obtained from microwave assisted hot water extraction

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance		TAC*
						λ_{574}	λ_{618}	
1	0 0 0	1:40	640	120	1	8.1205	8.3370	27.12
					2	7.3740	7.3530	28.17
					3	7.5170	7.5090	30.79
2	0 - +	1:40	480	180	1	11.0820	11.2870	37.74
					2	8.0045	7.8720	51.50
					3	8.1590	8.0825	47.39
3	- - 0	1:50	480	120	1	3.5625	3.7140	13.71
					2	3.4075	3.4015	14.24
					3	4.1570	4.0905	15.11
4	0 0 0	1:40	640	120	1	7.1840	7.2415	24.63
					2	6.0420	5.9075	23.45
					3	6.6575	6.4865	25.95
5	- 0 -	1:50	640	60	1	0.7565	0.8080	3.62
					2	1.1880	1.2145	3.54
					3	1.1490	1.1530	3.64
6	0 0 0	1:40	640	120	1	6.1015	6.0830	33.55
					2	5.5850	5.5115	30.14
					3	7.8025	7.6820	34.04
7	+ - 0	1:30	480	120	1	5.4945	5.6850	28.74
					2	6.0360	6.0120	42.16
					3	4.1360	4.1100	37.86
8	+ + 0	1:30	800	120	1	11.1670	11.0850	55.16
					2	10.7340	10.5140	64.09
					3	12.5080	12.1520	56.02
9	+ 0 -	1:30	640	60	1	2.0670	2.0965	12.04

Trt.	Code	S:W* (g/mL)	Power (W)	Time (s)		Absorbance		TAC*
						λ_{574}	λ_{618}	
					2	4.4840	4.4225	20.59
					3	3.4270	3.4065	10.05
10	0 - -	1:40	480	60	1	1.5050	1.6280	9.00
					2	1.7800	1.7935	5.99
					3	2.1195	2.1255	7.92
11	0 + -	1:40	800	60	1	3.2200	3.2200	46.53
					2	3.0485	3.0940	44.83
					3	3.5890	3.5870	50.56
12	0 + +	1:40	800	180	1	18.9700	18.7270	61.13
					2	22.2020	21.9520	72.62
					3	21.9570	21.6680	77.30
13	- + 0	1:50	800	120	1	4.6530	4.8535	19.00
					2	3.9245	3.8950	22.06
					3	3.4030	3.3970	25.53
14	+ 0 +	1:30	640	180	1	22.2420	21.9110	77.97
					2	17.1890	16.7930	77.92
					3	19.1860	18.9790	91.26
15	- 0 +	1:50	640	180	1	6.6450	6.8240	23.23
					2	4.6735	4.7500	24.14
					3	5.4490	5.4870	24.45

*Trt. = Treatment; S:W = Sample to water ratio; A = Absorbance; TAC = Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L)

Least Squares Fit
Response Abs 574
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.943238
RSquare Adj	0.928642
Root Mean Square Error	1.569065
Mean of Response	7.325767
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1431.9064	159.101	64.6235
Error	35	86.1688	2.462	**Prob > F**
C. Total	44	1518.0752		<.0001*

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	44.489762	14.8299	11.3860
Pure Error	32	41.679014	1.3025	**Prob > F**
Total Error	35	86.168777		<.0001*
				Max RSq
				0.9725

Parameter Estimates

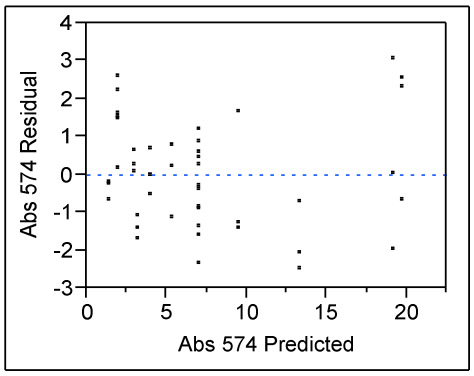
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-14.50932	1.524857	-9.52	<.0001*
Ratio	3.15425	0.320284	9.85	<.0001*
Power	0.0156074	0.002002	7.80	<.0001*
Time	5.7260625	0.320284	17.88	<.0001*
Ratio*Ratio	-1.13234	0.471445	-2.40	0.0218*
Ratio*(Power-640)	0.0093172	0.002831	3.29	0.0023*
(Power-640)*(Power-640)	0.0000117	1.842e-5	0.64	0.5296
Ratio*(Time-2)	2.91375	0.45295	6.43	<.0001*
(Power-640)*(Time-2)	0.0163701	0.002831	5.78	<.0001*
(Time-2)*(Time-2)	1.5721181	0.471445	3.33	0.0020*

Effect Tests

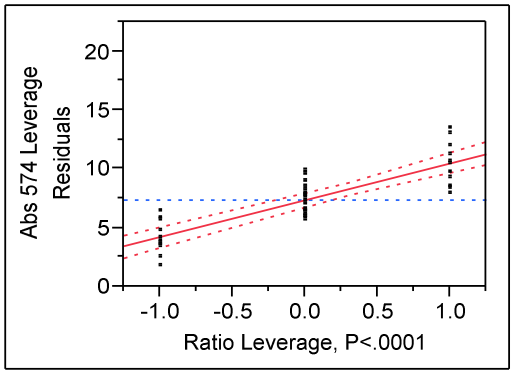
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Ratio	1	1	238.78303	96.9888	<.0001*
Power	1	1	149.66269	60.7899	<.0001*
Time	1	1	786.90700	319.6256	<.0001*
Ratio*Ratio	1	1	14.20277	5.7689	0.0218*
Ratio*Power	1	1	26.66803	10.8320	0.0023*
Power*Power	1	1	0.99273	0.4032	0.5296
Ratio*Time	1	1	101.87927	41.3813	<.0001*
Power*Time	1	1	82.32303	33.4379	<.0001*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Time*Time	1	1	27.37723	11.1201	0.0020*

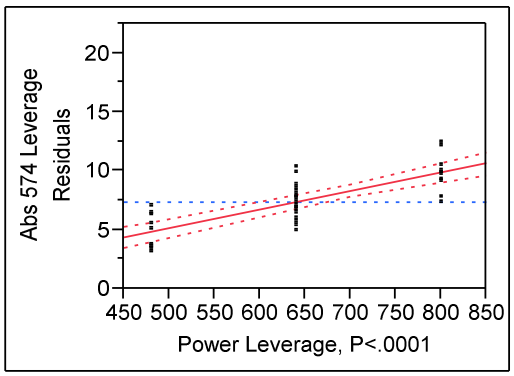
Residual by Predicted Plot



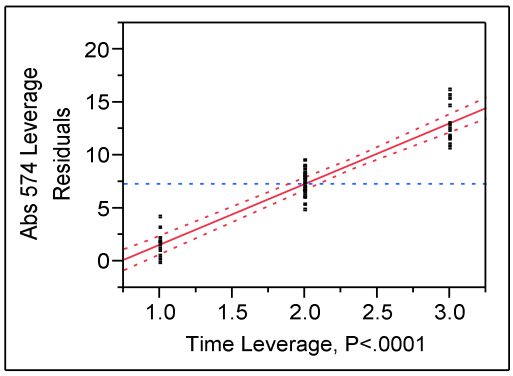
Ratio Leverage Plot



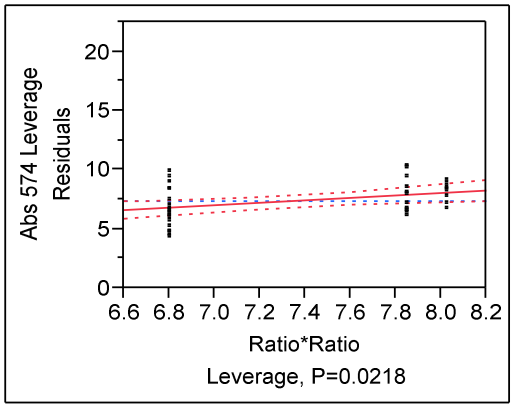
Power Leverage Plot



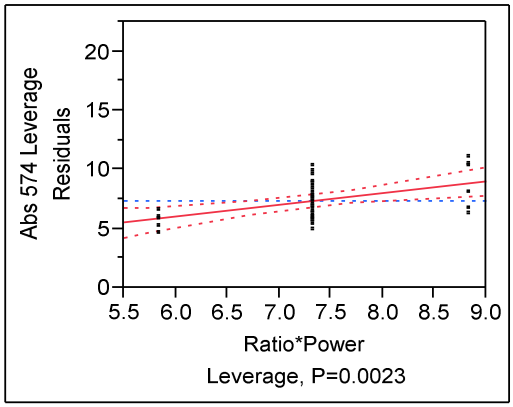
Time Leverage Plot



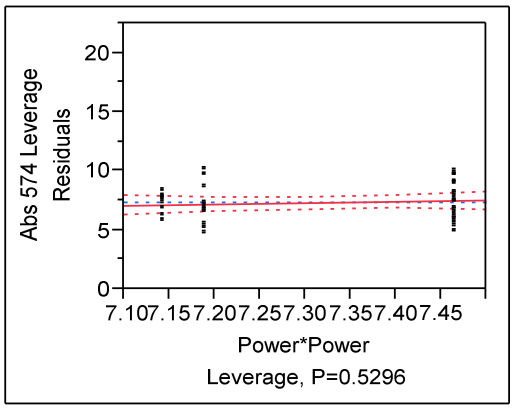
Ratio*Ratio Leverage Plot



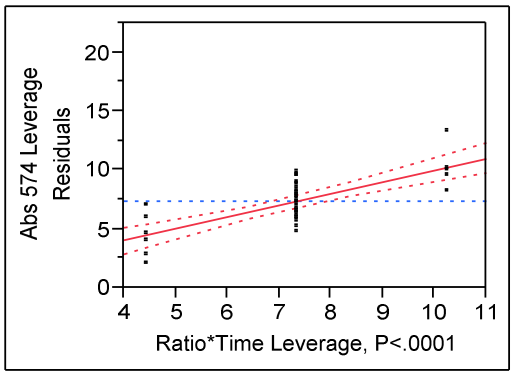
Ratio*Power Leverage Plot



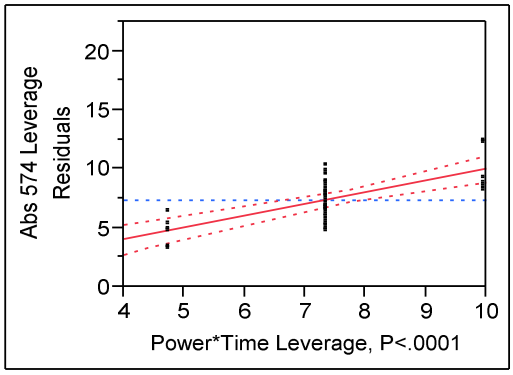
Power*Power Leverage Plot



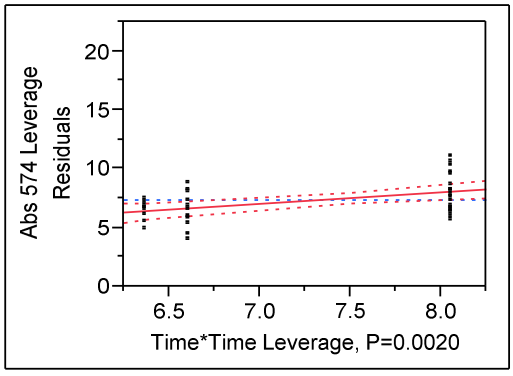
Ratio*Time Leverage Plot



Power*Time Leverage Plot



Time*Time Leverage Plot



Response Surface

Coef	Ratio	Power	Time	Abs 574
Ratio	-1.13234	0.0093172	2.91375	3.15425
Power	.	0.0000117	0.0163701	0.0156074
Time	.	.	1.5721181	5.7260625

Solution

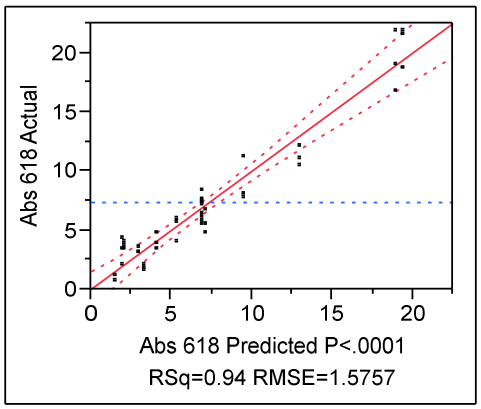
Variable	Critical Value
Ratio	-0.18311
Power	427.63313
Time	1.4542175

Solution is a
SaddlePoint

Critical values outside data range

Predicted Value at Solution
3.4229262

Response Abs 618
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.940716
RSquare Adj	0.925471
Root Mean Square Error	1.575684
Mean of Response	7.286711
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1378.8776	153.209	61.7085
Error	35	86.8973	2.483	**Prob > F**
C. Total	44	1465.7750		<.0001*

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	42.814070	14.2714	10.3596
Pure Error	32	44.083275	1.3776	**Prob > F**
Total Error	35	86.897345		<.0001*
				Max RSq
				0.9699

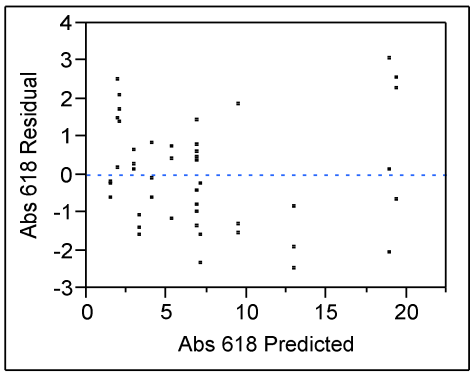
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-14.1379	1.53129	-9.23	<.0001*
Ratio	3.0657708	0.321635	9.53	<.0001*
Power	0.0151935	0.00201	7.56	<.0001*
Time	5.6576458	0.321635	17.59	<.0001*
Ratio*Ratio	-1.129028	0.473434	-2.38	0.0226*
Ratio*(Power-640)	0.0088565	0.002843	3.12	0.0037*
(Power-640)*(Power-640)	1.1859e-5	1.849e-5	0.64	0.5255
Ratio*(Time-2)	2.8226667	0.454861	6.21	<.0001*
(Power-640)*(Time-2)	0.0160164	0.002843	5.63	<.0001*
(Time-2)*(Time-2)	1.5482222	0.473434	3.27	0.0024*

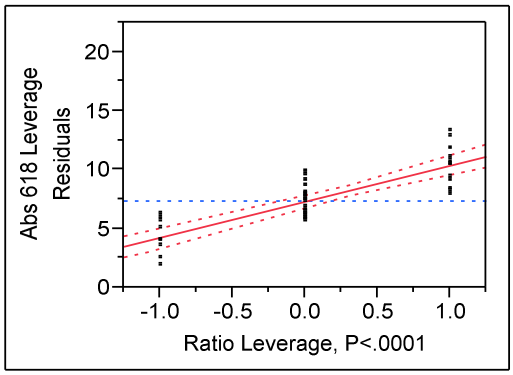
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Ratio	1	1	225.57482	90.8557	<.0001*
Power	1	1	141.82940	57.1252	<.0001*
Time	1	1	768.21495	309.4171	<.0001*
Ratio*Ratio	1	1	14.11980	5.6871	0.0226*
Ratio*Power	1	1	24.09609	9.7053	0.0037*
Power*Power	1	1	1.02097	0.4112	0.5255
Ratio*Time	1	1	95.60937	38.5090	<.0001*
Power*Time	1	1	78.80456	31.7404	<.0001*
Time*Time	1	1	26.55130	10.6942	0.0024*

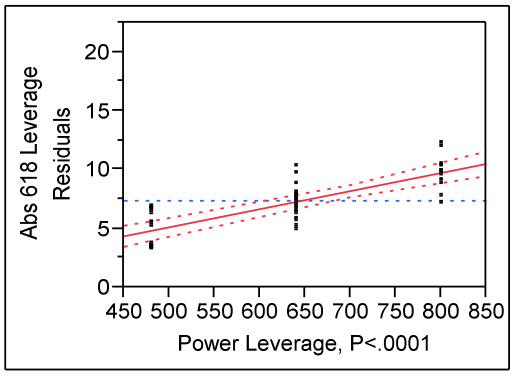
Residual by Predicted Plot



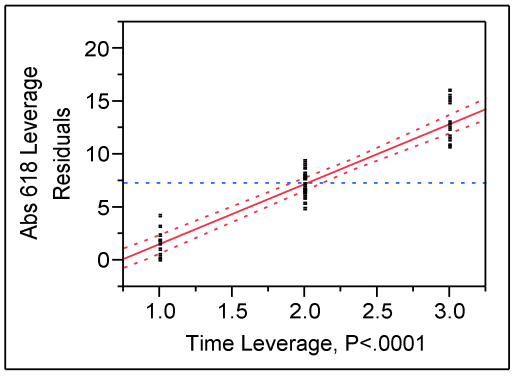
Ratio Leverage Plot



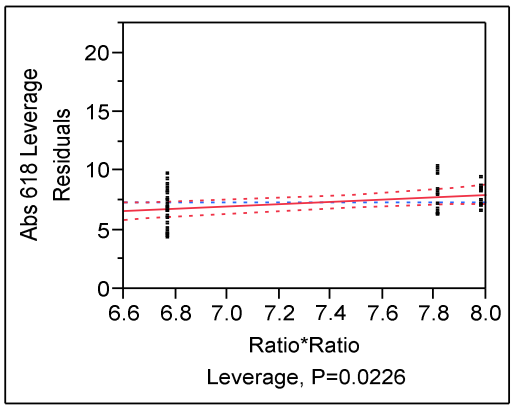
Power Leverage Plot



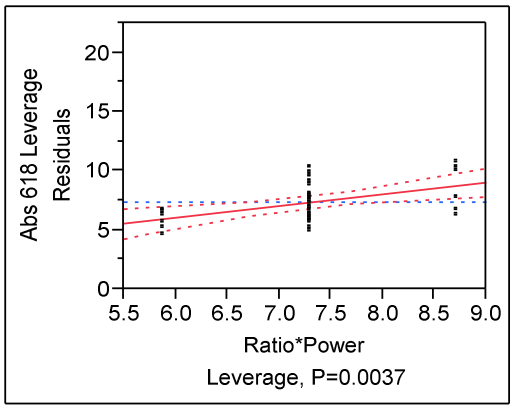
Time Leverage Plot



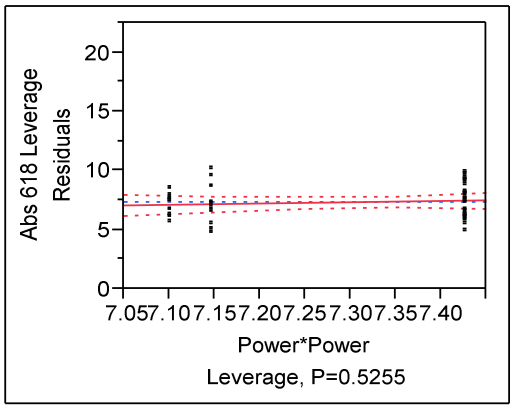
Ratio*Ratio Leverage Plot



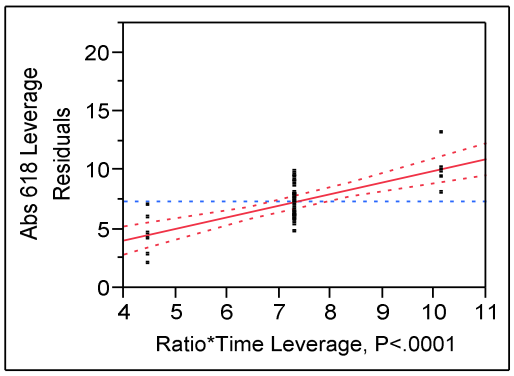
Ratio*Power Leverage Plot



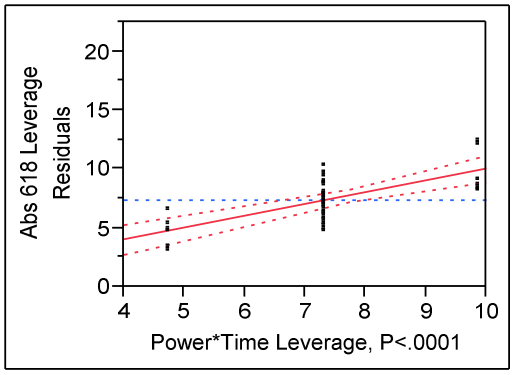
Power*Power Leverage Plot



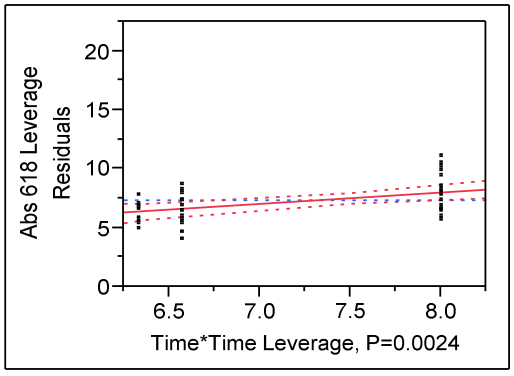
Ratio*Time Leverage Plot



Power*Time Leverage Plot



Time*Time Leverage Plot



Response Surface

Coef	Ratio	Power	Time	Abs 618
Ratio	-1.129028	0.0088565	2.8226667	3.0657708
Power	.	1.1859e-5	0.0160164	0.0151935
Time	.	.	1.5482222	5.6576458

