



**Effect of starter culture addition on Thai
fermented sausage production**

By

Miss Wiramsri Sripochanart

A Thesis Submitted in Partial Fulfillment of the Requirements

For the Degree of Doctoral of Engineering

Department of Chemical Engineering

Faculty of Engineering

Thammasat University

2009

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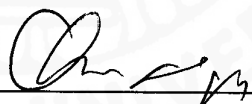
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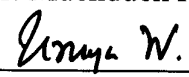
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บทคัดย่อ

งานวิจัยนี้ได้ปรับปรุงการผลิตไส้กรอกอีสานแบบดั้งเดิม โดยวิธีการเติมต้นเชื้อ 6 ชนิด ได้แก่ *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Weissella cibaria*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, และ *Lactobacillus sakei* ผลการทดลองเปรียบเทียบระหว่างไส้กรอกที่เติมต้นเชื้อกับไส้กรอกหมักธรรมชาติ (ชุดควบคุม) ผลการวิเคราะห์จุลินทรีย์พบว่าจำนวนประชากรของแบคทีเรียแลคติกที่มีปริมาณมากสามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียที่ทำให้เกิดโรคและเกิดการเน่าเสียได้ ไส้กรอกที่เติมต้นเชื้อมีค่า pH (pH = 4.51-4.67) ต่ำกว่าไส้กรอกชุดควบคุม pH ที่ลดลงอาจเป็นผลเนื่องมาจากการผลิตกรดอินทรีย์ โดยเฉพาะการผลิตกรดแลคติกจากเชื้อแบคทีเรียแลคติก อัตราการผลิตกรดแลคติกเริ่มต้นในไส้กรอกที่เติมต้นเชื้อมีค่าสูงกว่าชุดควบคุม ไส้กรอกที่เติมต้นเชื้อ *P. acidilactici* มีอัตราการผลิตกรดแลคติกเริ่มต้น (4.45×10^{-2} g/100g·h) และค่าผลได้ของกรดแลคติก (0.89) สูงสุด ขณะที่ชุดควบคุมมีอัตราเร็วเริ่มต้นการผลิตกรดแลคติก และค่าผลได้ของกรดแลคติกเท่ากับ 2.66×10^{-2} g/100g·h และ 0.63 ตามลำดับ การศึกษาผลของการเติมต้นเชื้อต่อกระบวนการย่อยสลายด้วยโปรตีเอส (Proteolysis) พบว่าระหว่างกระบวนการหมัก ไมโอไฟบริลลารีโปรตีน (Myofibrillar protein) และซาร์โคพลาสมิคโปรตีน (Sarcoplasmic protein) มีปริมาณลดลง และไนโตรเจนที่ไม่ใช่โปรตีนและกรดอะมิโนทั้งหมดมีปริมาณเพิ่มขึ้น ไส้กรอกที่มีการเติมต้นเชื้อแบคทีเรียแลคติกมีความเข้มข้นของไนโตรเจนที่ไม่ใช่โปรตีนและกรดอะมิโนอิสระสูงสุด การแยกโปรตีนโดยวิธี sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) แสดงให้เห็นว่ารูปแบบการเกิดโปรตีนโอไลซิสของซาร์โคพลาสมิคโปรตีนของไส้กรอกอีสานเหมือนกันทุกการทดลอง ในขณะที่ไส้กรอกที่มีการเติมต้นเชื้อ *L. plantarum* *L. pentosus* และ *L. sakei* มีการสลายตัวของไมโอไฟบริลลารีโปรตีนแถบที่ 200 และ 45 kDa นอกจากนี้งานวิจัยได้ศึกษาผลของการเติมต้นเชื้อต่อกลิ่นของไส้กรอกอีสาน สารประกอบกลิ่นของไส้กรอก เช่น 3-methyl-butanal 3-methyl-butanoic acid และ 3-methyl-butanol ซึ่งสังเคราะห์จากกรดอะมิโนลิซีน กิจกรรมของต้นเชื้อแบคทีเรียแลคติกที่เติมในไส้กรอกมีผลทำให้ความเข้มข้นของสารระเหย 3-methyl-butanoic acid มีความเข้มข้นมากกว่าไส้กรอกชุดควบคุม จากการสำรวจความพึงพอใจพบว่า ไส้กรอกอีสานที่เติมต้นเชื้อ *P. acidilactici* ได้รับความนิยมความพอใจด้านภาพรวมทั้งหมดสูงที่สุด

