

# SMART PACKAGING FOR FISH SPOILAGE INDICATOR



Acharee Seephueng

A Thesis Submitted in Partial Fulfilment of the Requirements  
for the Degree of Master of Science  
The Petroleum and Petrochemical College, Chulalongkorn University  
in Academic Partnership with  
The University of Michigan. The University of Oklahoma,  
and Case Western Reserve University

2008

511992

**Thesis Title:** Smart Packaging for Fish Spoilage Indicator  
**By:** Acharee Seephueng  
**Program:** Polymer Science  
**Thesis Advisors:** Asst. Prof. Hathaikarn Manuspiya  
Assoc. Prof. Rathanawan Magaraphan  
Asst. Prof. Manit Nithitanakul

---

Accepted by the Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Master of Science.

*Nantaya Yanumet*  
..... College Director  
(Assoc. Prof. Nantaya Yanumet)

**Thesis Committee:**

*Hathaikarn Manuspiya*  
.....  
(Asst. Prof. Hathaikarn Manuspiya)

*R. Magaraphan*  
.....  
(Assoc. Prof. Rathanawan Magaraphan)

*Manit Nithitanakul*  
.....  
(Asst. Prof. Manit Nithitanakul)

*Thanyalak Chaisuwan*  
.....  
(Dr. Thanyalak Chaisuwan)

*H. Kiratisaevee*  
.....  
(Dr. Harittapak Kiratisaevee)

## บทคัดย่อ

อัจฉริ สีสั่ง : บรรจุภัณฑ์ฉลาดสำหรับการบ่งบอกความสดของเนื้อปลา (Smart Packaging for Fish Spoilage Indicator) อ. ที่ปรึกษา: ศศ.ดร.หทัยกานต์ มนต์ปิยะ รศ.ดร. รัตนาวรรณ มกรพันธุ์ และ ศศ.ดร.มานิตย์ นิธิธนากุล 118 หน้า

การผลิตวัสดุที่ไวต่อการเปลี่ยนแปลงความเป็นกรด-เบสเพื่อใช้ในบรรจุภัณฑ์สำหรับปลา โดยใช้พอลิพรอพิลีนเคลย์นาโนคอมโพสิตร่วมกับการใส่เม็ดสีอินดิเคเตอร์ เพื่อใช้เป็นเครื่องชี้วัดความเสียของเนื้อปลา ช่วยให้ผู้บริโภคสะดวกที่จะทราบว่าปลานั้นเสียหรือไม่ เพียงแค่ดูการเปลี่ยนสีของฟิล์มบนบรรจุภัณฑ์เท่านั้น ฟิล์มนาโนคอมโพสิตชนิดไวต่อความเป็นกรดต่างเพื่อตรวจวัดความสดของเนื้อปลาสามารถทำได้โดยการนำแผ่นเซ็นเซอร์ (sensor disc) ประกบติดกับแผ่นฟิล์มพอลิพรอพิลีนนาโนคอมโพสิต โดยใช้เครื่องเคลือบให้ประกบติดกันที่อุณหภูมิ 160°C โดยฟิล์มพอลิพรอพิลีนเคลย์นาโนคอมโพสิตผลิตโดยใช้เครื่องอัดรีดแบบเกลียวคู่ โดยมีเซอร์ลีนเป็นตัวเชื่อมประสาน และขึ้นรูปเพื่อใช้ในการทดสอบเชิงกลและทางความร้อน เมื่อเนื้อปลาเกิดการเน่าเสีย จะมีการผลิตก๊าซเน่าเสียที่ประกอบด้วยสารประกอบไนโตรเจน (TVB-N) เช่น ก๊าซไตรเมทิลเอมีน ไดเมทิลเอมีน และก๊าซแอมโมเนียขึ้น ทำให้ค่าความเป็นกรด-เบส (pH) เพิ่มขึ้น ซึ่งค่าความเสียของเนื้อปลาถูกทดสอบโดยค่าความเป็นกรด-เบส (pH) จำนวนรวมของแบคทีเรีย (APC) ปริมาณรวมของ TVB-N จากการไตเตรทและการเปลี่ยนแปลงของสีถูกทดสอบโดยค่าเปลี่ยนแปลงของสีทั้งหมด โดยสีของฟิล์มชนิดโบรโมครีซอล กรีน จะเปลี่ยนจากสีเหลืองเป็นสีน้ำเงิน ซึ่งฟิล์มนี้สามารถบอกความสัมพันธ์ระหว่างการเปลี่ยนแปลงที่เกิดขึ้นภายในบรรจุภัณฑ์กับการเปลี่ยนแปลงสีของอินดิเคเตอร์ได้ และเมื่อใช้ฟิล์มอินดิเคเตอร์ดังกล่าวร่วมกับพอลิพรอพิลีนเคลย์นาโนคอมโพสิตฟิล์มยังช่วยยืดอายุการเก็บรักษาปลาสดให้ยาวนานขึ้นอีกด้วย ดังนั้นฟิล์มนาโนคอมโพสิตอินดิเคเตอร์จึงสามารถใช้เป็นวัสดุที่ไวต่อการเปลี่ยนแปลงความเป็นกรด-เบสรวมทั้งยืดอายุการเก็บรักษาเพื่อใช้ในบรรจุภัณฑ์สำหรับปลาสดได้อย่างมีประสิทธิภาพ