Solution

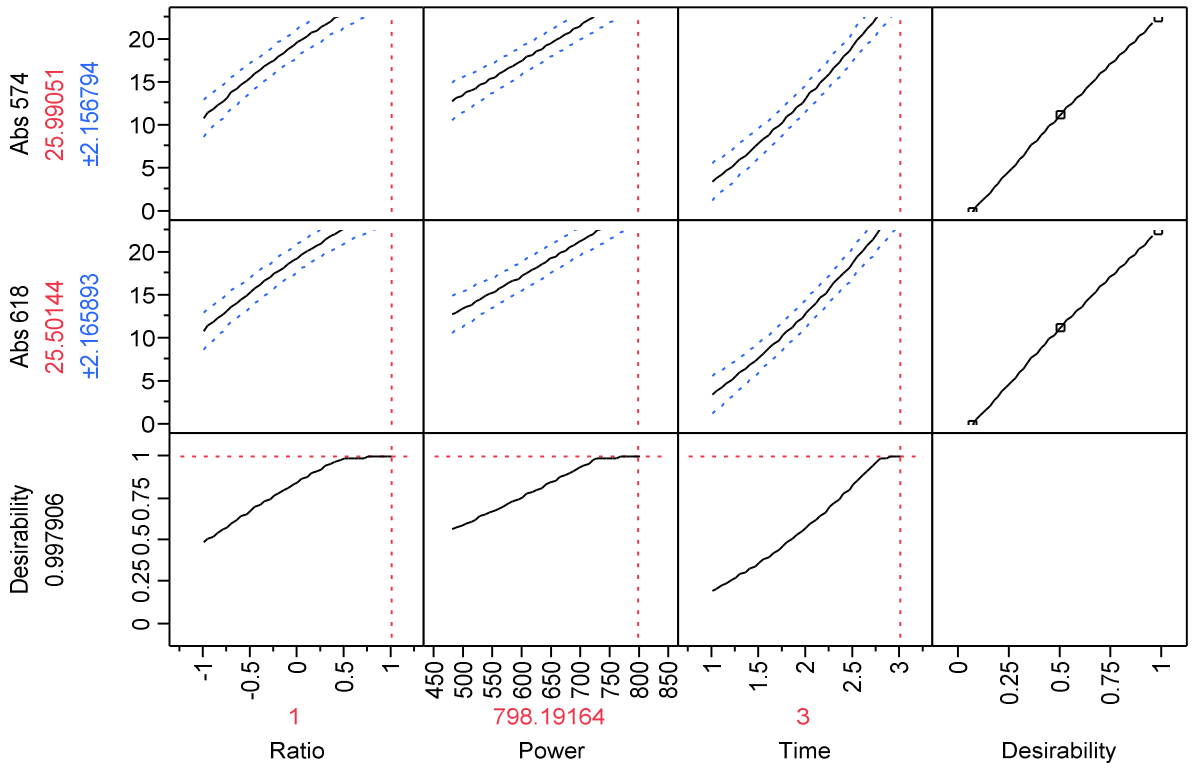
Variable	Critical Value
Ratio	-0.169606
Power	418.49907
Time	1.4731842

Solution is a SaddlePoint

Critical values outside data range

Predicted Value at Solution
3.4682811

Prediction Profiler



Appendix 3 – Hot water extraction of plant extracts at 80ºC

3.1. Hot water extraction of red cabbage

Table A3.1: Absorbance at λ_{max} = 553 nm of red cabbage extract obtained from hot water extraction at constant 80^{O}C and sample to water ratio of 1:3 g/mL

Time (min)		Absorbance
10	1	0.8164
	2	0.7626
	3	0.7704
	Ave. ± S.D.	0.7831±0.0291
20	1	1.0550
	2	1.0064
	3	1.0728
	Ave. ± S.D.	1.0447±0.0344
30	1	1.0692
	2	1.0876
	3	1.0632
	Ave. ± S.D.	1.0733±0.0127
40	1	1.0716
	2	1.0782
	3	1.0800
	Ave. ± S.D.	1.0766±0.0044
50	1	1.1392
	2	1.1240
	3	1.1566
	Ave. ± S.D.	1.1399±0.0163
60	1	1.1822
	2	1.1524
	3	1.1618
	Ave. ± S.D.	1.1655±0.0152
70	1	1.1442
	2	1.0980

Time (min)		Absorbance
	3	1.1098
	Ave. ± S.D.	1.1173±0.0240
80	1	1.0768
	2	1.0468
	3	1.0490
	Ave. ± S.D.	1.0575±0.0167
90	1	1.0710
	2	1.1070
	3	1.0640
	Ave. ± S.D.	1.0807±0.0231
100	1	1.0664
	2	1.0794
	3	1.0724
	Ave. ± S.D.	1.0727±0.0065
110	1	1.0382
	2	1.0554
	3	1.0208
	Ave. ± S.D.	1.0381±0.0173
120	1	1.0412
	2	1.0392
	3	1.0596
	Ave. ± S.D.	1.0467±0.0112
130	1	1.0470
	2	1.0756
	3	1.0434
	Ave. ± S.D.	1.0553±0.0176
140	1	1.0052
	2	1.0056
	3	0.9994
	Ave. ± S.D.	1.0034±0.0035
150	1	1.0154
	2	1.0156

Time (min)		Absorbance
	3	1.0026
	Ave. ± S.D.	1.0112±0.0074
160	1	1.0404
	2	1.0028
	3	1.0628
	Ave. ± S.D.	1.0353±0.0303
170	1	0.9770
	2	0.9826
	3	0.9742
	Ave. ± S.D.	0.9779±0.0043
180	1	0.9928
	2	0.9774
	3	1.0000
	Ave. ± S.D.	0.9901±0.0115

*TAC = Total monomeric anthocyanin content (cyd-3-glu equivalents, mg/L)

3.2. Hot water extraction of Dendrobium orchid

Table A3.2: Absorbance at λ_{max} = 543 and 583 nm of Dendrobium orchid extract obtained from hot water extraction at constant 80^{O}C and sample to water ratio of 1:3 g/mL

Time (min)		Absorbance	
		λ_{543}	λ_{583}
10	1	0.7536	0.7864
	2	0.6962	0.7346
	3	0.7090	0.7486
	Ave. ± S.D.	0.7196±0.0301	0.7565±0.0268
20	1	1.4860	1.4882
	2	1.5578	1.5592
	3	1.5184	1.4628
	Ave. ± S.D.	1.5207±0.0360	1.5034±0.0500
30	1	2.1370	2.1160
	2	2.2244	2.1996
	3	2.2004	2.0018
	Ave. ± S.D.	2.1873±0.0452	2.1058±0.0993
40	1	2.4850	2.4478
	2	2.5302	2.4914
	3	2.3844	2.4068
	Ave. ± S.D.	2.4665±0.0746	2.4487±0.0423
50	1	2.7746	2.7270
	2	2.8902	2.8360
	3	2.7194	2.7822
	Ave. ± S.D.	2.7947±0.0872	2.7817±0.0545
60	1	3.0610	2.9980
	2	2.9698	2.9086
	3	2.9394	2.8736
	Ave. ± S.D.	2.9901±0.0633	2.9267±0.0642
70	1	3.0012	3.0202
	2	3.0990	3.0338
	3	3.1088	3.0090

Time (min)		Absorbance	
		λ_{543}	λ_{583}
	Ave. ± S.D.	3.0697±0.0595	3.0210±0.0124
80	1	3.0830	3.0156
	2	3.2056	3.0176
	3	3.2754	3.1064
	Ave. ± S.D.	3.1880±0.0974	3.0465±0.0519
90	1	3.2418	3.1656
	2	3.2442	3.1682
	3	3.2616	3.2348
	Ave. ± S.D.	3.2492±0.0108	3.1895±0.0392
100	1	3.2872	3.2088
	2	3.2882	3.2098
	3	3.3018	3.2398
	Ave. ± S.D.	3.2924±0.0082	3.2195±0.0176
110	1	3.3062	3.2286
	2	3.3786	3.2958
	3	3.3456	3.3012
	Ave. ± S.D.	3.3435±0.0362	3.2752±0.0404
120	1	3.3626	3.2808
	2	3.4520	3.3630
	3	3.4632	3.4010
	Ave. ± S.D.	3.4259±0.0551	3.3483±0.0614
130	1	3.2812	3.2040
	2	3.4076	3.3264
	3	3.3528	3.3470
	Ave. ± S.D.	3.3472±0.0634	3.2925±0.0773
140	1	3.2145	3.1230
	2	3.2760	3.1986
	3	3.2878	3.2444
	Ave. ± S.D.	3.2594±0.0394	3.1887±0.0613
150	1	3.1520	3.0828
	2	3.1994	3.1894

Time (min)		Absorbance	
		λ_{543}	λ_{583}
	3	3.1104	3.1502
	Ave. ± S.D.	3.1539±0.0445	3.1408±0.0539
160	1	3.2450	3.0788
	2	3.2048	3.1881
	3	3.2088	3.1388
	Ave. ± S.D.	3.2195±0.0221	3.1352±0.0547
170	1	3.1958	3.0586
	2	3.1944	3.1644
	3	3.1684	3.1024
	Ave. ± S.D.	3.1862±0.0154	3.1085±0.0532
180	1	3.1632	3.0586
	2	3.1996	3.1322
	3	3.1186	3.0736
	Ave. ± S.D.	3.1605±0.0406	3.0881±0.0389

3.3. Hot water extraction of butterfly pea

Table A3.3: Absorbance at λ_{max} = 574 and 618 nm of butterfly pea extract obtained from hot water extraction at constant 80^{o}C and sample to water ratio of 1:30 g/mL

Time (min)		Absorbance	
		λ_{574}	λ_{618}
10	1	2.2255	2.6270
	2	2.2855	2.6465
	3	2.1325	2.5520
	Ave. ± S.D.	2.2145±0.0771	2.6085±0.0499
20	1	6.8720	7.6520
	2	5.6670	6.4800
	3	7.0540	6.8950
	Ave. ± S.D.	6.5310±0.7538	7.0090±0.5943
30	1	6.9970	7.7960
	2	7.8070	8.5540
	3	8.0110	7.5180
	Ave. ± S.D.	7.6050±0.5363	7.9560±0.5362
40	1	9.2020	9.9150
	2	9.0440	9.7850
	3	9.5170	10.9140
	Ave. ± S.D.	9.2543±0.2408	10.2047±0.6177
50	1	8.9550	8.9450
	2	8.9510	9.7090
	3	9.3390	9.9630
	Ave. ± S.D.	9.0817±0.2229	9.5390±0.5299
60	1	8.9910	8.9180
	2	8.9710	8.8530
	3	9.1010	9.2750
	Ave. ± S.D.	9.0210±0.0700	9.0153±0.2272
70	1	8.5440	8.7530
	2	8.2900	8.9020
	3	9.0320	9.2420

Time (min)		Absorbance	
		λ_{574}	λ_{618}
	Ave. ± S.D.	8.6220±0.3771	8.9657±0.2506
80	1	8.4830	8.2140
	2	8.1730	8.0350
	3	9.0990	9.0300
	Ave. ± S.D.	8.5850±0.4714	8.4263±0.5304
90	1	8.3356	8.5690
	2	8.3960	8.1840
	3	8.5640	8.7190
	Ave. ± S.D.	8.4319±0.1183	8.4907±0.2760
100	1	8.1038	8.3710
	2	8.0960	8.0920
	3	8.0550	8.4220
	Ave. ± S.D.	8.0849±0.0262	8.2950±0.1776
110	1	8.0630	7.9080
	2	8.0730	7.8420
	3	7.4610	8.5000
	Ave. ± S.D.	7.8657±0.3505	8.0833±0.3623
120	1	8.1390	7.9780
	2	7.9280	7.8520
	3	7.5940	8.0410
	Ave. ± S.D.	7.8870±0.2748	7.9570±0.0962
130	1	8.1038	7.8560
	2	7.9110	7.9490
	3	7.3000	8.1640
	Ave. ± S.D.	7.7716±0.4196	7.9897±0.1580
140	1	8.1309	7.8720
	2	8.0240	7.8010
	3	7.4620	8.0080
	Ave. ± S.D.	7.8723±0.3593	7.8937±0.1052
150	1	8.1420	7.7660
	2	8.0430	7.7730

Time (min)		Absorbance	
		λ_{574}	λ_{618}
	3	7.5540	7.9910
	Ave. ± S.D.	7.9130±0.3148	7.8433±0.1279
160	1	7.5480	7.3500
	2	7.9920	7.7650
	3	7.4040	7.8520
	Ave. ± S.D.	7.6480±0.3065	7.6557±0.2683
170	1	7.5060	7.2570
	2	6.3110	7.1520
	3	7.2540	7.5480
	Ave. ± S.D.	7.0237±0.6299	7.3190±0.2052
180	1	7.6000	6.3840
	2	6.7150	6.5650
	3	7.0150	7.0690
	Ave. ± S.D.	7.1100±0.4501	6.6727±0.3550

Appendix 4 – RSM and desirability analysis for development of colorimetric film

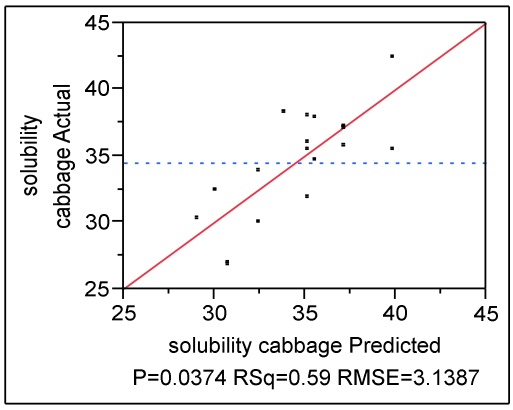
4.1. Optimization of colorimetric film with red cabbage extract (using $\Delta E_{3,7}$ and film solubility as responses)

Table A4.1: $\Delta E_{3,7}$ and film solubility of prepared colorimetric film with red cabbage extract

Formula	Code	Banana Flour (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
1	0.167, 0.167, 0.667	0.50	0.50	2.00	1	24.92	14.72	4.32	24.87	9.01	3.34	5.80	37.12
					2	28.81	8.13	3.34	28.30	3.43	2.02	4.91	38.00
					3	22.27	11.09	4.89	24.12	7.32	4.86	4.19	42.29
2	0,1,0	0.00	3.00	0.00	1	25.41	10.48	2.71	29.86	5.17	2.46	6.94	35.80
					2	29.03	12.84	4.16	29.42	5.62	2.69	7.37	42.47
					3	23.03	15.21	5.86	23.10	10.37	3.55	5.36	63.53
3	0.167, 0.667, 0.167	0.50	2.00	0.50	1	25.75	10.87	3.81	28.45	6.97	3.78	4.75	35.50
					2	23.19	17.20	2.19	25.14	10.45	-0.57	7.55	30.66
					3	24.08	17.61	5.91	24.02	11.86	2.74	6.57	47.02
4	0.333, 0.333, 0.333	1.00	1.00	1.00	1	27.77	11.36	2.79	27.22	7.25	2.08	4.21	26.59
					2	23.75	15.89	1.23	26.01	11.32	0.01	5.24	100.00
					3	23.88	12.30	5.69	24.27	7.49	4.02	5.11	44.28
5	0.5,0.5,0	1.50	0.00	2.00	1	28.77	11.33	3.66	26.89	7.95	3.83	3.87	30.07
					2	24.49	19.15	2.26	27.09	12.01	-0.17	7.98	35.49

Formula	Code	Banana Flour (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
					3	23.70	15.59	1.40	23.45	15.07	-0.14	1.64	37.95
6	0.667, 0.167, 0.167	2.00	0.50	0.50	1	26.99	10.12	2.72	27.34	6.59	1.77	3.67	37.14
					2	22.17	13.65	1.64	25.16	10.15	1.54	4.61	37.32
					3	24.43	20.88	4.73	26.70	12.09	1.22	9.73	28.58
7	1,0,0	3.00	0.00	0.00	1	26.93	13.08	3.56	27.76	8.49	2.90	4.71	34.00
					2	27.98	12.84	5.38	26.85	7.04	5.54	5.91	34.67
					3	24.68	13.91	2.64	28.75	9.69	2.51	5.86	31.88
8	0,0.5,0.5	0.00	1.50	1.50	1	26.37	11.78	4.34	25.57	6.40	2.78	5.66	159.47
					2	24.40	15.90	5.60	25.44	8.97	2.98	7.48	30.27
					3	22.24	21.23	5.35	22.18	15.05	0.19	8.05	16.99
9	0.5,0,0.5	1.50	0.00	1.50	1	24.62	18.10	6.71	22.92	9.73	2.13	9.69	32.43
					2	21.51	22.19	7.50	25.25	14.88	-1.63	12.28	-7.17
					3	25.39	15.35	3.76	25.32	6.29	0.82	9.53	26.98
10	0,0,1	0.00	0.00	3.00	1	28.52	8.74	7.16	27.43	5.41	6.59	3.55	36.01
					2	22.77	17.03	3.38	23.71	14.72	1.40	3.18	26.88
					3	24.92	14.72	4.32	24.87	9.01	3.34	5.80	37.12

Least Squares Fit
Response solubility cabbage
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.588006
RSquare Adj	0.416342
Root Mean Square Error	3.138729
Mean of Response	34.51079
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	168.72502	33.7450	3.4253
Error	12	118.21941	9.8516	**Prob > F**
C. Total	17	286.94443		0.0374*

Tested against reduced model: Y=mean

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	4	67.63820	16.9095	2.6744
Pure Error	8	50.58121	6.3227	**Prob > F**
Total Error	12	118.21941		0.1102
				Max RSq
				0.8237

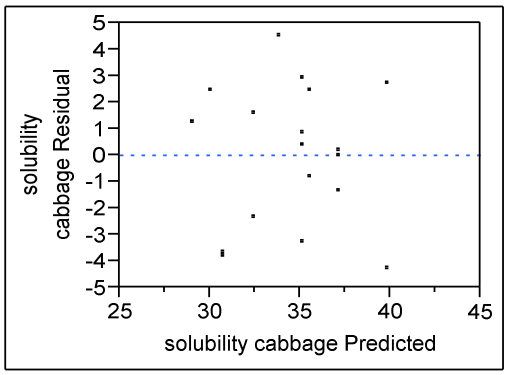
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
BF	39.75346	2.166984	18.35	<.0001*
CMC	37.13331	1.773224	20.94	<.0001*
Carrageenan	29.971784	2.935932	10.21	<.0001*
BF*CMC	-13.32983	9.699962	-1.37	0.1945
BF*Carrageenan	-23.43886	12.43932	-1.88	0.0840
CMC*Carrageenan	7.6904462	10.50658	0.73	0.4782

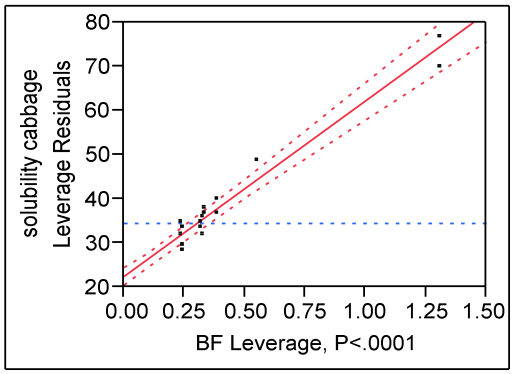
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
BF	1	1	3315.4777	336.5415	<.0001*
CMC	1	1	4320.2390	438.5309	<.0001*
Carrageenan	1	1	1026.6930	104.2157	<.0001*
BF*CMC	1	1	18.6045	1.8885	0.1945
BF*Carrageenan	1	1	34.9774	3.5504	0.0840
CMC*Carrageenan	1	1	5.2782	0.5358	0.4782

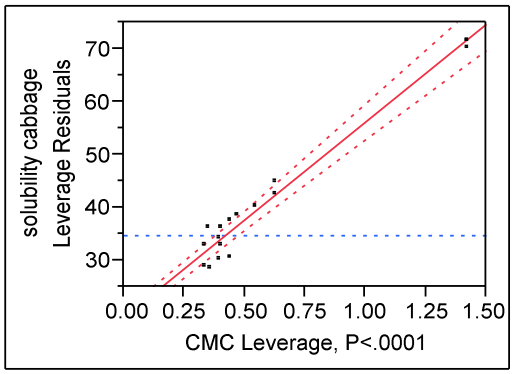
Residual by Predicted Plot



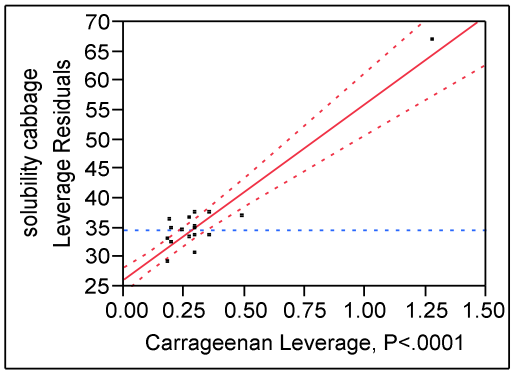
BF Leverage Plot



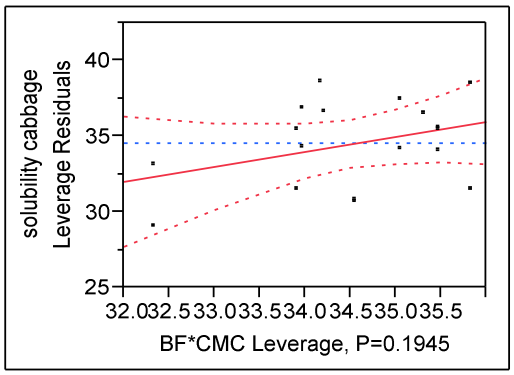
CMC Leverage Plot



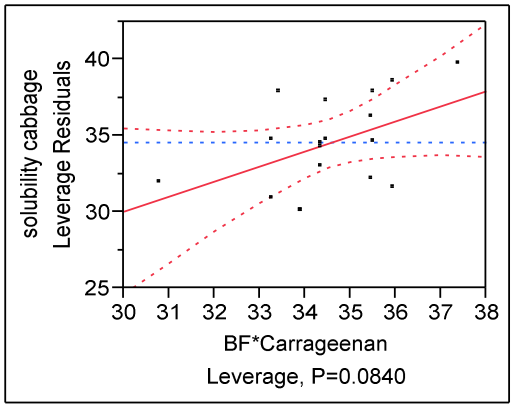
Carrageenan Leverage Plot



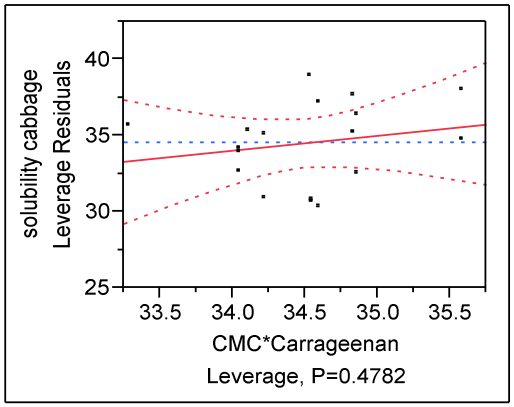
BF*CMC Leverage Plot



BF*Carrageenan Leverage Plot



CMC*Carrageenan Leverage Plot



Response Surface

Coef	BF	CMC	Carrageenan	solubility cabbage
BF	0	-13.32983	-23.43886	39.75346
CMC	.	0	7.6904462	37.13331
Carrageenan	.	.	0	29.971784