การพัฒนาแบบจำลองทางคณิตศาสตร์เพื่อประยุกต์ใช้กับจลนพลศาสตร์ของการเจริญเติบโตของแบคทีเรียแลคติก การใช้โปรตีนและน้ำตาลกลูโคส และการผลิตไนโตรเจนที่ไม่ใช่โปรตีนกรดแลคติก และกรดฟอร์มิก การทดลองที่เลือกมาทดสอบแบบจำลองมี 2 ชุด ได้แก่ชุดควบคุมและชุดที่มีการเติมต้นเชื้อ *P. acidilactici* โดยหาค่าพารามิเตอร์ด้วยวิธีสุ่มหาค่าพารามิเตอร์ของกิบบส์ (Gibbs parameter sampling approach) การทดสอบความสัมพันธ์ระหว่างตัวแปรที่เป็นอิสระต่อกันโดยใช้สถิติไค-สแควร์ที่ระดับความเชื่อมั่น 95% พบว่าผลจากแบบจำลองสอดคล้องกับผลการทดลองของทั้งชุดควบคุม และชุดที่มีการเติมต้นเชื้อ *P. acidilactici*

Abstract

The conventional Thai fermented sausage, Sai Kork E Sarn, production process was improved by introducing six different external starter cultures including *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Weissella cibaria*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, and *Lactobacillus sakei*. The experimental results with the starter cultures were compared with naturally fermented sausage (control). The results of microbiological analysis indicated that the dominance of lactic acid bacteria (LAB) could inhibit the growth of pathogens and spoilage. Sausages inoculated with starters exhibited lower pH (pH = 4.51-4.67) than the uninoculated control. The decrease of pH was attributed to organic acids, mainly by lactic acid production by LAB. The initial rate of lactic acid production in sausages inoculated with starter cultures was higher than the control. Addition of *P. acidilactici* showed the highest initial rate of lactic acid production (4.45×10^{-2} g/100g·h) and lactic acid yield (0.89), while the initial rate of lactic acid production and lactic acid yield were 2.66×10^{-2} g/100g·h and 0.63 respectively in control experiments. The effect of addition of starter cultures on proteolysis of Thai fermented sausages was investigated. Proteolysis was observed during fermentation by the reduction of myofibrillar and sarcoplasmic proteins and the increase in non-protein nitrogen (NPN) and total free amino acids. The highest increase in concentration of NPN and free amino acids was obtained from sausages inoculated with LAB. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showed a similar pattern of proteolysis of sarcoplasmic proteins in all sausages, while that of the inoculated sausages with *L. plantarum*, *L. pentosus* and *L. sakei* exhibited the most degradation of myofibrillar protein bands at 200 and 45 kDa. In addition, the effect of starter culture addition on flavor generation in Thai fermented sausages was evaluated. The sausage aroma compounds such as 3-methyl-butanal, 3-methyl-butanoic acid and 3-methyl-butanol were derived from leucine. The activity of LAB resulted in higher concentration of the volatile compound, 3-methyl-butanoic acid than in the control sample. For sensory test, Thai fermented sausage inoculated with *P. acidilactici* received the highest scores for overall preference.

Mathematical models were developed to fit the experimental data including the growth of LAB, the consumption of protein and glucose, and the production of NPN, lactic acid and formic acid concentration. Two experimental data sets, i.e., control and *P. acidilactici* inoculated experiments, were selected to estimate parameter values and to calibrate the model using the Gibbs parameter sampling approach. The goodness of fit was evaluated non-parametrically by estimating the χ^2 statistic at 95% confidence interval. It signifies the quality of agreement between predicted and observed results of naturally fermented sausage (control) and sausage inoculated with *P. acidilactici*.

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Wiramsri Sriphochanart

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