Solution

Variable	Critical Value
BF	0.2395736
CMC	1.0032838
Carrageenan	-0.242857

Solution is a
SaddlePoint

Assuming the following mixture sum: BF+CMC+Carrageenan=1

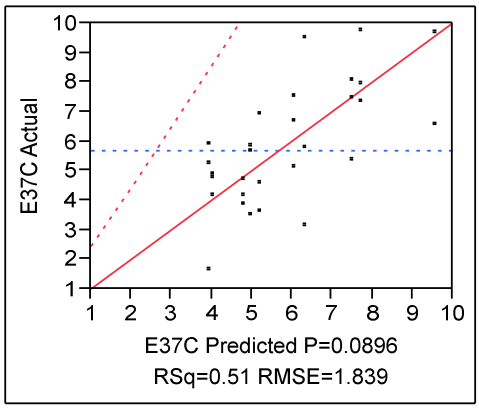
Critical values outside data range

Predicted Value at Solution
35.786205

Canonical Curvature
Eigenvalues and Eigenvectors

Eigenvalue	23.4858	-7.7374
BF	0.99925	-0.03876
CMC	0.03876	0.99925

Response E37C
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.510504
RSquare Adj	0.306547
Root Mean Square Error	1.838962
Mean of Response	5.684444
Observations (or Sum Wgts)	18

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	42.323050	8.46461	2.5030
Error	12	40.581394	3.38178	**Prob > F**
C. Total	17	82.904444		0.0896

Tested against reduced model: Y=mean

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	4	2.452794	0.61320	0.1287
Pure Error	8	38.128600	4.76607	**Prob > F**
Total Error	12	40.581394		0.9677
				Max RSq

Source	DF	Sum of Squares	Mean Square	F Ratio
				0.5401

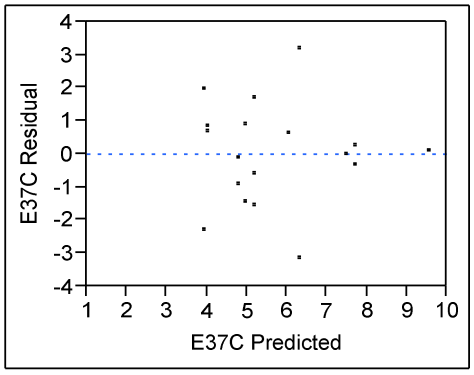
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
BF	7.7101676	1.269623	6.07	<.0001*
CMC	5.2009181	1.038921	5.01	0.0003*
Carrageenan	9.5673563	1.720145	5.56	0.0001*
BF*CMC	-5.929169	5.68315	-1.04	0.3174
BF*Carrageenan	-4.550636	7.288122	-0.62	0.5441
CMC*Carrageenan	-13.7495	6.155743	-2.23	0.0453*

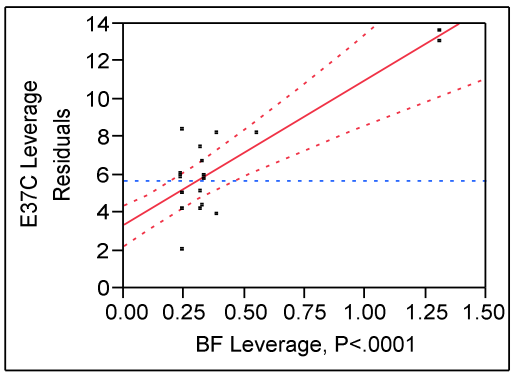
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
BF	1	1	124.71649	36.8789	<.0001*
CMC	1	1	84.75015	25.0608	0.0003*
Carrageenan	1	1	104.61629	30.9352	0.0001*
BF*CMC	1	1	3.68091	1.0885	0.3174
BF*Carrageenan	1	1	1.31843	0.3899	0.5441
CMC*Carrageenan	1	1	16.87170	4.9890	0.0453*

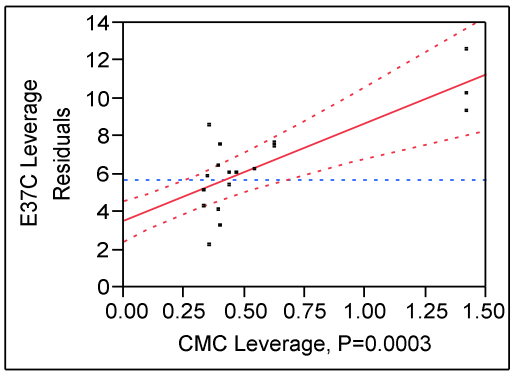
Residual by Predicted Plot



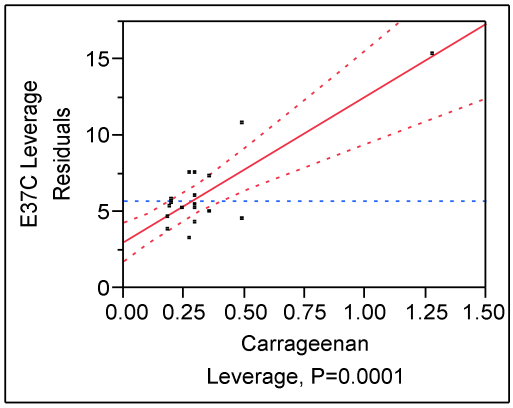
BF Leverage Plot



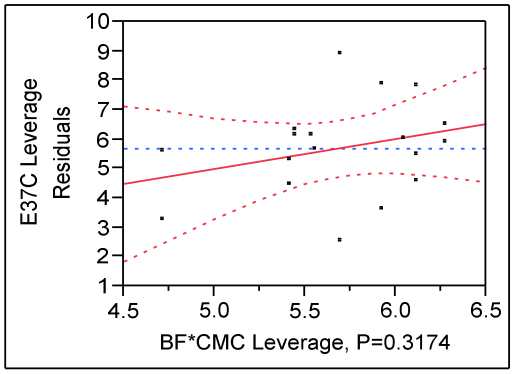
CMC Leverage Plot



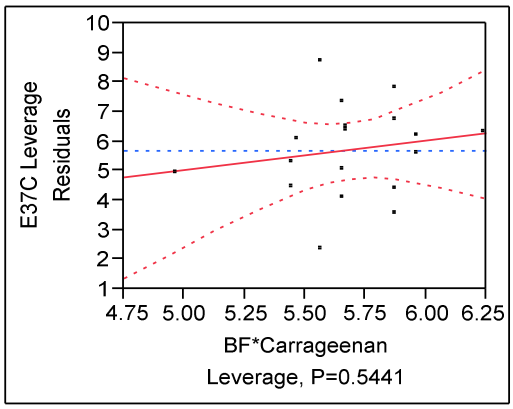
Carrageenan Leverage Plot



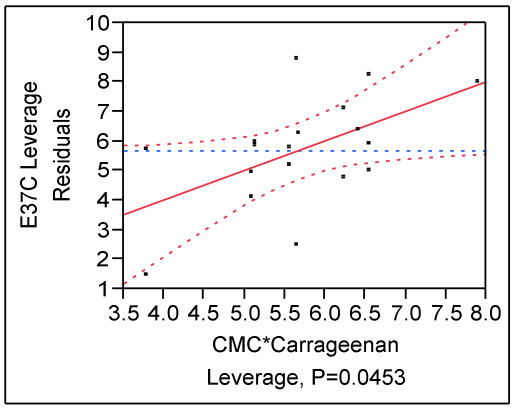
BF*CMC Leverage Plot



BF*Carrageenan Leverage Plot



CMC*Carrageenan Leverage Plot



Response Surface

Coef	BF	CMC	Carrageenan	E37C
BF	0	-5.929169	-4.550636	7.7101676
CMC	.	0	-13.7495	5.2009181
Carrageenan	.	.	0	9.5673563

Solution

Variable	Critical Value
BF	-0.492651
CMC	0.8804143
Carrageenan	0.6122372

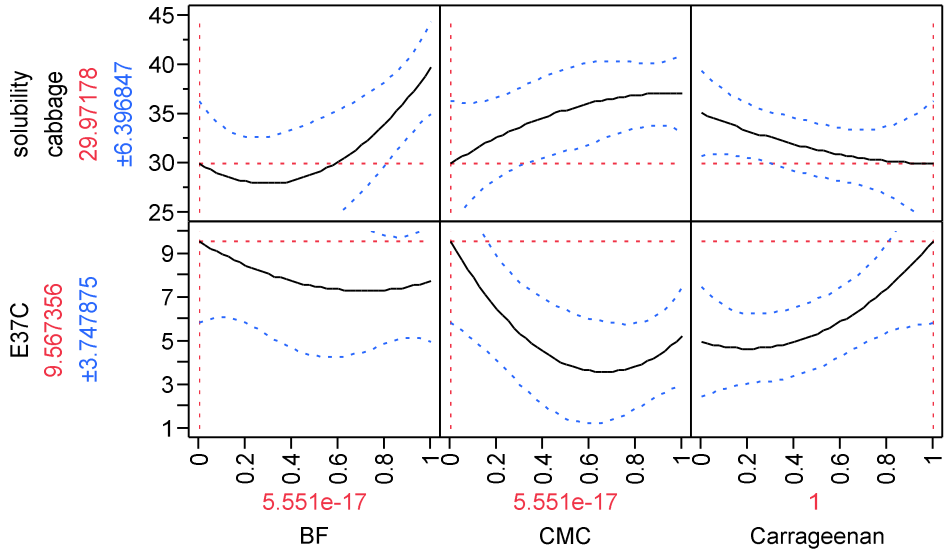
Solution is a
Minimum

Assuming the following mixture sum: BF+CMC+Carrageenan=1

Critical values outside data range

Predicted Value at Solution
3.1710011

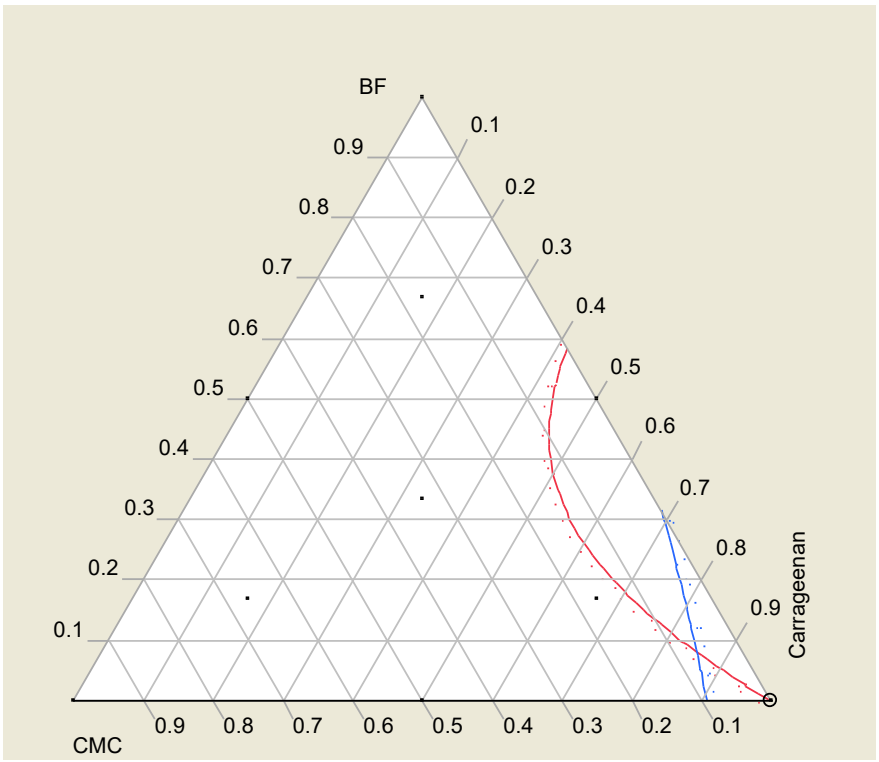
Prediction Profiler



Mixture Profiler

T L R Factor	Current X	Lo Limit	Hi Limit
BF	0	0	1
CMC	0	0	1
Carrageenan	1	0	1

Response	Contour	Current Y	Lo Limit	Hi Limit
solubility cabbage	30	29.971784	.	.
E37C	8	9.5673563	.	.



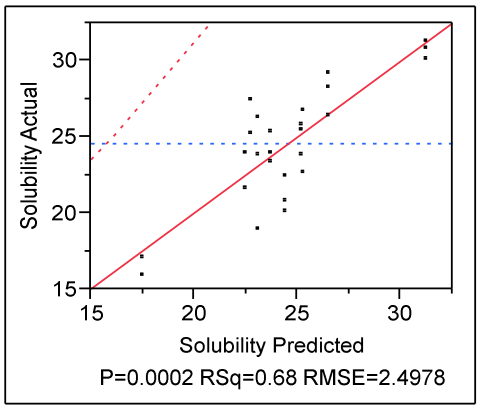
4.2. Optimization of colorimetric film with Dendrobium orchid extract (using $\Delta E_{3,7}$ and film solubility as responses)

Table A4.2: $\Delta E_{3,7}$ and film solubility of prepared colorimetric film with Dendrobium orchid extract

Formula	Code	Banana Flour (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
1	0.167, 0.167, 0.667	0.50	0.50	2.00	1	24.65	17.32	2.72	22.39	13.91	-2.75	6.84	36.99
					2	22.66	17.11	-1.24	21.51	15.97	-4.24	3.41	23.93
					3	23.85	13.13	-0.59	21.61	15.26	-3.84	4.49	25.33
2	0,1,0	0.00	3.00	0.00	1	28.14	21.47	-0.86	23.02	17.70	-2.12	6.49	18.91
					2	23.49	20.41	1.20	23.65	16.69	-6.37	8.45	30.22
					3	27.12	18.67	3.05	22.01	17.47	-3.67	8.53	19.89
3	0.167, 0.667, 0.167	0.50	2.00	0.50	1	25.93	18.41	-0.37	21.39	15.21	-3.87	6.57	23.36
					2	26.47	20.30	-0.03	25.21	15.00	-6.61	8.55	28.31
					3	26.26	18.90	4.13	24.21	15.05	-3.61	8.89	17.09
4	0.333, 0.333, 0.333	1.00	1.00	1.00	1	23.92	18.75	0.73	21.93	15.50	-3.85	5.96	21.24
					2	23.35	18.92	0.36	23.42	15.81	-3.79	5.18	25.54
					3	28.91	16.89	0.27	24.79	14.42	-4.36	6.68	26.40
5	0.5,0.5,0	1.50	0.00	2.00	1	20.91	17.98	0.60	18.25	15.73	-4.10	5.85	20.19
					2	15.43	16.87	1.18	25.50	14.90	-5.66	12.33	30.83
					3	29.12	18.46	0.89	25.92	14.86	-5.28	7.83	23.84
6	0.667,	2.00	0.50	0.50	1	21.15	18.70	-1.34	17.28	17.61	-5.23	5.59	26.32

Formula	Code	Banana Flour (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
	0.167, 0.167				2	23.90	22.22	-2.49	20.44	17.75	-3.51	5.75	23.88
					3	26.40	17.32	3.01	26.04	15.54	-2.32	5.64	31.37
7	1,0,0	3.00	0.00	0.00	1	23.46	17.69	-0.21	20.93	17.00	-5.31	5.73	20.08
					2	29.52	14.39	1.01	26.32	14.22	-0.65	3.61	25.87
					3	22.78	15.28	1.15	21.55	13.18	-1.41	3.53	23.99
8	0,0.5,0.5	0.00	1.50	1.50	1	20.41	20.85	0.06	20.35	19.67	-6.19	6.37	21.66
					2	33.49	15.64	2.75	29.43	11.28	-4.09	9.08	26.80
					3	26.96	18.54	4.35	24.79	15.82	-3.19	8.30	16.05
9	0.5,0,0.5	1.50	0.00	1.50	1	25.53	18.27	0.39	25.14	15.23	-4.86	6.08	29.22
					2	27.95	16.78	4.88	23.83	15.06	-3.25	9.28	25.75
					3	25.63	20.61	3.64	19.70	18.67	-2.63	8.85	15.88
10	0,0,1	0.00	0.00	3.00	1	23.15	17.94	1.84	20.31	16.87	-3.15	5.84	31.66
					2	34.55	19.38	-0.97	25.02	13.38	-4.15	11.70	21.61
					3	21.18	20.34	2.74	19.88	16.64	-2.65	6.66	25.27

Least Squares Fit
Response Solubility
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.683078
RSquare Adj	0.603847
Root Mean Square Error	2.497771
Mean of Response	24.51923
Observations (or Sum Wgts)	26

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	268.93845	53.7877	8.6214
Error	20	124.77720	6.2389	**Prob > F**
C. Total	25	393.71566		0.0002*

Tested against reduced model: Y=mean

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	4	69.63423	17.4086	5.0512
Pure Error	16	55.14297	3.4464	**Prob > F**
Total Error	20	124.77720		0.0080*
				Max RSq
				0.8599

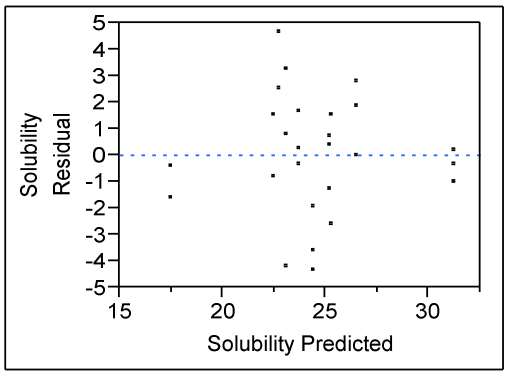
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
BF	31.189582	1.392442	22.40	<.0001*
CMC	23.08315	1.394184	16.56	<.0001*
Carrageenan	17.481825	1.696735	10.30	<.0001*
BF*CMC	-18.77518	7.103095	-2.64	0.0156*
BF*Carrageenan	3.7520712	7.403333	0.51	0.6178
CMC*Carrageenan	19.329844	6.80328	2.84	0.0101*

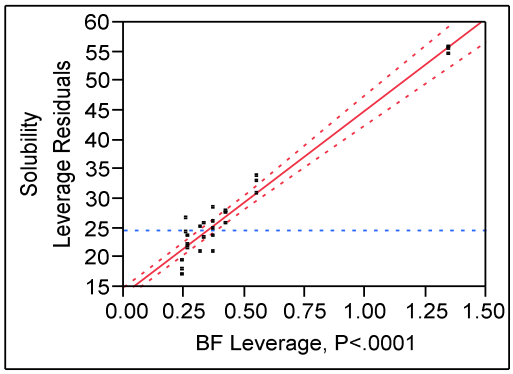
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
BF	1	1	3130.1873	501.7242	<.0001*
CMC	1	1	1710.2328	274.1258	<.0001*
Carrageenan	1	1	662.2938	106.1562	<.0001*
BF*CMC	1	1	43.5891	6.9867	0.0156*
BF*Carrageenan	1	1	1.6025	0.2569	0.6178
CMC*Carrageenan	1	1	50.3646	8.0727	0.0101*

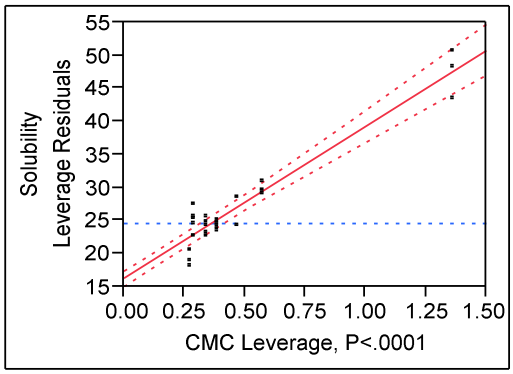
Residual by Predicted Plot



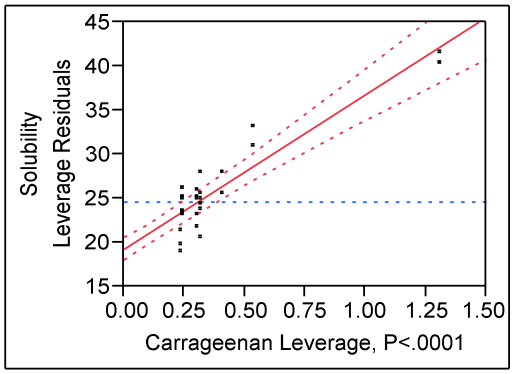
BF Leverage Plot



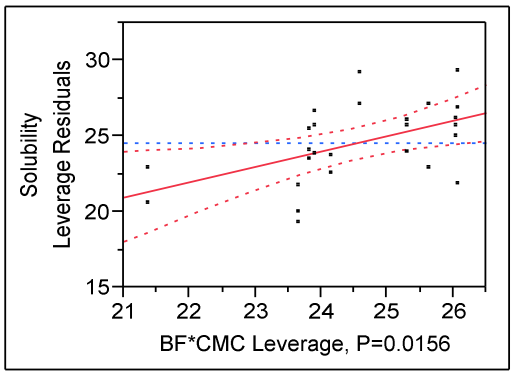
CMC Leverage Plot



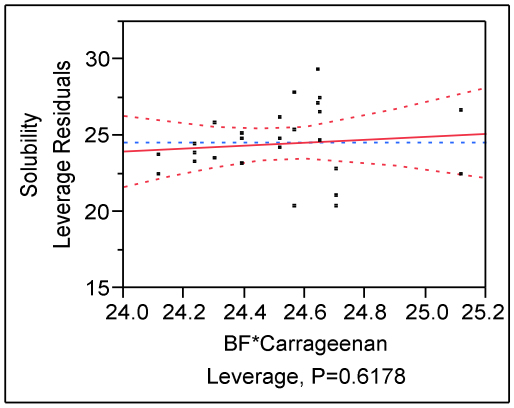
Carrageenan Leverage Plot



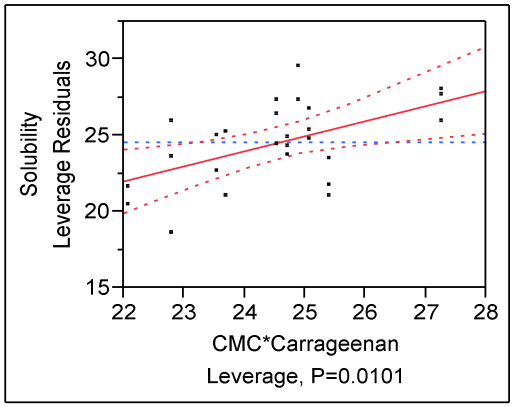
BF*CMC Leverage Plot



BF*Carrageenan Leverage Plot



CMC*Carrageenan Leverage Plot



Response Surface

Coef	BF	CMC	Carrageenan	Solubility
BF	0	-18.77518	3.7520712	31.189582
CMC	.	0	19.329844	23.08315
Carrageenan	.	.	0	17.481825

Solution

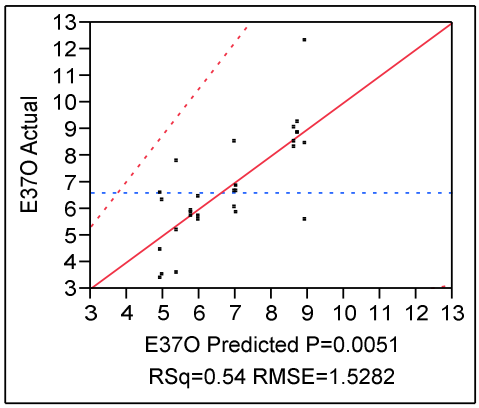
Variable	Critical Value
BF	0.2521052
CMC	0.371932
Carrageenan	0.3759627

Solution is a
SaddlePoint

Assuming the following mixture sum: BF+CMC+Carrageenan=1

Predicted Value at Solution
24.319032

Response E37O
Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.542052
RSquare Adj	0.427565
Root Mean Square Error	1.528235
Mean of Response	6.610769
Observations (or Sum Wgts)	26

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	55.28855	11.0577	4.7346
Error	20	46.71004	2.3355	**Prob > F**
C. Total	25	101.99858		0.0051*

Tested against reduced model: Y=mean

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	4	1.579204	0.39480	0.1400
Pure Error	16	45.130833	2.82068	**Prob > F**
Total Error	20	46.710037		0.9649
				Max RSq
				0.5575

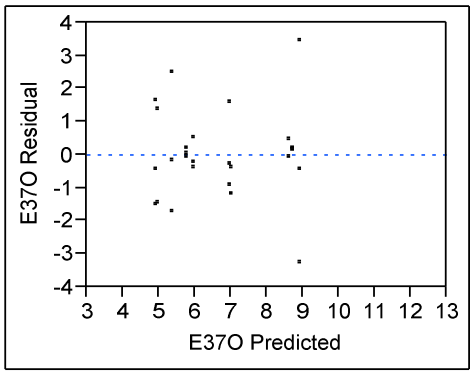
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
BF	8.8868546	0.851951	10.43	<.0001*
CMC	5.9605833	0.853017	6.99	<.0001*
Carrageenan	8.6770715	1.038129	8.36	<.0001*
BF*CMC	-9.887477	4.345954	-2.28	0.0341*
BF*Carrageenan	-0.755667	4.529651	-0.17	0.8692
CMC*Carrageenan	-7.972272	4.162515	-1.92	0.0699

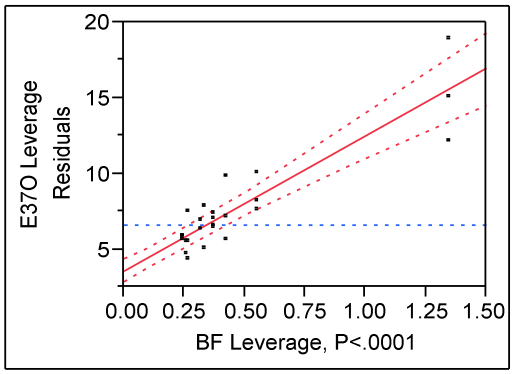
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
BF	1	1	254.12498	108.8096	<.0001*
CMC	1	1	114.03617	48.8273	<.0001*
Carrageenan	1	1	163.16370	69.8624	<.0001*
BF*CMC	1	1	12.08874	5.1761	0.0341*
BF*Carrageenan	1	1	0.06500	0.0278	0.8692
CMC*Carrageenan	1	1	8.56708	3.6682	0.0699

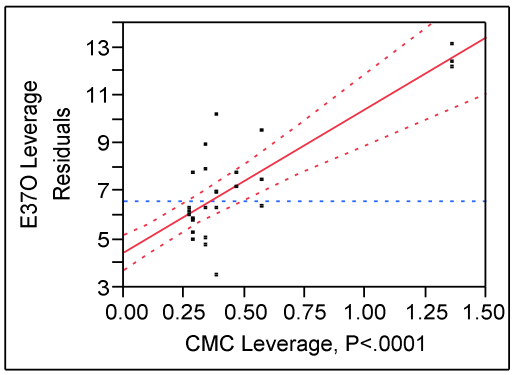
Residual by Predicted Plot



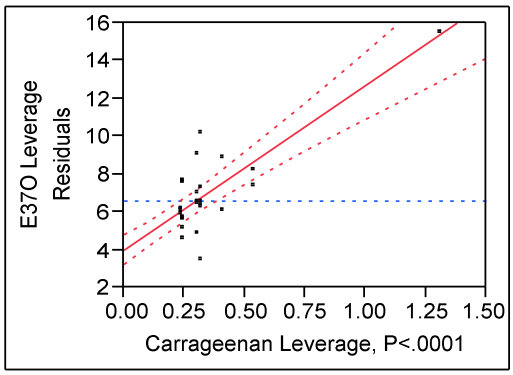
BF Leverage Plot



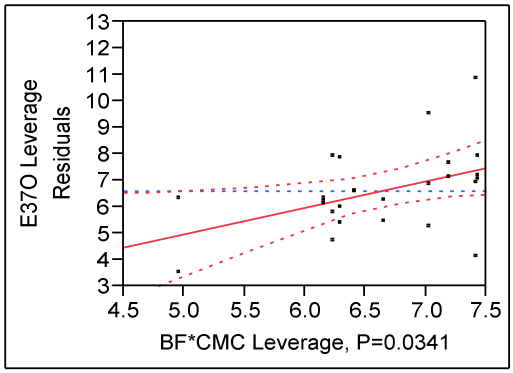
CMC Leverage Plot



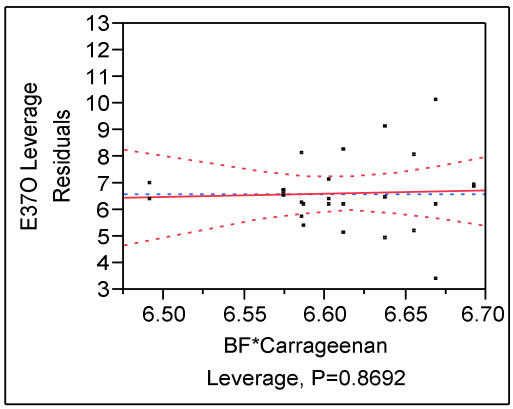
Carrageenan Leverage Plot



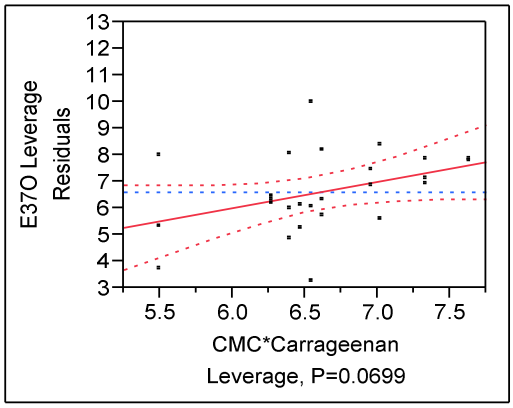
BF*CMC Leverage Plot



BF*Carrageenan Leverage Plot



CMC*Carrageenan Leverage Plot



Response Surface

Coef	BF	CMC	Carrageenan	E37O
BF	0	-9.887477	-0.755667	8.8868546
CMC	.	0	-7.972272	5.9605833
Carrageenan	.	.	0	8.6770715

Solution

Variable	Critical Value
BF	0.9272574
CMC	0.7378041
Carrageenan	-0.665062

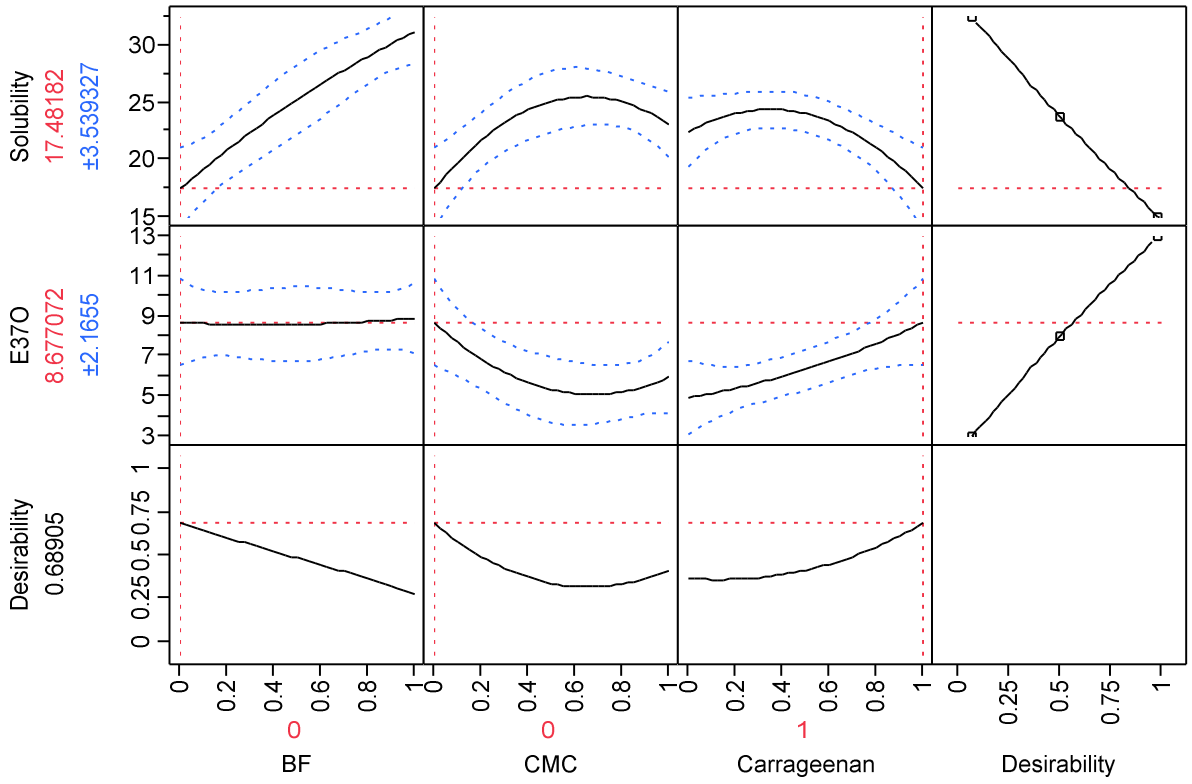
Solution is a
Minimum

Assuming the following mixture sum: BF+CMC+Carrageenan=1

Critical values outside data range

Predicted Value at Solution
4.4808782

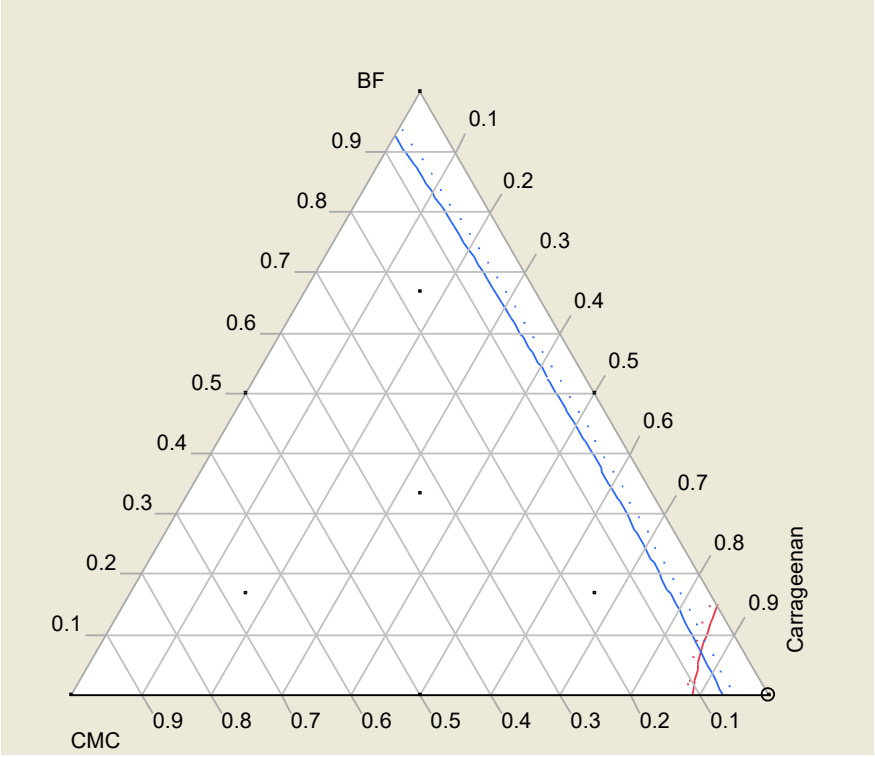
Prediction Profiler



Mixture Profiler

T L R	Factor	Current X	Lo Limit	Hi Limit
	BF	0	0	1
	CMC	0	0	1
	Carrageenan	1	0	1

Response	Contour	Current Y	Lo Limit	Hi Limit
Solubility	20	17.481825	.	.
E37O	8	8.6770715	.	.


BF
0.1
0.9
0.2
0.8
0.3
0.7
0.4
0.6
0.5
0.5
0.6
0.4
0.7
0.3
0.8
0.2
0.9
0.1
Carrageenan
CMC
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1

4.3. Optimization of colorimetric film with butterfly pea extract (using $\Delta E_{3,7}$ and film solubility as responses)

Table A4.3: $\Delta E_{3,7}$ and film solubility of prepared colorimetric film with butterfly pea extract

Formula	Code	Pectin (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
1	+ + 0	4.00	2.00	1.00	1	27.37	19.14	-6.42	27.24	13.20	-9.51	6.70	98.76
					2	26.69	19.44	-7.49	25.82	13.10	-11.28	7.44	100.87
					3	29.44	18.38	-7.76	28.13	12.13	-11.05	7.18	102.30
2	- - 0	1.00	0.00	1.00	1	34.82	15.08	-21.36	34.09	-1.70	-17.35	17.27	71.83
					2	29.81	15.12	-19.15	30.40	-0.27	-18.34	15.42	69.06
					3	32.14	17.86	-23.75	31.48	-0.38	-18.34	19.03	76.58
3	0 - +	2.50	0.00	2.00	1	31.81	14.78	-15.13	30.99	7.04	-16.25	7.86	51.42
					2	30.21	16.24	-16.25	29.37	7.72	-16.87	8.58	52.25
					3	31.09	15.14	-15.46	30.14	8.14	-16.31	7.12	55.61
4	0 0 0	2.50	1.00	1.00	1	32.57	18.19	-12.44	29.89	6.74	-15.38	12.12	98.59
					2	31.20	18.97	-13.31	26.30	10.82	-16.62	10.07	101.58
					3	32.58	18.64	-12.98	27.71	8.31	-15.82	11.76	103.65
5	0 - -	2.50	0.00	0.00	1	28.10	14.65	-9.19	28.51	9.17	-10.33	5.61	58.34
					2	27.25	16.20	-10.44	27.18	10.60	-11.02	5.63	61.49
					3	29.48	14.17	-9.68	27.64	9.83	-11.01	4.91	65.36
6	+ 0 -	4.00	1.00	0.00	1	32.32	14.45	-14.76	31.80	6.90	-15.63	7.61	52.87

Formula	Code	Pectin (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
					2	29.73	16.43	-16.01	29.14	8.46	-16.82	8.03	52.99
					3	33.46	14.64	-15.61	29.99	7.16	-16.20	8.27	58.31
7	- 0 +	1.00	1.00	2.00	1	31.81	13.53	-9.83	30.99	7.92	-10.88	5.77	58.93
					2	30.22	14.65	-11.53	31.32	8.10	-13.11	6.83	57.00
					3	31.21	14.29	-10.35	31.90	7.30	-11.45	7.11	60.80
8	- 0 -	1.00	1.00	0.00	1	34.74	14.72	-20.78	34.50	-2.70	-17.04	17.82	61.06
					2	31.72	16.82	-22.33	31.39	-0.15	-19.00	17.30	51.48
					3	34.92	15.55	-21.51	35.24	-3.57	-17.13	19.62	58.76
9	0 + +	2.50	2.00	2.00	1	33.12	15.69	-22.68	34.34	-2.12	-17.72	18.52	75.48
					2	35.72	12.81	-20.03	34.99	-2.01	-16.79	15.19	67.96
					3	32.97	15.14	-21.53	33.40	-0.36	-17.45	16.03	73.42
10	0 + -	2.50	2.00	0.00	1	33.55	12.69	-16.21	33.46	4.14	-16.29	8.55	48.31
					2	33.16	12.37	-14.53	36.83	2.72	-13.61	10.37	45.53
					3	33.00	12.29	-14.36	31.89	5.54	-14.50	6.84	51.16
11	+ - 0	4.00	0.00	1.00	1	29.40	23.88	-23.67	28.25	0.83	-20.19	23.34	101.78
					2	29.97	22.33	-21.87	30.78	-1.58	-18.67	24.13	102.41
					3	27.69	24.18	-21.92	27.93	1.26	-19.05	23.10	101.01
12	+ 0 +	4.00	1.00	2.00	1	29.81	16.51	-17.16	28.08	8.06	-17.26	8.62	56.47
					2	32.24	14.31	-14.86	33.31	4.71	-15.37	9.67	53.66

Formula	Code	Pectin (g)	CMC (g)	Carrageenan (g)		pH = 3			pH = 7			$\Delta E_{3,7}$	Solubility (%)
						L*	a*	b*	L*	a*	b*		
					3	30.98	15.49	-15.57	31.61	6.24	-15.56	9.27	58.91
13	0 0 0	2.50	1.00	1.00	1	31.52	13.28	-12.45	28.79	8.49	-12.95	5.53	53.26
					2	33.79	9.29	-10.40	32.75	6.48	-11.21	3.11	52.08
					3	32.54	11.99	-10.86	33.32	6.09	-10.97	5.96	55.98
14	0 0 0	2.50	1.00	1.00	1	30.22	15.43	-17.81	29.98	7.35	-17.02	8.11	63.20
					2	34.02	11.67	-14.63	30.83	6.08	-14.58	6.44	60.68
					3	35.11	12.00	-14.70	34.98	3.63	-13.58	8.44	65.42
15	- + 0	1.00	2.00	1.00	1	29.51	19.62	-14.58	28.94	8.99	-19.33	11.67	100.74
					2	32.88	18.00	-13.68	31.68	6.47	-10.36	12.05	100.89
					3	28.16	19.87	-12.89	27.38	11.21	-16.39	9.37	100.61

Appendix 5 – Properties of developed colorimetric layers

Table A5.1: Properties of developed colorimetric layer with red cabbage, Dendrobium orchid, and butterfly pea extracts

Properties*		Base film		Indicator type		
		For cabbage/orchid	For butterfly pea	Red cabbage	Dendrobium orchid	Butterfly pea
Thickness (mm)	1	0.59	0.615	0.67	0.65	0.62
	2	0.63	0.505	0.58	0.51	0.47
	3	0.52	0.575	0.54	0.70	0.58
	Ave. ± S.D.	0.58±0.06	0.57±0.06	0.59±0.07	0.62±0.10	0.55±0.08
a_w	1	0.42	0.38	0.44	0.42	0.46
	2	0.38	0.41	0.42	0.41	0.38
	3	0.39	0.44	0.36	0.41	0.40
	Ave. ± S.D.	0.40±0.02	0.41±0.03	0.41±0.04	0.41±0.01	0.41±0.04
Moisture content (%)	1	10.55	10.52	11.13	12.03	10.75
	2	9.95	10.26	10.57	11.25	9.59
	3	9.36	9.33	9.57	9.38	10.05
	Ave. ± S.D.	9.95±0.60	10.03±0.62	10.42±0.79	10.89±1.36	10.13±0.58
Solubility (%)	1	16.61	19.05	21.53	17.90	16.50
	2	15.25	16.56	18.14	16.81	14.69
	3	13.55	15.30	16.30	16.45	12.43
	Ave. ± S.D.	15.14±1.53	16.97±1.91	18.66±2.65	17.05±0.76	14.54±2.04

Properties*		Base film		Indicator type		
		For cabbage/orchid	For butterfly pea	Red cabbage	Dendrobium orchid	Butterfly pea
Volume swelling ratio	1	1.08	2.10	1.72	1.33	2.02
	2	1.86	1.52	1.62	1.75	1.57
	3	1.44	1.15	1.31	0.94	1.40
	Ave. ± S.D.	1.46±0.39	1.59±0.48	1.55±0.21	1.34±0.41	1.66±0.32
Tensile strength (MPa)	1	0.50	0.49	0.39	0.31	0.43
	2	0.52	0.36	0.27	0.39	0.43
	3	0.33	0.36	0.39	0.23	0.28
	4	0.30	0.46	0.23	0.28	0.40
	5	0.40	0.38	0.42	0.38	0.38
	6	0.52	0.46	0.48	0.41	0.36
	7	0.41	0.37	0.25	0.36	0.26
	8	0.52	0.35	0.44	0.32	0.37
	9	0.45	0.49	0.50	0.36	0.29
	10	0.51	0.33	0.47	0.27	0.25
	11	0.32	0.29	0.25	0.26	0.37
	12	0.45	0.50	0.36	0.37	0.48
	Ave. ± S.D.	0.43±0.08	0.40±0.07	0.37±0.10	0.33±0.06	0.36±0.07
Elongation (%)	1	97.37	94.75	88.83	84.22	98.93
	2	98.37	85.71	83.02	90.46	99.23

Properties*		Base film		Indicator type		
		For cabbage/orchid	For butterfly pea	Red cabbage	Dendrobium orchid	Butterfly pea
	3	83.49	87.37	85.28	82.99	86.77
	4	85.35	97.44	82.42	84.88	96.57
	5	89.47	89.07	89.37	92.89	97.37
	6	97.24	98.40	93.05	91.59	98.96
	7	91.96	87.27	82.89	90.20	86.18
	8	95.18	87.67	87.80	85.35	97.64
	9	95.38	99.20	93.09	91.06	89.47
	10	98.30	87.07	92.82	83.69	87.11
	11	85.78	84.88	83.39	84.02	95.91
	12	95.84	94.85	87.94	85.45	103.85
	Ave. ± S.D.	92.81±5.45	91.14±5.35	87.49±4.10	87.23±3.66	94.83±5.89
T_g (ºC)	1	77.54	78.06	79.48	78.28	79.51
	2	81.02	76.06	77.52	76.75	72.01
	3	75.13	72.05	74.13	71.80	73.07
	Ave. ± S.D.	77.90±2.96	75.39±3.06	77.04±2.71	75.61±3.39	74.89±4.06
Decomposition temperature (ºC)	1	203.64	202.09	194.88	207.48	204.11
	2	199.07	195.04	201.62	204.56	193.11
	3	198.34	199.01	200.56	194.13	199.09
	Ave. ± S.D.	200.35±2.87	198.71±3.53	199.02±3.62	202.06±7.02	198.77±5.51

Appendix 6 – pH sensitivity of developed colorimetric layers

6.1. Color change in response to pH of colorimetric film with red cabbage extract

Table A6.1: Color change in response to pH at pH = 2 to 7 of colorimetric film with red cabbage extract

pH		L*	a*	b*
2	1	37.39	53.38	33.50
	2	35.44	54.87	32.04
	3	37.33	55.44	32.60
	Ave. ± S.D.	36.72±1.11	54.56±1.07	32.72±0.74
3	1	34.29	51.93	21.07
	2	36.35	53.27	20.51
	3	35.77	52.53	20.81
	Ave. ± S.D.	35.47±1.06	52.58±0.67	20.80±0.28
4	1	34.94	39.99	10.31
	2	34.73	39.90	9.82
	3	33.65	39.66	10.72
	Ave. ± S.D.	34.44±0.69	39.85±0.17	10.28±0.45
5	1	36.29	35.70	-0.81
	2	34.71	35.26	-0.34
	3	34.63	36.02	0.43
	Ave. ± S.D.	35.21±0.94	35.66±0.38	-0.24±0.63
6	1	29.41	38.55	10.19
	2	29.10	39.33	11.05
	3	30.93	39.34	10.14
	Ave. ± S.D.	29.81±0.98	39.07±0.45	10.46±0.51
7	1	36.76	25.86	-4.17
	2	37.11	25.62	-3.43
	3	37.29	25.35	-3.63
	Ave. ± S.D.	37.05±0.27	25.61±0.25	-3.74±0.39

6.2. Color change in response to pH of colorimetric film with Dendrobium orchid extract

Table A6.2: Color change in response to pH at pH = 2 to 7 of colorimetric film with Dendrobium orchid extract

pH		L*	a*	b*
2	1	44.52	65.41	45.44
	2	44.12	65.22	45.44
	3	42.74	64.27	45.75
	Ave. ± S.D.	43.79±0.93	64.97±0.61	45.55±0.18
3	1	42.98	63.79	30.94
	2	41.83	62.85	32.48
	3	41.64	61.92	31.76
	Ave. ± S.D.	42.15±0.72	62.85±0.93	31.73±0.77
4	1	36.17	57.95	26.11
	2	36.64	58.19	24.79
	3	36.15	57.30	25.62
	Ave. ± S.D.	36.32±0.28	57.81±0.46	25.51±0.67
5	1	29.97	47.30	11.68
	2	31.71	52.00	21.09
	3	32.81	53.88	22.30
	Ave. ± S.D.	31.50±1.43	51.06±3.39	18.35±5.81
6	1	32.46	50.80	5.57
	2	32.19	48.44	3.91
	3	32.30	49.40	4.06
	Ave. ± S.D.	32.32±0.14	49.55±1.19	4.51±0.92
7	1	20.02	34.12	-6.76
	2	20.31	33.59	-7.53
	3	19.13	33.59	-7.79
	Ave. ± S.D.	19.82±0.61	33.77±0.31	-7.36±0.54

6.3. Color change in response to pH of colorimetric film with butterfly pea extract

Table A6.3: Color change in response to pH at pH = 2 to 7 of colorimetric film with butterfly pea extract

pH		L*	a*	b*
2	1	32.74	20.62	-5.11
	2	31.42	20.33	-4.74
	3	33.58	21.99	-5.13
	Ave. ± S.D.	32.58±1.09	20.98±0.89	-5.00±0.22
3	1	32.92	14.79	-14.55
	2	33.78	15.96	-15.56
	3	33.54	15.99	-14.65
	Ave. ± S.D.	33.41±0.45	15.58±0.68	-14.92±0.56
4	1	35.29	11.66	-16.68
	2	34.72	11.72	-16.85
	3	35.39	11.68	-17.11
	Ave. ± S.D.	35.13±0.36	11.69±0.03	-16.88±0.22
5	1	25.91	8.46	-15.36
	2	27.06	7.97	-15.81
	3	28.33	8.10	-15.37
	Ave. ± S.D.	27.10±1.21	8.18±0.26	-15.51±0.26
6	1	33.62	8.38	-15.41
	2	32.79	7.60	-14.77
	3	33.18	8.32	-15.29
	Ave. ± S.D.	33.20±0.41	8.10±0.44	-15.16±0.34
7	1	36.00	-4.91	-16.87
	2	36.31	-4.96	-16.45
	3	33.72	-4.86	-17.13
	Ave. ± S.D.	35.34±1.42	-4.91±0.05	-16.82±0.34

6.4. Sensory evaluation (Difference test – Two out of five)

6.4.1. Ballot

แบบสอบถามการแยกแยะความแตกต่างของผู้บริโภคต่อฟิล์มเปลี่ยนสีได้

วันที่ ...../...../.....
ชุดที่ .....

ส่วนที่ 1: ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

คำแนะนำ: โปรดทำเครื่องหมาย ✓ ลงใน ☐ หน้าคำตอบที่ท่านเห็นว่าเหมาะสมและตรงตามความคิดเห็นของท่านมากที่สุด

1. เพศ ☐ ชาย ☐ หญิง
2. อายุ ☐ 15-19 ปี ☐ 20-24 ปี ☐ 25-29 ปี ☐ 30 ปีขึ้นไป
3. ระดับการศึกษา
   ☐ ประถมศึกษา ☐ มัธยมศึกษา ☐ ปริญญาตรี ☐ สูงกว่าปริญญาตรี
4. รายได้เฉลี่ยต่อเดือน
   ☐ น้อยกว่า 10,000 บาท ☐ 10,000-15,000 บาท
   ☐ 15,001-25,000 บาท ☐ มากกว่า 25,000 บาท ขึ้นไป

ส่วนที่ 2: ข้อมูลพฤติกรรมการบริโภคที่เกี่ยวข้องกับความเป็นกรด-ด่างของอาหาร

5. โปรดทำเครื่องหมาย ✓ ลงใน ☐ หน้าผลิตภัณฑ์อาหารที่ท่านคิดว่า ระดับความเปรี้ยวมีผลเป็นอย่างมากต่อการตัดสินใจซื้อผลิตภัณฑ์ (ตอบได้มากกว่า 1 ข้อ)
   ☐ แหนม ☐ ปลาส้ม ☐ โยเกิร์ต
   ☐ กิมจิ ☐ ลูกอมรสเปรี้ยว เช่น รสมะนาว รสส้ม เป็นต้น
   ☐ เครื่องดื่มรสเปรี้ยว เช่น น้ำมะนาว เครื่องดื่มผสมวิตามินซี เป็นต้น
   ☐ อื่น ๆ ระบุ.....

ส่วนที่ 3: ข้อมูลเกี่ยวกับการแยกแยะความแตกต่างของผู้บริโภคต่อฟิล์มเปลี่ยนสีได้

วันที่ ...../...../.....
ชุดที่ .....

ผลิตภัณฑ์: ฟิล์มเปลี่ยนสีได้ (ส่วนประกอบ: สารสกัดจากกล้วยไม้หวาย กะหล่ำม่วงหรือดอกอัญชัน เพคติน คาร์บอกซีเมททิลเซลลูโลส คาราจีแนน และเซลลูโลสผง)

คำแนะนำ: พิจารณาตัวอย่างที่ได้รับด้วยสายตา ตามลำดับจาก ซ้าย ไป ขวา ในแต่ละชุดมี ตัวอย่าง 2 กลุ่ม กลุ่มแรกมี 2 ตัวอย่าง กลุ่มที่สองมี 3 ตัวอย่าง กรุณา**<u>วงกลมล้อมรอบรหัสตัวอย่าง 2 ตัวอย่างที่เหมือนกัน</u>** (กลุ่มแรก)

ชุดที่	**ตัวอย่าง**				
1	.....	.....	.....	.....	.....
2	.....	.....	.....	.....	.....
3	.....	.....	.....	.....	.....
4	.....	.....	.....	.....	.....
5	.....	.....	.....	.....	.....
6	.....	.....	.....	.....	.....
7	.....	.....	.....	.....	.....
8	.....	.....	.....	.....	.....
9	.....	.....	.....	.....	.....
10	.....	.....	.....	.....	.....

ข้อเสนอแนะ: .....

.....

.....

ขอบคุณที่ให้ความร่วมมือ

6.4.2. Examples of colorimetric film at different pH values and their corresponding 3-digit codes for sensory evaluation (Actual pH values displayed here were not shown to the panelists)

– Colorimetric film with red cabbage extract

#1					
3-digit code	785	318	934	159	451
(**Actual pH**	**5**	**4**	**4**	**4**	**5**)
#2					
3-digit code	958	746	172	853	516
(**Actual pH**	**6**	**4**	**6**	**4**	**6**)
#3					
3-digit code	713	497	502	391	365
(Actual pH	**5**	**7**	**5**	**7**	**7**)

– Colorimetric film with Dendrobium orchid extract

#1					
3-digit code	536	293	105	927	862
(**Actual pH**	**4**	**4**	**3**	**4**	**3**)
#2					
3-digit code	486	251	375	421	592
(**Actual pH**	**5**	**4**	**4**	**5**	**5**)

– Colorimetric film with butterfly pea extract extract

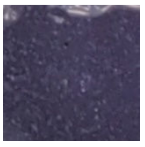
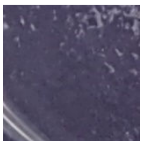
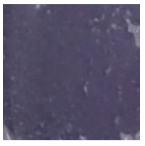
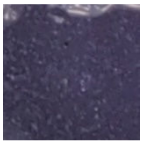
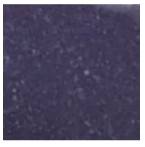
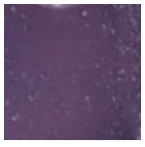
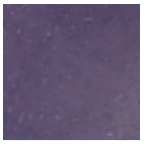
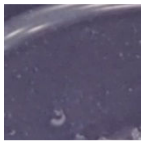
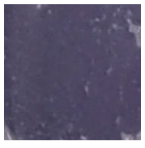
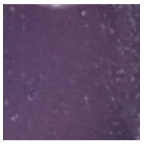
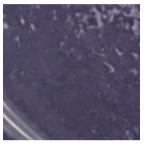
#1					
3-digit code	307	318	934	451	159
(**Actual pH**	**2**	**2**	**3**	**2**	**3**)
#2					
3-digit code	625	549	724	142	219
(**Actual pH**	**6**	**5**	**6**	**5**	**6**)
#3					
3-digit code	406	157	619	529	790
(**Actual pH**	**4**	**4**	**6**	**6**	**4**)
#4					
3-digit code	168	759	946	490	326
(**Actual pH**	**4**	**5**	**4**	**5**	**5**)
#5					
3-digit code	927	293	185	536	862
(**Actual pH**	**4**	**3**	**3**	**3**	**4**)
#6					
3-digit code	486	592	375	961	251
(**Actual pH**	**3**	**6**	**6**	**3**	**6**)

Table A6.4: Answers of ‘Two out of five’ sensory test on colorimetric layer with red cabbage, from 50 panelists

Panelist	#1		#2		#3	
1	785	451	746	853	713	502
2	785	451	746	853	713	502
3	785	451	746	853	713	502
4	785	451	746	853	713	502
5	785	451	746	853	713	502
6	785	451	746	853	713	502
7	785	451	172	516	713	502
8	785	451	746	853	713	502
9	318	159	746	853	713	502
10	785	451	746	853	713	502
11	785	451	746	853	497	391
12	785	451	746	853	713	502
13	785	451	746	853	713	502
14	785	451	746	853	713	502
15	785	451	746	853	713	502
16	785	451	746	853	713	502
17	785	451	746	853	713	502
18	318	934	958	516	497	391
19	785	451	746	853	713	502
20	785	451	958	516	713	502
21	785	451	746	853	713	502
22	318	159	958	516	497	365
23	318	159	958	516	497	365
24	785	451	958	516	497	391
25	785	451	958	516	497	391
26	318	159	746	853	713	365
27	318	934	172	853	713	391
28	318	159	958	516	497	365
29	785	451	958	853	497	391
30	785	451	172	516	497	391

Panelist	#1		#2		#3	
31	785	451	172	516	497	391
32	159	451	958	516	713	502
33	318	451	958	516	497	391
34	785	451	746	853	713	502
35	785	451	958	516	497	391
36	159	451	172	516	391	365
37	785	451	958	516	497	391
38	318	159	746	853	713	365
39	785	451	958	516	713	502
40	785	318	958	516	497	391
41	785	451	746	853	713	502
42	785	451	746	853	713	502
43	758	451	746	853	713	502
44	785	451	746	853	713	502
45	785	451	746	853	713	502
46	785	451	746	853	713	502
47	785	451	746	853	713	391
48	785	451	746	853	713	502
49	785	451	746	853	713	502
50	785	451	746	853	713	502

Correct Answer	38	30	30
%	76.00	60.00	60.00

Table A6.5: Answers of 'Two out of five' sensory test on colorimetric layer with Dendrobium orchid, from 50 panelists

Panelist	#1		#2	
1	105	862	251	375
2	105	862	486	592
3	105	862	251	375
4	105	862	251	375
5	105	862	251	375
6	105	862	486	592
7	105	862	251	375
8	105	862	486	592
9	105	862	486	592
10	105	862	251	375
11	105	862	486	592
12	105	862	421	592
13	105	862	251	375
14	105	862	251	375
15	105	862	251	375
16	105	862	251	375
17	105	862	251	375
18	105	862	251	375
19	105	862	251	375
20	536	293	486	592
21	105	862	251	375
22	536	927	486	592
23	105	862	251	375
24	105	862	486	592
25	105	862	486	592
26	536	293	486	592
27	105	862	486	592
28	105	862	486	592
29	536	927	486	421
30	536	927	486	421

Panelist	#1		#2	
31	536	927	486	592
32	536	927	486	592
33	536	293	486	592
34	105	862	421	592
35	536	293	421	592
36	105	862	251	375
37	536	293	486	592
38	293	927	421	592
39	105	862	486	592
40	105	862	251	375
41	105	862	251	375
42	105	862	251	375
43	105	862	251	375
44	105	862	251	375
45	105	862	251	375
46	105	862	251	375
47	105	862	251	375
48	105	862	251	375
49	105	862	251	375
50	105	862	251	375

Correct Answer	39	27
%	78.00	54.00

Table A6.6: Answers of ‘Two out of five’ sensory test on colorimetric layer with butterfly pea, from 50 panelists

Panelist	#1		#2		#3		#4		#5		#6	
1	934	159	549	142	619	529	168	946	927	862	486	961
2	934	159	549	142	619	529	168	946	927	862	486	961
3	934	159	549	142	619	529	168	946	927	862	486	961
4	934	159	549	142	619	529	168	946	927	862	486	961
5	934	159	625	724	619	529	168	946	927	862	486	961
6	318	451	549	142	619	529	168	946	927	862	486	961
7	934	159	549	142	619	529	168	946	927	862	486	961
8	934	159	549	142	619	529	168	946	927	862	486	961
9	934	159	549	142	619	529	168	946	927	862	486	961
10	934	159	549	142	406	790	759	326	293	185	486	961
11	318	451	549	142	157	790	168	946	185	536	486	961
12	934	159	625	724	619	529	168	946	927	862	486	961
13	934	159	625	724	406	157	168	946	927	862	486	961
14	934	159	625	724	406	157	168	946	927	862	486	961
15	318	451	549	142	619	529	168	946	927	862	486	961
16	934	159	625	549	406	157	168	946	927	862	486	961
17	934	159	625	724	406	157	168	946	927	862	486	961
18	318	451	549	142	406	790	759	326	927	185	486	961
19	934	159	625	724	619	529	168	946	293	536	486	961

Panelist	#1		#2		#3		#4		#5		#6	
20	934	159	549	142	619	529	168	946	927	862	486	961
21	934	159	549	142	406	790	168	946	293	536	486	961
22	934	159	625	724	619	529	168	946	927	862	486	961
23	934	159	625	219	406	157	168	946	185	536	486	961
24	934	159	549	142	406	790	759	326	293	185	375	251
25	934	159	625	219	406	157	168	946	185	536	486	961
26	934	159	625	219	406	157	168	946	185	536	486	961
27	934	159	625	219	406	157	168	326	293	862	486	961
28	934	159	625	142	406	157	168	946	293	536	486	961
29	934	159	549	142	157	790	168	759	293	185	486	961
30	318	451	549	142	406	790	759	326	185	536	375	251
31	934	159	625	549	157	790	759	326	293	536	486	961
32	934	159	724	219	619	529	168	946	927	862	486	961
33	934	159	625	219	406	790	168	759	293	862	486	961
34	934	159	549	142	406	157	168	946	927	862	375	251
35	307	318	625	219	406	790	759	326	927	862	592	251
36	934	159	625	549	529	790	759	326	293	185	486	961
37	318	451	625	549	406	790	168	946	927	862	486	961
38	934	159	549	142	157	790	168	946	293	185	486	961
39	934	159	625	549	406	157	759	326	293	185	486	961

Panelist	#1		#2		#3		#4		#5		#6	
40	934	159	549	142	619	529	168	946	927	862	486	961
41	934	159	625	724	619	529	168	946	927	862	486	961
42	934	159	549	142	619	529	168	946	927	862	486	961
43	934	159	549	142	619	529	168	946	927	862	486	961
44	934	159	724	219	619	529	168	946	927	862	486	961
45	934	159	625	724	619	529	168	946	927	862	486	961
46	934	159	625	724	619	529	168	946	927	862	486	961
47	934	159	625	724	619	529	168	946	927	862	486	961
48	934	159	549	142	619	529	168	946	927	862	486	961
49	934	159	625	724	619	529	168	946	927	862	486	961
50	934	159	549	142	619	529	168	946	927	862	486	961

Correct Answer	43	25	26	39	32	46
%	86.00	50.00	52.00	78.00	64.00	92.00

Appendix 7 – Performance of pH indicator

Table 7.1: pH value and total plate count of fermented fish, and color value of pH indicator, during storage

Sample	Properties		Day 0	Day 3	Day 5
Fermented fish	pH	1	6.63	4.69	4.03
		2	6.32	4.72	3.51
		3	6.92	4.24	3.5
		Ave. ± S.D.	6.62±0.30	4.55±0.27	3.68±0.30
	TPC (log CFU/g)	1	4.53	7.21	8.57
		2	4.73	7.47	8.89
		3	4.80	7.42	9.03
		Ave. ± S.D.	4.69±0.14	7.37±0.14	8.83±0.23
Indicator with red cabbage extract	L*	1	25.94	28.64	37.12
		2	30.30	32.03	36.35
		3	26.78	39.81	36.70
		Ave. ± S.D.	27.67±2.31	33.49±5.73	36.72±0.39
	a*	1	32.06	37.75	43.88
		2	34.43	37.29	40.62
		3	26.10	30.46	42.38
		Ave. ± S.D.	30.86±4.29	35.17±4.08	42.29±1.63
	b*	1	12.45	9.18	17.65
		2	10.48	8.44	17.66
		3	8.37	13.80	18.96
		Ave. ± S.D.	10.43±2.04	10.47±2.90	18.09±0.75
Indicator with Dendrobium orchid extract	L*	1	44.01	46.32	44.61
		2	38.25	37.66	45.34
		3	56.14	42.17	41.90
		Ave. ± S.D.	46.13±9.13	42.05±4.33	43.95±1.81
	a*	1	44.83	43.19	46.39
		2	40.47	48.35	47.16
		3	31.99	45.68	47.85
		Ave. ± S.D.	39.10±6.53	45.74±2.58	47.13±0.73
	b*	1	-9.42	5.02	20.26

Sample	Properties		Day 0	Day 3	Day 5
		2	-7.95	11.85	20.77
		3	-7.24	7.40	22.64
		Ave. ± S.D.	-8.20±1.11	8.09±3.47	21.22±1.25
Indicator with butterfly pea extract	L*	1	20.56	33.34	33.80
		2	21.57	26.78	25.78
		3	34.48	23.93	30.08
		Ave. ± S.D.	25.54±7.76	28.02±4.83	29.89±4.01
	a*	1	4.06	7.40	14.85
		2	4.38	8.11	14.65
		3	4.30	11.94	12.36
		Ave. ± S.D.	4.25±0.17	9.15±2.44	13.95±1.38
	b*	1	-10.97	-12.55	-10.13
		2	-12.06	-13.24	-9.12
		3	-12.98	-16.36	-10.11
		Ave. ± S.D.	-12.00±1.01	-14.05±2.03	-9.79±0.58

Table 7.2: pH value and total plate count of egg tofu, and color value of pH indicator, during storage

Sample	Properties		Day 1	Day 7
Egg tofu	pH	1	7.59	6.22
		2	7.64	5.72
		3	7.23	6.63
		Ave. ± S.D.	7.49±0.22	6.19±0.46
	TPC (log CFU/g)	1	0.00	3.71
		2	0.00	3.83
		3	1.48	3.95
		Ave. ± S.D.	0.49±0.85	3.83±0.12
Indicator with red cabbage extract	L*	1	29.75	48.09
		2	42.98	40.49
		3	50.68	41.66
		Ave. ± S.D.	41.14±10.59	43.41±4.09
	a*	1	11.33	32.65
		2	9.25	34.94
		3	8.46	40.23
		Ave. ± S.D.	9.68±1.48	35.94±3.89
	b*	1	-4.46	21.44
		2	0.54	25.71
		3	2.12	25.76
		Ave. ± S.D.	-0.60±3.43	24.30±2.48
Indicator with Dendrobium orchid extract	L*	1	21.85	28.54
		2	33.10	29.22
		3	23.64	33.59
		Ave. ± S.D.	26.20±6.05	30.45±2.74
	a*	1	32.55	43.20
		2	33.24	43.65
		3	33.43	45.36
		Ave. ± S.D.	33.07±0.46	44.07±1.14
	b*	1	-19.50	9.49
		2	-20.67	9.10
		3	-19.58	9.75

Sample	Properties		Day 1	Day 7
		Ave. ± S.D.	-19.92±0.65	9.45±0.33
Indicator with butterfly pea extract	L*	1	34.64	21.07
		2	27.96	39.48
		3	30.53	22.14
		Ave. ± S.D.	31.04±3.37	27.56±10.33
	a*	1	-2.24	4.65
		2	2.78	5.55
		3	1.30	5.82
		Ave. ± S.D.	0.61±2.58	5.34±0.61
	b*	1	2.53	-3.45
		2	-5.22	0.03
		3	-5.92	-4.04
		Ave. ± S.D.	-2.87±4.69	-2.49±2.20

Table 7.3: pH value and total plate count of fermented mushroom, and color value of pH indicator, during storage

Sample	Properties		Day 0	Day 3	Day 9
Fermented mushroom	pH	1	7.27	4.50	4.34
		2	7.02	4.48	4.23
		3	7.04	4.35	4.15
		Ave. ± S.D.	7.11±0.14	4.44±0.08	4.24±0.10
	TPC (log CFU/g)	1	4.41	7.41	10.67
		2	4.59	7.51	10.79
		3	4.88	7.60	10.79
		Ave. ± S.D.	4.63±0.23	7.51±0.10	10.75±0.07
Indicator with red cabbage extract	L*	1	28.84	22.65	39.51
		2	30.22	31.14	29.62
		3	29.70	33.82	34.88
		Ave. ± S.D.	29.59±0.70	29.20±5.83	34.67±4.95
	a*	1	26.37	25.24	63.07
		2	24.15	53.78	27.01
		3	26.89	19.41	68.64
		Ave. ± S.D.	25.80±1.46	32.81±18.39	52.91±22.60
	b*	1	12.86	17.32	47.23
		2	16.46	38.25	9.75
		3	16.03	11.50	56.40
		Ave. ± S.D.	15.12±1.97	22.36±14.07	37.79±24.72
Indicator with Dendrobium orchid extract	L*	1	29.68	22.16	29.27
		2	21.24	35.30	36.95
		3	23.20	41.53	28.32
		Ave. ± S.D.	24.71±4.42	33.00±9.89	31.51±4.73
	a*	1	23.03	33.45	28.42
		2	23.05	46.00	60.45
		3	24.75	35.69	27.08
		Ave. ± S.D.	23.61±0.99	38.38±6.69	38.65±18.89
	b*	1	-2.94	-1.34	3.48
		2	0.86	4.10	21.70
		3	-3.29	-2.72	2.79

		Ave. ± S.D.	-1.79±2.30	0.01±3.61	9.32±10.72
Indicator with butterfly pea extract	L*	1	39.27	35.86	44.20
		2	30.87	37.94	34.59
		3	39.34	24.09	33.18
		Ave. ± S.D.	36.49±4.87	32.63±7.47	37.32±6.00
	a*	1	-2.61	6.55	9.15
		2	2.04	7.40	8.02
		3	-1.06	7.28	10.42
		Ave. ± S.D.	-0.54±2.37	7.08±046	9.20±1.20
	b*	1	-2.08	-6.30	-8.41
		2	-7.15	-6.61	-9.44
		3	-5.48	-7.73	-5.12
		Ave. ± S.D.	-4.90±2.58	-6.88±0.75	-7.66±2.26

Table 7.4: pH value of fruit and color value of pH indicator

Sample	Properties		Lemon	Yellow kiwi
	pH	1	2.35	3.03
		2	2.23	3.67
		3	2.51	3.11
		Ave. ± S.D.	2.36±0.14	3.27±0.35
Indicator with red cabbage extract	L*	1	27.61	41.34
		2	41.28	20.74
		3	43.14	43.90
		Ave. ± S.D.	37.34±8.48	35.33±12.70
	a*	1	43.77	43.25
		2	56.05	36.98
		3	58.60	40.42
		Ave. ± S.D.	52.81±7.93	40.22±3.14
	b*	1	26.34	33.60
		2	34.93	29.34
		3	38.11	30.70
		Ave. ± S.D.	33.13±6.09	31.21±2.18
Indicator with Dendrobium orchid extract	L*	1	37.38	38.02
		2	37.65	36.69
		3	40.20	39.58
		Ave. ± S.D.	38.41±1.56	38.10±1.45
	a*	1	50.71	60.27
		2	49.04	55.40
		3	49.52	56.89
		Ave. ± S.D.	49.76±0.86	57.52±2.50
	b*	1	45.26	38.03
		2	45.14	32.75
		3	45.75	30.64
		Ave. ± S.D.	45.38±0.32	33.81±3.81
Indicator with butterfly pea extract	L*	1	31.50	36.73
		2	54.00	31.68
		3	33.57	36.25
		Ave. ± S.D.	39.69±12.44	34.89±2.79

Sample	Properties		Lemon	Yellow kiwi
	a*	1	15.78	3.07
		2	21.06	8.64
		3	20.45	8.29
		Ave. ± S.D.	19.10±2.89	6.67±3.12
	b*	1	0.22	-9.81
		2	3.23	-9.29
		3	2.08	-9.14
		Ave. ± S.D.	1.84±1.52	-9.41±0.35

Table 7.5: pH value and total plate count of whole coconut fruit, and color value of pH indicator, during storage

Sample	Properties		Day 1	Day 3	Day 5
Coconut fruit	pH	1	6.45	5.57	4.57
		2	6.54	5.80	4.75
		3	6.04	5.96	4.82
		Ave. ± S.D.	6.34±0.27	5.78±0.20	4.71±0.13
	TPC (log CFU/g)	1	2.18	2.18	5.30
		2	1.78	3.60	6.79
		3	1.00	3.96	6.93
		Ave. ± S.D.	1.65±0.60	3.25±0.95	6.34±0.90
Indicator with red cabbage extract	L*	1	30.16	43.20	48.92
		2	41.48	49.67	55.06
		3	43.05	38.96	47.38
		Ave. ± S.D.	38.23±7.03	43.94±5.39	50.45±4.06
	a*	1	19.76	31.51	10.57
		2	18.60	26.60	4.16
		3	19.34	30.74	11.33
		Ave. ± S.D.	19.23±0.59	29.62±2.64	8.69±3.94
	b*	1	2.39	11.63	11.08
		2	3.54	10.22	4.91
		3	2.80	13.37	11.74
		Ave. ± S.D.	2.91±0.58	11.74±1.58	9.24±3.77
Indicator with Dendrobium orchid extract	L*	1	27.53	42.32	41.11
		2	30.87	48.06	37.05
		3	39.96	41.75	49.72
		Ave. ± S.D.	32.79±6.43	44.04±3.49	42.63±6.47
	a*	1	34.08	25.78	21.90
		2	31.02	19.50	24.77
		3	23.31	26.43	16.85
		Ave. ± S.D.	29.47±5.55	23.90±3.83	21.17±4.01
	b*	1	7.27	8.14	7.49
		2	7.34	9.03	7.03
		3	6.50	8.68	5.34

Sample	Properties		Day 1	Day 3	Day 5
		Ave. ± S.D.	7.04±0.47	8.62±0.45	6.62±1.13
Indicator with butterfly pea extract	L*	1	28.90	30.66	33.18
		2	22.18	31.04	40.61
		3	27.32	42.27	36.48
		Ave. ± S.D.	26.13±3.51	34.66±6.60	36.76±3.72
	a*	1	0.05	3.18	5.34
		2	5.40	3.94	4.78
		3	2.08	1.06	5.69
		Ave. ± S.D.	2.51±2.70	2.73±1.49	5.27±0.46
	b*	1	-5.46	-1.24	-1.82
		2	-4.35	0.23	1.66
		3	-3.98	3.95	1.43
		Ave. ± S.D.	-4.60±0.77	0.98±2.68	0.42±1.95

Table 7.6: pH value and total plate count of sweet orange, and color value of pH indicator, during storage

Sample	Properties		Day 1	Day 3	Day 5
Sweet orange	pH	1	5.48	5.40	4.56
		2	5.06	5.08	4.87
		3	5.86	5.71	4.67
		Ave. ± S.D.	5.47±0.40	5.40±0.32	4.70±0.16
	TPC (log CFU/g)	1	4.71	6.29	7.37
		2	4.76	6.30	6.99
		3	4.80	6.25	7.64
		Ave. ± S.D.	4.75±0.05	6.28±0.03	7.34±0.33
Indicator with red cabbage extract	L*	1	37.50	30.18	38.45
		2	38.26	37.23	31.72
		3	38.15	33.39	43.86
		Ave. ± S.D.	37.97±0.41	33.60±3.53	38.01±6.08
	a*	1	23.59	21.46	15.80
		2	23.80	11.09	19.06
		3	22.57	15.44	11.19
		Ave. ± S.D.	23.32±0.66	16.00±5.21	15.35±3.95
	b*	1	9.07	7.35	11.24
		2	10.94	12.42	9.55
		3	12.80	11.87	11.37
		Ave. ± S.D.	10.94±1.87	10.55±2.78	10.72±1.02
Indicator with Dendrobium orchid extract	L*	1	19.98	15.23	21.34
		2	30.41	21.70	21.77
		3	26.33	20.05	17.85
		Ave. ± S.D.	25.57±5.26	18.99±3.36	20.32±2.15
	a*	1	22.05	27.14	26.80
		2	26.74	24.89	19.43
		3	31.08	25.19	21.52
		Ave. ± S.D.	26.62±4.52	25.74±1.22	22.58±3.80
	b*	1	9.76	5.29	9.40
		2	7.92	5.34	7.67
		3	10.48	6.75	10.75

Sample	Properties		Day 1	Day 3	Day 5
		Ave. ± S.D.	9.39±1.32	5.79±0.83	9.27±1.54
Indicator with butterfly pea extract	L*	1	31.24	23.08	34.65
		2	34.38	26.89	30.25
		3	35.70	31.66	38.81
		Ave. ± S.D.	33.77±2.29	27.21±4.30	34.57±4.28
	a*	1	4.72	2.45	2.49
		2	3.45	2.76	2.91
		3	3.26	1.09	3.20
		Ave. ± S.D.	3.81±0.79	2.10±0.89	2.87±0.36
	b*	1	-3.02	0.06	0.14
		2	-4.76	-1.05	2.36
		3	-5.35	0.30	3.54
		Ave. ± S.D.	-4.38±1.21	-0.23±0.72	2.01±1.73

Table 7.7: pH value and total plate count of pineapple, and color value of pH indicator, during storage

Sample	Properties		Day 1	Day 3	Day 5
pineapple	pH	1	4.55	4.49	4.32
		2	4.68	4.58	4.48
		3	4.02	3.98	3.75
		Ave. ± S.D.	4.42±0.35	4.35±0.32	4.18±0.38
	TPC (log CFU/g)	1	3.30	5.27	7.00
		2	4.00	5.32	6.67
		3	4.11	5.50	7.37
		Ave. ± S.D.	3.80±0.44	5.36±0.12	7.01±0.35
Indicator with red cabbage extract	L*	1	29.46	31.95	26.43
		2	33.88	28.27	36.21
		3	33.67	41.00	26.37
		Ave. ± S.D.	32.34±2.49	33.74±6.55	29.67±5.66
	a*	1	31.18	29.64	27.20
		2	30.54	28.46	19.06
		3	30.04	19.47	26.58
		Ave. ± S.D.	30.59±0.57	25.86±5.56	24.28±4.53
	b*	1	19.55	19.03	16.57
		2	18.03	17.78	14.15
		3	16.22	13.21	17.06
		Ave. ± S.D.	17.93±1.67	16.67±3.06	15.93±1.56
Indicator with Dendrobium orchid extract	L*	1	23.17	25.67	25.93
		2	23.49	25.33	27.94
		3	29.31	25.09	25.76
		Ave. ± S.D.	25.32±3.46	25.36±0.29	26.54±1.21
	a*	1	30.97	34.25	33.05
		2	30.45	32.01	33.75
		3	27.06	34.64	33.61
		Ave. ± S.D.	29.49±2.12	33.63±1.42	33.47±0.37
	b*	1	9.65	11.08	13.78
		2	9.15	12.27	13.06
		3	7.74	10.84	11.20

Sample	Properties		Day 1	Day 3	Day 5
		Ave. ± S.D.	8.85±0.99	11.40±0.77	12.68±1.33
Indicator with butterfly pea extract	L*	1	40.15	32.58	39.31
		2	36.89	31.33	32.45
		3	34.56	36.00	46.96
		Ave. ± S.D.	37.20±2.81	33.30±2.42	39.57±7.26
	a*	1	5.28	5.40	7.52
		2	3.21	7.29	8.45
		3	4.62	5.75	4.02
		Ave. ± S.D.	4.37±1.06	6.15±1.01	6.66±2.34
	b*	1	-2.07	3.56	0.24
		2	-2.73	1.40	-3.74
		3	-3.06	4.05	1.15
		Ave. ± S.D.	-2.62±0.50	3.00±1.41	-0.78±2.60

Table 7.8: pH value and total plate count of watermelon, and color value of pH indicator, during storage

Sample	Properties		Day 1	Day 3	Day 5
Watermelon	pH	1	5.74	5.70	4.75
		2	5.23	5.21	4.59
		3	5.39	5.35	4.58
		Ave. ± S.D.	5.45±0.26	5.42±0.25	4.64±0.10
	TPC (log CFU/g)	1	4.38	6.34	8.21
		2	4.49	5.98	8.27
		3	4.51	6.03	8.16
		Ave. ± S.D.	4.46±0.07	6.12±0.19	8.21±0.05
Indicator with red cabbage extract	L*	1	25.07	21.52	24.36
		2	27.55	35.41	31.01
		3	28.30	24.85	25.05
		Ave. ± S.D.	26.97±1.69	27.26±7.25	26.81±3.66
	a*	1	33.05	26.74	26.02
		2	31.60	21.38	19.43
		3	31.36	24.05	27.59
		Ave. ± S.D.	32.00±0.91	24.06±2.68	24.35±4.33
	b*	1	15.94	11.69	18.49
		2	16.02	7.05	15.75
		3	17.86	12.44	18.82
		Ave. ± S.D.	16.61±1.09	10.39±2.92	17.69±1.69
Indicator with Dendrobium orchid extract	L*	1	26.10	22.89	29.12
		2	26.88	31.24	38.04
		3	28.45	23.83	32.19
		Ave. ± S.D.	27.14±1.20	25.99±4.57	33.12±4.53
	a*	1	23.65	23.76	33.57
		2	22.07	18.95	22.64
		3	21.92	22.61	28.40
		Ave. ± S.D.	22.55±0.96	21.77±2.51	28.20±5.47
	b*	1	6.75	10.03	18.06
		2	6.13	11.66	15.29
		3	10.57	7.49	15.31

Sample	Properties		Day 1	Day 3	Day 5
		Ave. ± S.D.	7.82±2.40	9.73±2.10	16.22±1.59
Indicator with butterfly pea extract	L*	1	29.78	21.22	35.95
		2	35.98	27.59	29.32
		3	36.06	35.24	39.75
		Ave. ± S.D.	33.94±3.60	28.02±7.02	35.01±5.28
	a*	1	3.99	3.15	2.67
		2	4.48	2.30	2.43
		3	2.27	2.09	3.11
		Ave. ± S.D.	3.58±1.16	2.51±0.56	2.74±0.34
	b*	1	-4.35	-2.29	2.41
		2	-4.80	-3.08	0.60
		3	-6.01	1.85	6.83
		Ave. ± S.D.	-5.05±0.86	-1.17±2.65	3.28±3.20

Appendix 8 – pH sensitivity of developed pH indicator during storage

Table A8.1: Color values of pH indicator with red cabbage extract at pH = 3, 5, and 7, during storage at 25ºC

Week		pH = 3			pH = 5			pH = 7		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
0	1	34.29	51.93	21.07	36.29	35.70	-0.81	36.76	25.86	-4.17
	2	36.35	53.27	20.51	34.71	35.26	-0.34	37.11	25.62	-3.43
	3	35.77	52.53	20.81	34.63	35.96	0.43	37.29	25.35	-3.63
	Ave. ± S.D.	35.47±1.06	52.57±0.67	20.80±0.28	35.21±0.94	35.64±0.36	-0.24±0.63	37.05±0.27	25.61±0.26	-3.74±0.39
2	1	33.17	54.08	20.98	33.81	36.82	-0.59	35.86	26.38	-2.70
	2	32.39	55.38	20.54	32.65	36.24	0.28	36.15	26.52	-3.53
	3	31.64	52.98	20.40	32.64	37.70	-0.19	37.01	25.44	-3.80
	Ave. ± S.D.	32.39±0.76	54.14±1.20	20.64±0.30	33.03±0.67	36.92±0.74	-0.17±0.44	36.34±0.60	26.11±0.58	-3.34±0.58
3	1	30.92	54.39	22.72	28.27	36.90	-0.03	35.24	27.46	-3.52
	2	30.27	53.62	22.17	28.23	34.30	-1.24	34.29	26.68	-3.51
	3	29.81	53.69	21.49	30.60	39.25	0.54	32.44	25.41	-3.29
	Ave. ± S.D.	30.33±0.55	53.90±0.43	22.13±0.62	29.03±1.35	36.82±2.48	-0.24±0.91	33.99±1.43	26.52±1.03	-3.44±0.13
4	1	26.11	49.80	25.87	23.41	37.04	-1.28	30.26	27.12	-4.61
	2	25.67	49.79	23.73	28.50	34.70	1.08	32.21	28.81	-4.12
	3	25.23	50.94	24.85	24.49	36.21	-0.74	30.01	25.26	-3.32
	Ave. ± S.D.	25.67±0.44	50.18±0.66	24.82±1.07	25.46±2.68	35.99±1.19	-0.31±1.24	30.83±1.21	27.06±1.77	-4.02±0.65

Table A8.2: Color values of pH indicator with Dendrobium orchid extract at pH = 3, 5, and 7, during storage at 25ºC

Week		pH = 3			pH = 5			pH = 7		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
0	1	42.98	63.79	30.94	29.97	47.30	11.68	20.02	34.12	-6.76
	2	41.83	62.85	32.48	31.71	52.00	21.09	20.31	33.59	-7.53
	3	41.64	61.92	31.76	32.81	53.88	22.30	19.13	33.59	-7.79
	Ave. ± S.D.	42.15±0.72	62.85±0.93	31.73±0.77	31.50±1.43	51.06±3.39	18.35±5.81	19.82±0.61	33.77±0.31	-7.36±0.54
2	1	42.45	63.82	31.21	30.52	53.01	17.01	16.63	32.15	-8.04
	2	38.22	59.88	33.27	32.04	49.61	17.26	19.37	34.86	-4.43
	3	44.08	64.59	28.51	28.52	54.07	21.30	15.90	33.11	-8.85
	Ave. ± S.D.	41.58±3.03	62.76±2.53	30.99±2.39	30.36±1.77	52.23±2.33	18.52±2.41	17.30±1.83	33.37±1.38	-7.11±2.35
4	1	39.09	64.21	32.58	28.03	57.69	11.11	14.63	31.08	-9.15
	2	35.90	61.40	30.01	26.54	47.75	17.45	15.64	31.68	-7.45
	3	43.46	64.20	28.70	35.63	53.90	23.47	15.40	34.09	-6.35
	Ave. ± S.D.	39.48±3.80	63.27±1.62	30.43±1.98	30.07±4.87	53.12±5.02	17.35±6.18	15.23±0.53	32.28±1.59	-7.65±1.41
5	1	36.26	62.36	28.80	27.05	50.59	17.09	13.88	27.63	-7.88
	2	34.70	59.69	33.02	33.55	51.90	17.81	16.90	30.83	-7.41
	3	39.44	64.66	29.00	23.69	55.93	15.38	11.39	28.54	-6.19
	Ave. ± S.D.	36.80±2.41	62.24±2.49	30.27±2.38	28.10±5.01	52.81±2.78	16.76±1.25	14.06±2.76	29.00±1.65	-7.16±0.87

Table A8.3: Color values of pH indicator with butterfly pea extract at pH = 3, 5, and 7, during storage at 25ºC

Week		pH = 3			pH = 5			pH = 7		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
0	1	32.92	14.79	-14.55	25.91	8.46	-15.36	36.00	-4.91	-16.87
	2	33.78	15.96	-15.56	27.06	7.97	-15.81	36.31	-4.96	-16.45
	3	33.54	15.99	-14.65	28.33	8.10	-15.37	33.72	-4.86	-17.13
	Ave. ± S.D.	33.41±0.45	15.58±0.68	-14.92±0.56	27.10±1.21	8.18±0.26	-15.51±0.26	35.34±1.42	-4.91±0.05	-16.81±0.34
2	1	32.27	15.15	-14.75	26.11	7.53	-14.64	34.99	-5.21	-15.79
	2	33.02	15.51	-15.91	26.24	7.53	-15.23	37.54	-4.54	-16.68
	3	34.12	16.90	-16.24	26.53	8.47	-15.23	36.25	-3.72	-16.44
	Ave. ± S.D.	33.14±0.93	15.85±0.92	-15.63±0.79	26.29±0.22	7.84±0.54	-15.03±0.34	36.26±1.28	-4.45±0.75	-16.30±0.46
4	1	32.82	15.64	-15.35	24.34	7.81	-14.80	38.62	-5.25	-15.73
	2	31.32	16.14	-15.65	23.87	7.28	-14.92	37.76	-4.65	-15.28
	3	30.79	16.05	-16.25	26.46	8.37	-15.45	37.35	-3.22	-16.85
	Ave. ± S.D.	31.64±1.05	15.94±0.26	-15.75±0.46	24.89±1.38	7.82±0.55	-15.05±0.35	37.91±0.65	-4.37±1.04	-15.95±0.81
6	1	25.43	15.62	-16.66	24.44	8.80	-15.36	34.29	-4.77	-16.40
	2	27.49	18.57	-17.36	25.04	9.06	-15.72	36.53	-5.41	-14.75
	3	26.54	19.01	-16.15	27.86	9.34	-15.13	33.75	-2.17	-15.22
	Ave. ± S.D.	26.49±1.03	17.73±1.84	-16.72±0.61	25.78±1.82	9.07±0.27	-15.40±0.30	34.86±1.47	-4.12±1.72	-15.46±0.85
7	1	23.85	18.05	-18.26	25.57	9.96	-15.36	31.48	-4.11	-16.25
	2	23.27	18.49	-18.57	23.71	9.95	-15.81	32.13	-3.31	-15.07
	3	21.69	18.08	-17.25	24.97	11.33	-15.37	31.63	-3.06	-14.96
	Ave. ± S.D.	22.94±1.12	18.21±0.24	-18.03±0.69	24.75±0.95	10.41±0.79	-15.51±0.26	31.75±0.34	-3.49±0.55	-15.43±0.71

Research output (ผลผลิตของงานวิจัย)

Expected Output 1 – ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

ผลงานตามที่คาดไว้ในสัญญาโครงการของโครงการวิจัยนี้ ได้แก่ ผลงานตีพิมพ์ในวารสารวิชาการ จำนวน 2 เรื่อง ซึ่งมีรายละเอียดดังนี้

1.1. ชื่อเรื่อง (อาจมีการเปลี่ยนแปลง):

Development of Colorimetric Bio-based Indicator from Dendrobium Orchid (*Dendrobium* Sonia 'Earsaku')

ชื่อวารสารนานาชาติ: Packaging Technology and Science

ความคืบหน้าของงาน: ในขณะนี้ ได้ผลการทดลองที่เกี่ยวข้องกับการเขียน manuscript ทั้งหมดแล้ว คาดว่าจะสามารถส่งต้นฉบับสมบูรณ์ให้กับผู้ร่วมวิจัย ได้ภายในเดือน มิถุนายน พ.ศ. 2562

1.2. ชื่อเรื่อง (อาจมีการเปลี่ยนแปลง)

Development of Colorimetric Bio-based Indicator from Red Cabbage (*Brassica oleracea* var. *capitata* f *rubra*) and Butterfly pea (*Clitoria ternatea* L.)

ชื่อวารสารนานาชาติ: Packaging Technology and Science

ความคืบหน้าของงาน: ในขณะนี้ ได้ผลการทดลองที่เกี่ยวข้องกับการเขียน manuscript ทั้งหมดแล้ว คาดว่าจะสามารถส่งต้นฉบับสมบูรณ์ให้กับผู้ร่วมวิจัย ได้ภายในเดือน มิถุนายน พ.ศ. 2562

Output 2 – การนำผลงานวิจัยไปใช้ประโยชน์

ผลผลิตด้านการนำไปใช้ประโยชน์ของโครงการวิจัยนี้ ได้แก่

2.1. เชิงสาธารณะ ได้มีการสร้างเครือข่ายความร่วมมือด้านงานวิจัยที่เกี่ยวข้องกับบรรจุภัณฑ์ กับ School of Industrial Technology, Universiti Sains Malaysia ในรูปแบบของการผลิตงานวิจัยร่วมกัน และการแลกเปลี่ยนนักศึกษาระดับปริญญาตรี

2.2. เชิงวิชาการ ได้ส่งนักศึกษาระดับปริญญาตรี จำนวน 1 คน ได้แก่ น.ส.พิมนพัศย์ ศิรินุพงศ์ หลักสูตรเทคโนโลยีการอาหาร มหาวิทยาลัยมหิดล วิทยาเขตกาญจนบุรี ไปทำงานวิจัยในหัวข้อ “Development of Colorimetric Indicator for pH from Bio-based materials, and Red Cabbage (*Brassica oleracea* var. *capitata* f *rubra*) and Dendrobium orchid (*Dendrobium* Sonia ‘Earsaku’)” ภายใต้การดูแลของ Assistant Professor Hayati Samsudin, School of Industrial Technology, Universiti Sains Malaysia ในระหว่างวันที่ 19 เมษายน – 31 พฤษภาคม พ.ศ. 2560 (เอกสารแนบ O2.1)

Output 3 – อื่น ๆ

ผลผลิตอื่นๆ ของโครงการวิจัยนี้ ได้แก่

3.1. การนำเสนอผลงานในที่ประชุมวิชาการ The 2nd CU-MU Juint Symposium 2018 ที่มีขึ้นในระหว่างวันที่ 11-12 ตุลาคม พ.ศ. 2561 ณ Chiba University ประเทศญี่ปุ่น ในหัวข้อ “Farm to Fork: Reducing Food Wastage along the Agricultural Product’s Value Chain” (เอกสารแนบ O3.1)

3.2. Full paper proceeding และการนำเสนอผลงานในที่ประชุมวิชาการ Food Innovation Asia Conference 2019 ที่จะมีขึ้นในระหว่างวันที่ 13-15 มิถุนายน พ.ศ. 2562 ณ ศูนย์ประชุม BITEC กรุงเทพมหานคร ในหัวข้อ “Enhanced Extraction of Anthocyanins from Red Cabbage (*Brassica oleraces*) Using Microwave Assisted Extraction” ซึ่งอยู่ในระหว่างการแก้ไข manuscript ในลักษณะ Minor Revision (รายละเอียด ดังเอกสารแนบ O3.1) โดยได้ทำการส่ง manuscript ฉบับแก้ไขไปแล้วในวันที่ 25 เมษายน พ.ศ. 2562 (เอกสารแนบ O3.2)

นางสาว พิมนพัศษ์ ศิรินุพงศ์

นักศึกษาหลักสูตรเทคโนโลยีการอาหาร ชั้นปีที่ 4

ได้รับทุนโครงการทุนแลกเปลี่ยนด้านวิชาการ ณ ต่างประเทศ สำหรับนักศึกษาระดับปริญญาตรีของมหาวิทยาลัยมหิดล ประจำปี 2560 (Mahidol University Scholarships for Undergraduate Student Exchange Program 2017) โดยเดินทางเข้าร่วมกิจกรรมแลกเปลี่ยน ณ Universiti Sains Malaysia ประเทศมาเลเซีย ระหว่างวันที่ 19 เมษายน – 31 พฤษภาคม 2560 รวม 43 วัน

โดยไปทำงานวิจัยระดับปริญญาตรีในหัวข้อเรื่อง Development of Colorimetric Indicator for pH from Bio-based materials, and Red Cabbage (Brassica oleraceavar.capitatafrubra) and Dendrobium orchid (Dendrobium Sonia 'Earsaku') (การพัฒนาฟิล์มบ่งชี้ค่าความเป็นกรด-ด่างจากกะหล่ำปลีม่วงและกล้วยไม้หวาย)ภายใต้การดูแลของ Prof. Hayati Samsudin, School of Industrial Technology, Universiti Sains Malaysia ร่วมด้วย ดร. สิริยุภา เนตรมัย และ ดร. ฐิติศิลป์ กิจเชวงกุล หลักสูตรเทคโนโลยีการอาหาร สาขาวิชาอุตสาหกรรมอาหาร มหาวิทยาลัยมหิดล วิทยาเขตกาญจนบุรี

Mahidol University Kanchanaburi Campus

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" Wisdom Of The Land " นักศึกษาหลักสูตรFT ได้รับทุนแลกเปลี่ยนด้านวิชาการไปประเทศมาเลเซีย

นางสาว พิมนพัศษ์ ศิรินุพงศ์ นักศึกษาชั้นปีที่ 4 หลักสูตรเทคโนโลยีการอาหาร มหาวิทยาลัยมหิดล วิทยาเขตกาญจนบุรี ได้รับทุนโครงการทุนแลกเปลี่ยนด้านวิชาการ ณ ต่างประเทศ สำหรับนักศึกษาระดับปริญญาตรีของมหาวิทยาลัยมหิดล ประจำปี 2560 (Mahidol University Scholarships for Undergraduate Student Exchange Program 2017) โดยเดินทางเข้าร่วมกิจกรรมแลกเปลี่ยน ณ Universiti Sains Malaysia ประเทศมาเลเซีย ระหว่างวันที่ 19 เมษายน – 31 พฤษภาคม 2560 รวม 43 วัน
ทั้งนี้ นักศึกษาได้ไปทำงานวิจัยระดับปริญญาตรีในหัวข้อเรื่อง Development of Colorimetric Indicator for pH from Bio-based materials, and Red Cabbage (Brassica oleraceavar.capitatafrubra) and Dendrobium orchid (Dendrobium Sonia 'Earsaku') (การพัฒนาฟิล์มบ่งชี้ค่าความเป็นกรด-ด่างจากกะหล่ำปลีม่วงและกล้วยไม้หวาย)
ภายใต้การดูแลของ Prof. Hayati Samsudin, School of Industrial Technology, Universiti Sains Malaysia ร่วมด้วย ดร. สิริยุภา เนตรมัย และ ดร. ฐิติศิลป์ กิจเชวงกุล หลักสูตรเทคโนโลยีการอาหาร สาขาวิชาอุตสาหกรรมอาหาร มหาวิทยาลัยมหิดล วิทยาเขตกาญจนบุรี

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10th September, 2018

Invitation to participate in "The 2nd CU-MU Joint Symposium 2018" เอกสารแนบ O3.1

Dear Assoc. Prof. Kanyaratt Supaibulwattana,

It is our great honor to invite you and your colleagues as the representative from Mahidol University from 10th to 13th October 2018 to participate in "The 2nd CU-MU Joint Symposium 2018", during 11th -12th October 2018 at Graduate School of Horticulture in Matsudo campus and Center for Environment, Health and Field Sciences in Kashiwanoha campus of Chiba University.

In accordance with the initiative of CU-MU Joint Symposium 2018, the goals of the symposium are to promote technology and innovation for food production and agriculture in Asia, to provide an opportunity for participants to exchange knowledge and experiences related to horticulture, as well as, to strengthen the collaboration on research and training center under the MoU between Faculty of Science, Mahidol University (MUSC) and Graduate School of Horticulture and Center for Environment, Health and Field Sciences, Chiba University (CUHORT).

On behalf of a local organizer, CUHORT is very pleased to invite you to be a Keynote speaker and your colleagues to attend the symposium.
Please kindly find the attached name list below:

Asst. Prof. Aussanee Pichakam
Asst. Prof. Charturong Chanseetis
Asst. Prof. Watcharra Chintakovid
Asst. Prof. Panida Kongsawadworakul
Dr. Siriyupa Netramai
Dr. Thitisilp Kijchavengkul
Ms. Rungrat Suriyin

We are looking forward to welcoming you at Chiba University.

Sincerely yours,

T. Kobayashi

Tatsuaki Kobayashi
Dean, Professor
Graduate School of Horticulture
Chiba University, Japan

Farm to Fork: Reducing Food Wastage along the Agricultural Product's Value Chain

เอกสารแนบ O3.1

Siriyupa Netramai[1], Thitisilp Kijchavengkul[1]

[1]*School of Bioinnovation and Bio-based Product Intelligence,*

Faculty of Science, Mahidol University, Thailand

Abstract. 100 – 200 words

Three research studies outlining different approaches to reduce food wastage along the agricultural product's value chain were discussed: 1) utilization of chlorine dioxide (ClO_2) gas in disinfecting pathogenic microorganisms during fumigation and as part of antimicrobial packaging for leafy greens, ensuring food safety and reducing microbial spoilage; 2) investigation on effects of distribution hazards and suitability of packaging system on quality and shelf-life of food products delivered via Thailand postal service, outlining potential factors that could cause food loss during multiple-day, ground delivery in Thailand; and 3) development of bio-based materials from agricultural produce and waste, reducing agricultural waste and lowering the use of non-renewable resources.

***Key words*:** Chlorine dioxide; Packaging; Parcel delivery; Bio-based material; Cultivated banana

Topic/Case Submission

1.	Ref No.	BPJ90 Admin...
	Topic	Enhanced Extraction of Anthocyanins from Red Cabbage (Brassica oleraces) Using Microwave Assisted Extraction
	Category	Division (B) Food Processing and Engineering
	Type	Poster - Asia-Pacific Journal of Science and Technology
	File upload	Folder for Full paper manuscript, Figures, and Abstracts (Thai and English) (zip) Full paper(docx)
	Status	Minor revision
	Edited file	Edited-Full Paper with track change(docx) Edited-Response to Reviewers(pdf) Edited-Full paper(docx) Edited-Abstract-ENG(docx) Edited-Abstract-TH(docx) Edit...

Abstract Guideline

- Use English language (American spelling and usage) and the SI system for measurements and units.
- The title should clearly define the topic and contain no abbreviations.
- List ALL authors. Spell out completely the names of authors, including first and last names.
- **Single paragraph abstract** is required.
- Abstract text **not exceeding 350 words**, define all acronyms and abbreviations; do not cite references.
- The abstract should be informative and detailed. The content of the abstract should contain:
  - Introduction:*explain the importance of the topic and the objective(s) of your work.*
  - Materials and Methods: *State what was done and how it was done.*
  - Results: *A summary of the main findings.* ***Abstract without any results will not be considered.***
  - Conclusion:*Indicate the significance and application of the research findings.*
  - Provide **up to 5 keywords** for indexing purposes
- **All Thai participants please provide abstract(s) both in English and Thai language.**

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Enhanced Extraction of Anthocyanins from Red Cabbage (*Brassica oleraces*) Using Microwave Assisted Extraction

Siriyupa Netramai[1*], Thitisilp Kijchavengkul[1], Hayati Samsudin[2], and Sittiwat Lertsiri[3]

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ABSTRACT: This study was aimed to 1) investigate and compare efficacy of conventional hot water extraction (HWE) and microwave assisted extraction (MAE) of anthocyanin-rich plant, *i.e.* red cabbage (*Brassica oleraces*); and 2) identify optimal extraction condition for MAE of red cabbage. Color extracts were obtained from fresh red cabbage through HWE at 80°C, for 0-180 min; and MAE using household microwave oven. The MAE process parameters included in the study were red cabbage to water ratio (1:5 to 1:3 g/mL), extraction power (480-800 W), and extraction time (120-480 s). The color extracts were determined their total monomeric anthocyanin content. Red cabbage extracts from HWE and MAE were slightly acidic and had λ_{max} of 553 nm. Mathematical model of red cabbage MAE was constructed using response surface methodology (R^2 = 0.8066). Red cabbage to water ratio and extraction time significantly influenced the efficacy of MAE ($P \leq 0.001$). The condition that gave color extract with highest anthocyanin content (1023.39±36.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage) was red cabbage to water ratio of 1:3 g/mL, extraction power of 800 W, and extraction time of 8 min. The use of MAE significantly reduced extraction time and increased yield (by 3.42%) of color extraction, as compared to those from HWE procedure ($P < 0.001$). Based on prediction profilers, it was possible that higher yield could be obtained at the higher extraction conditions. At 4°C storage, there was no significant change in anthocyanin level of the extract for the first 2 h of storage, but the anthocyanin level decreased to 418.42±255.78 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage after 12 h of storage ($P < 0.05$).

Keyword: Microwave assisted extraction; Anthocyanin, Red cabbage, RSM

INTRODUCTION

Utilization of natural pigments as coloring agents in local products has been practiced well before the invention of synthetic dyes; and, recently, the shifted preference of the consumers towards the use of natural ingredients has renewed and increased the demand of natural colorants, especially, for clothing, cosmetics, and food products [1]. Pigments can be extracted from various parts of plants, *e.g.* flower, leaf, fruit peel, tree bark, or root. The sources of colorants are often agricultural produce of low economic values or agricultural waste [2, 3].

Color extract from red cabbage (*Brassica oleraces*) has been used in various applications, for example, as coloring agent in food products, *e.g.* beverages, ice-cream, and confectioneries; as natural dye for textile items; or as pH indicator [3, 4]. Red cabbage contains high concentration of anthocyanins, more specifically, mono- or diacylated cyanidin anthocyanins. Color characteristics of anthocyanins are influenced by many factors, including their chemical composition and changes in pH of the environment [3, 4]. Ahmadiani *et al.* (2014) reported that anthocyanin content in 7 red cabbage cultivars ranged from 1000 to 1880 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage. At the harvest time of 13 and 21 weeks, color extracts from selected cultivars had similar λ_{max} at ~520 and ~610 nm, respectively. At different pH, color extracts from different harvest times were found to have colors similar to those of commercial coloring agent, *i.e.* FD&C Red No. 3 (week 13 extract, at pH 3.5) and FD&C Blue No. 2 (week 21 extract, at pH 7.0)0 [3]. Stability of anthocyanins depends on several factors, *e.g.* pH, processing and storage temperature, degree of complexation or copigmentation with other anthocyanins or other chemical species [5].

At household- or small-scale production level, conventional extraction method using hot water or acidic solution as solvent is often used to prepare crude color extracts from anthocyanin-rich plants. However, the method is time-consuming and usually gives low yield. Microwave assisted extraction (MAE) is an alternative extraction method that exposes the plant to electromagnetic radiation in microwave frequency range, resulting in increased accessability of the solvent into the sample's struture. Typically, MAE uses lower amount of solvent, reduces extraction time, and is available for commercial use in affordable prices, ranging from household- to industrial scale. There are several factors affecting efficiency of MAE, *e.g.* type of solvent used, extraction time, temperature, microwave power and contact surface area between the plant and the solvent [2, 6].

Therefore, the objectives of this work were to 1) evaluate efficiency of conventional extraction and MAE (using hot water as solvent) of red cabbage; and 2) determine optimal extraction condition for MAE; parameters that included in the study were red cabbage to water ratio, and extraction power and time.

MATERIALS AND METHODS

The yields of hot water extraction of red cabbage were determined and compared between conventional HWE and MAE, using absorbance at λ_{max} and total monomeric anthocyanin content of color extracts as indicating factors. Then, the optimization of MAE for red cabbage was determined using response surface methodology (RSM). The color extract obtained using optimal condition of MAE was later studied its stability under storage at 4°C.

1. Materials

Fresh red cabbage (*Brassica oleraces* var. *capitate* f. *rubra*) was purchased from local supermarkets in Bangkok, Thailand. Cabbage was stored at 4±1°C until used and used within 3 days of purchase.

Colorless buffer solutions of pH 1.0 and 4.5 used in determination of total monomeric anthocyanin pigment content were prepared from potassium chloride (0.025M) (KCl, Ajax Finechem, New South Wales, Australia) and sodium acetate (0.4M) ($CH_3CO_2Na{\cdot}3H_2O$, Ajax Finechem), respectively. The buffer solutions were adjusted their final pH with hydrochloric acid (HCl, Fisher Scientific, MA, USA) [7]. Disposable plastic cuvette (Bibby Scientific Ltd., Staffordshire, UK) was used in UV-Vis spectrophotometry.

2. Hot water extraction

Based on preliminary experiment, red cabbage per water ratio used in conventional HWE conditions in the study was 1:3 g/mL of water. For both extraction methods, only leaves were used and the core was discarded. The leaves were then cut into small pieces; and used within 1 h of preparation [8]. Pre-determined amount of freshly cut sample was submersed in 80˚C distilled water installed in waterbath (Memmert Waterbath WNE [illegible]2, Schwabach, Germany). The extractions were carried out at 80°C, for 10-180 min (in increment of 10 min). After the extraction, the heated mixture was filtered using cheesecloth. The aliquot was then collected as color extract and left to cool to room temperature before further testing [8]. All experiments were performed in triplicate.

3. Microwave assisted extraction

To study the effects of sample to water ratio, and extraction power and time of microwave assisted extraction, preliminary experiments were conducted. The selected levels of all testing parameters were 1:5 to 1:3 g/mL, 480-800 W and 120-480 s, respectively. These conditions were sufficient to yield extracts with visible color and did not cause significant violent boiling.

Table 1 shows 15 extraction treatments for red cabbage conducted in random order according to Box-Behnken design of response surface methodology (RSM). All treatments listed were conducted in triplicate. For each treatment, known amount of cut red cabbage was submersed in distilled water at room temperature, for 60 s to ensure thorough submersion, before starting the microwave heating process using household microwave oven (LG MG-3937C Microwave Oven, LG Electronics, Bangkok, Thailand). After the treatment, the mixture was filtered, and color extract was collected and left to cool to room temperature [illegible].

4. UV-Vis spectroscopy

The visible spectra (400-700 nm), λ_{max}, and absorbance at λ_{max} of color extracts of red cabbage by HWE and MAE were obtained using UV-Vis spectrophotometer (LAMBDA 35 UV/Vis Spectrophotometer, PerkinElmer Inc., MA, USA).

5. Determination of total monomeric anthocyanin pigment content

The amount of total monomeric anthocyanin pigment in extracted color solution was determined, using pH differential method [7]. Briefly, the extract was mixed with pH 1.0 or pH 4.5 buffers (final concentration of the extract in the solution was 10% vol/vol). The solutions were then measured their absorbance at 520 and 700 nm, using UV-Vis spectrophotometer. Amount of anthocyanin pigment was calculated using Equation 1 and 2.

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad \text{Eq. 1}$$

Total monomeric anthocyanin (cyanidin-3-glucoside equivalents, mg/L)

$$= \frac{A \times M_w \times DF \times 10^3}{\varepsilon \times \iota} \quad \text{Eq. 2}$$

where M_w is molecular weight of cyanidin-3-glucoside (cyd-3-glu) = 449.2 g/mol; DF is

dilution factor; ι represents pathlength in cm = 1 cm; ε is molar extinction coefficient for cyd-3-glu = 26,900 L·mol^{-1}·cm^{-1}.

6. Determination of color extract stability

Preliminary experiment on storage stability of red cabbage extract showed that, at room temperature (25±1°C), level of total anthocyanin content significantly decreased within 1 h after extraction. The study of color extract stability was then focused on stability of anthocyanins during cold storage.

The extracts were prepared using optimal condition of MAE (3 replicates of extract were prepared), and then kept in air-tight glass container, covered with aluminium foil, at 4±1°C. The extracts were sampling periodically to monitor their total monomeric anthocyanin pigment content for 24 h.

7. Statistical analysis

All data obtained from the study were statistically analysed using JMP 8.0 program (SAS Institute Inc., NC, USA) at the confidence level of 95% (α = 0.05) with Tukey's adjustment for comparison of the means.

To obtain mathematical model of MAE of red cabbage, the response, i.e. absorbance at λ_{max} of the extracts was analyzed using JMP 8.0 program (SAS Institute Inc.). Equation 3 describes 2nd-order polynomial equation used to develop a predictive model for MAE of red cabbage:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \varepsilon \quad \text{Eq. 3}$$

where y is absorbance at λ_{max} (553 nm); x_1, x_2, and x_3 are coded values of studied parameters, *i.e.* sample to water ratio (g/mL), extraction power (W), and extraction time (s), respectively; β_0 is intercept; β_1, β_2, and β_3 are linear effects of sample to water ratio, extraction power, and extraction time, respectively; β_{11}, β_{22}, and β_{33} are quadratic effects of red cabbage water ratio, extraction power, and extraction time, respectively; β_{12}, β_{13}, and β_{23} are interaction effects of red cabbage to water ratio and extraction power, red cabbage to water ratio and extraction time, and extraction power and extraction time, respectively; and ε is residual error.

RESULTS AND DISCUSSION

pH values of red cabbage extracts from HWE and MAE were 6.51±0.28 and 6.47±0.04, respectively. Visible spectra (400-700 nm) of color extracts obtained from both extraction methods showed λ_{max} at 553 nm (spectra not shown).

Data on absorbance at λ_{max} of red cabbage extracts from both extraction methods at various conditions were plotted against their corresponding total monomeric anthocyanin contents (Figure 1). The plot shows positive correlation between two parameters. Absorbance at 553 nm was then used as indicator for efficacy of both extraction methods. Figure 2 shows absorbance at 553 nm of red cabbage extracts obtained from HWE, at constant 80°C.

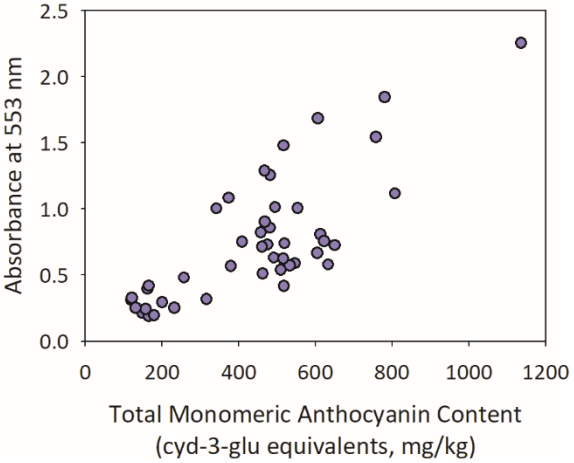

Figure 1 Total monomeric anthocyanin content (mg cyd-3-glu equivalents per kg fresh red cabbage) and absorbance at λ_{max} of red cabbage extracts

The red cabbage to water ratio used was similar to the highest ratio selected for MAE, *i.e.* 1:3 g/mL. Based on absorbance data, the extraction time of 60 min gave color extract with maximum absorbance at 553 nm for HWE of red cabbage. The extract obtained using this condition had anthocyanin content of 521.02±64.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage. This maximum level of anthocyanin content was similar to that of red cabbage extract obtained through HWE at 100°C, for 7 min (come-up time excluded) reported by Kham-ngam *et al.* (2015) [10]. After 60 min of extraction at 80° C, the absorbance at λ_{max} decreased.

Table 1 shows absorbance at 553 nm and total monomeric anthocyanin contents of the extracts at various MAE conditions. To study the effects of MAE on absorbance at λ_{max} of red cabbage, mathematical model was constructed (Table 2). The predictive model had R^2 of 0.8066. Based on obtained equation, both red cabbage to water ratio and extraction time had strong positive linear effects on amount of color compounds being extracted through MAE method (Table 2), as were indicated by the increases in absorbance at 553 nm, with red cabbage to water ratio being the most prominent factor. The weak synergistic effect of extraction power and time (x_1x_3) was also observed ($P < 0.005$).

Table 1 Conditions for microwave assisted hot water extraction of red cabbage and corresponding absorbance at 553 nm and total monomeric anthocyanin content of color extracts

Treatment	Code	Sample to Water Ratio (g/mL)	Extract Power (W)	Extraction Time (s)	Absorbance at 553 nm*	Total Monomeric Anthocyanin Content*,**
1	0 0 0	1:4	640	300	0.70±0.14^{c}	480.75±26.47^{c}
2	0 – –	1:4	480	120	0.24±0.06^{d,e}	144.77±23.28^{d}
3	0 – +	1:4	480	480	0.74±0.23^{b,c}	530.58±33.71^{c}
4	+ – 0	1:5	480	300	0.56±0.24^{c,d}	436.17±156.13^{c}
5	0 0 0	1:4	640	300	0.65±0.09^{c}	557.63±57.39^{b,c}
6	+ 0 –	1:5	640	120	0.23±0.03^{e}	189.76±38.14^{d}
7	+ 0 +	1:3	640	480	0.81±0.28^{b,c}	697.13±95.92^{a,b}
8	0 0 0	1:4	640	300	0.70±0.18^{c}	487.78±28.98^{c}
9	– – 0	1:5	480	300	1.00±0.25^{b,c}	411.01±69.74^{c}
10	– + 0	1:3	800	300	1.09±0.19^{b}	436.61±54.24^{c}
11	– 0 –	1:4	640	120	0.37±0.10^{d}	193.24±68.17^{d}
12	– 0 +	1:3	640	480	1.39±0.34^{a,b}	539.62±59.30^{c}
13	+ + –	1:4	800	120	0.36±0.09^{d}	153.15±19.09^{d}
14	0 + +	1:4	800	480	1.88±0.36^{a}	891.63±212.34^{a}
15	+ + 0	1:5	800	300	0.60±0.16^{c}	527.77±72.63^{c}

*Values with similar superscript, within the same column, are not statistically different at type I error (α) of 0.05

**Total monomeric anthocyanin content of red cabbage was calculated based on Eq. 1 and 2, with DF of 10, into mg cyanidin-3-glucoside equivalents per kg fresh red cabbage

Prediction profilers (Figure 3) indicated that even at the conditions where highest level of every testing parameter were applied, it might be possible to obtain higher yield if the levels were raised beyond the testing conditions, as could be implied by the on-going positive trends in the profilers. However, due to the risks of violent boiling from applying higher extraction power and/or longer extraction time, the MAE conditions that should be used to extract the color compounds from red cabbage is the

highest levels of every parameter, *i.e.* red cabbage per water ratio of 1:3 g/mL, extraction power of 800 W, and extraction time of 8 min. Color extract that was prepared using this optimal MAE condition contained anthocyanin content of 1023.39±36.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage. The extract had visible spectrum and λ_{max} (data not shown) similar to those of extracts yielded from MAE at less severe conditions, as well as those from HWE. This indicated that the qualities of the color extracts were not significantly affected.

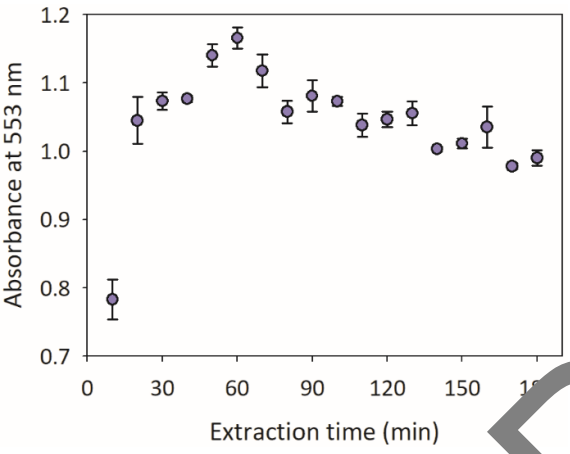

Figure 2 Absorbance at λ_{max} of red cabbage obtained from HWE at 90°C

Comparison between maximum anthocyanin content obtained from hot water extraction using HWE and MAE showed that MAE gave higher extraction yield of color extract from red cabbage, as compared to that from HWE method, i.e. 1023.39±36.62 and 521.02±4.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage, respectively. Thus, based on maximum anthocyanin contents in extracts obtained from both methods, MAE increased yield of color extraction by 96.42% (from HWE) [3].

High-temperature extraction can result in increased yield due to increases in solubility and/or mobility of compounds, or better accessibility from disruptions of cell or structure. However, prolonged exposure of anthocyanins, which are heat-sensitive compounds to high extraction temperature, as in the case of HWE, can cause significant degradation of anthocyanins [5, 6].

Table 2 Parameter estimates of RSM equation for microwave assisted extraction of red cabbage

Parameter	λ_{max} = 553 nm		
	Estimate	Standard Error	Prob > |t|
Intercept	-0.7618	0.218	0.0013
Water to sample ratio	0.2064	0.047	0001*
Extraction power	0.0011	0003	0.0008*
Extraction time	0.1	0.015	<.0001*
Ratio*Power	1667e-5	0.0004	0.8456
Ratio*Time	0374	0.0222	0.1012
Power*Time	0.00 5	0.0001	0.0005*
Ratio*Ratio	0.01	0.0693	0.8539
Power*Power	4.6086e-6	2.7070e-6	0.0976
Time*Time	0.0003	0.0077	0.9688

* indicates significance of the effects at type I error (α) = 0.05.

Figure 4 shows total monomeric anthocyanin content of red cabbage extracts stored at 4°C. The extracts were prepared using MAE at red cabbage to water ratio of 1:3 g/mL, 800 W, for 8 min (initial total anthocyanin content = 1023.39±36.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage). At 4° C, the level of anthocyanins did not significantly decrease in the first 2 h of storage, but significantly lowered after that ($P < 0.05$). The results coincided with findings reported by Ahmadiani *et al*. (2014). After refrigeration storage for 6 h, it was found that anthocyanin contents of extracts (adjusted pH values to 7) from 7 different red cabbage varieties decreased by 19.1±3.8 to 50.1±5.5% [3]. The pH of the extract also played an important role. Anthocyanins tends to degrade faster in aqueous solution and under basic condition as compared to that under acidic condition [3].

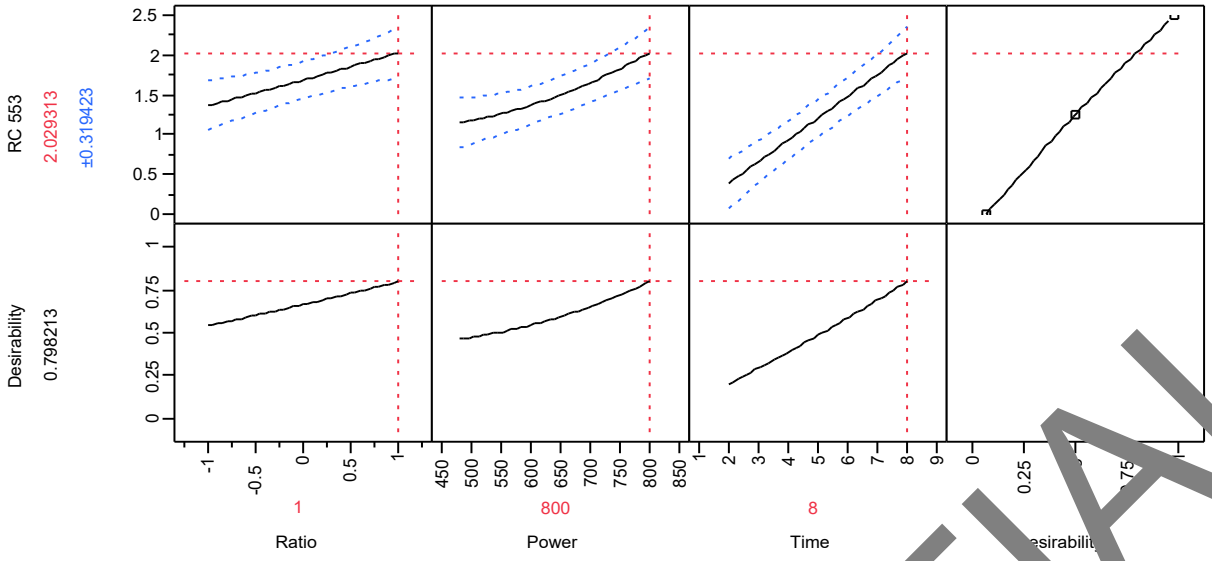

Figure 3 Prediction profiler of red cabbage extract; ranges of studied parameters were 1:5 (-1) to 1:3 (1) g/mL for red cabbage to water ratio, 480 (-1) to 800 (1) W for extraction power; and 2(-1) to 8 (1) min for extract time

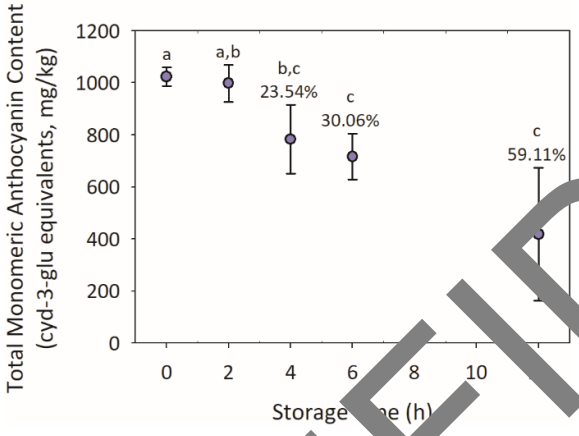

Figure 4 Total monomeric anthocyanin content (mg cyanidin-3-glucoside equivalents per kg fresh red cabbage) of red cabbage extract stored at 4°C

CONCLUSIONS

Red cabbage extracts obtained using HWE and MAE had pH value of 6.51±0.28 and 6.37±0.04, respectively; and λ_{max} of 553 nm. In comparison to HWE, MAE by household microwave oven increased extraction yield of red cabbage and lowered extraction time used. Based on mathematical model of MAE of red cabbage (R^2 = 0.8066), red cabbage to water ratio and extraction time strongly influenced efficacy of MAE ($P \leq 0.001$). Red cabbage to water ratio of 1:3 g/mL, extraction power of 800 W, and extraction time of 8 min resulted in color extract with highest anthocyanin content, *i.e.* 1023.39±36.62 mg cyanidin-3-glucoside equivalents per kg fresh red cabbage. Additionally, according to prediction profilers, it is also possible that higher yield could be obtained at the more severe condition. From storage stability study, it was recommended that red cabbage extract should be used within 2 h after preparation (if kept at 4°C) as anthocyanin level of the extract was stable for the first 2 h and significantly decreased after that.

NOMENCLATURE

HWE	Hot water extraction
MAE	Microwave assisted extraction
RSM	Response surface methodology

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REFERENCES

1. Saxena, S. and Raja, A.S.M. (2014). Natural dyes: Sources, chemistry, application and sustainability issues. In S.S. Muthu (Ed), Roadmap to Sustainable Textiles and Clothing. Singapore: Springer, 37-80.
2. Lasunon, C., Ponpesh, P. and Satirapipathkul, C. (2018). Pigment extraction from flower using microwave assisted extraction. International Journal of Science, Engineering and Management. 3(5): 37-39.
3. Ahmadiani, N., Robbins, R.J., Collins, T.M. and Giusti, M.M. (2014). Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. Journal of Agricultural and Food Chemistry. 62: 7524-7531.
4. Maftoonazad, N. and Ramaswamy, H. (2019). Design and testing of an electrospun nanofiber mat as a pH biosensor and monitor the pH associated quality in fresh date fruit (Rut[illegible]). Polymer Testing. 75: 76-84.
5. Limsitthichaikoon, S., Saodaeng, K., Priprem, A. and Damrongrungruang, P. (2015). 9(2):147-153.
6. Routray, W. and Orsat, V. (201[illegible] Microwave-assisted [illegible]traction of flavonoids: A review. Food and Bioprocess Technology. 5: 40[illegible]424.
7. Lee, J. (200[illegible] D[illegible]ination of total monomeric [illegible]thocyanin pigment conte[illegible]t of fru[illegible]juic[illegible] beverages, natural colo[illegible]ants, and wines by the pH diffe[illegible]ential meth[illegible]od: Collaborative study. Journal of AOAC International. 88(5): 1269-1278.
7. Netra[illegible]ai, S., Kijchavengkul, T., Tang[illegible]rimongkhon, P. and [illegible]achitpitak, R. (2015). The effects of citric acid and storage condition on color stability of butterfly pea (Clitoria ternatea L.) extract. In Book of Abstracts of IFT15 - IFT Annual Meeting & Food Expo. July 11-14, 2015. Chicago, IL: Institute of Food Technologists.
9. Sinha, K., Saha, D.P., Ramya, V. and Datta, S. (2012). Improved extraction of natural blue dye from butterfly pea using microwave assisted methodology to reduce the effect of synthetic blue dye. International Journal of Chemistry. 4(2): 57-65.
10. Kham-ngam, C., Netramai, S. and Kijchavengkul, T. (2015). The development of pH-indicating film from red cabbage (Brassica oleracea var. capitata f. rubra) and butterfly pea (Clitoria ternatea L.). In Book of Abstracts of Science & Management Project Exhibition. May 1[illegible] 2015. Kanchanaburi: Mahidol University.