## ABSTRACT

4972001063: Polymer Science Program

Acharee Seephueng: Smart Packaging for Fish Spoilage Indicator.

Thesis Advisors: Asst. Prof. Hathaikarn Manuspiya, Assoc.Prof.

Rathanawan Magaraphan, and Asst. Prof. Manit Nithitanakul 118 pp.

Keywords: Polypropylene/ Bentonite/ Montmorillonite/ Nanocomposite / Smart Packaging/ pH sensor/ Fish spoilage

A colored pH sensor for fresh fish packaging has been newly developed to evaluate the fresh fish spoilage during transport and storage. The processing of pH-sensitive film used for fish packaging based on the layer of organomodified clay nanocomposites laminated with the layer of indicator dye was focused. The nanoclay composites were prepared via melt compounding through a twin screw extruder by using Surlyn<sup>®</sup> as a reactive compatibilizer. Subsequently, the pH sensor was fabricated by using a spin-coater and was attached to PP/clay nanocomposite films using a laminating machine (at 160°C). Fish spoilage was assessed for aerobic plate count (APC) and total volatile basic nitrogen (TVB-N). The color changes of the pH sensor were measured and expressed as Hunter values and the total color difference (TCD). The TCD values of bromocresol green (BCG) type indicator also changed continuously with the response of the indicator. The color changes of the pH sensor correlated well with the APC and TVB-N values of fresh fish. According to the changes in Hunter color values of the indicator within the packages of fresh fish at room temperature, the color of the pH sensor turned from an initial yellow to a final green for BCG type. The color changes of the developed pH sensor film properly represented the spoilage of the fresh fish. By laminating pH sensor on nanocomposite film, the layer of naocomposite can facilitate the gas barrier properties and the leakage of indicator. As the final product, these pH sensors could be employed as an effective, smart packaging technology for evaluating fish freshness.

## ACKNOWLEDGEMENTS

This work is funded by National Research Council of Thailand (NRCT). The author thanks for the Scholarship that is partially supported by the Petroleum and Petrochemical College; the National Excellence Center for Petroleum, Petrochemicals and Advanced Materials, Thailand; and Polymer Processing and Polymer Nanomaterial Research Units. The authors would like to thank Thai Nippon Chemical Industry Co, Ltd., for providing the raw materials to carry out this research and Tang Packaging Co., Ltd. for tubular blown film extrusion machine.

The author would like to gratefully give special thanks to her advisors, Assist. Prof. Hathaikarn Manuspiya, Assoc. Prof. Rathawan Magaraphan and Assist. Prof. Manit Nithitanakul for their intensive suggestions, valuable guidance and vital help throughout this research. The author deeply thanks to Dr. Thanyalak Chaisuwan and Dr. Harittapak Kiratisaevae for serving on her thesis committee.

Special thanks go to all of the Petroleum and Petrochemical College's faculties who have tendered invaluable knowledge and go to the college staffs who give her invaluable assistance.

In addition, the author would like to take this opportunity to thank all her PPC friends for their friendly assistance, cheerfulness, creative suggestions, and encouragement.

Finally, the author is greatly indebted to her parents and family for their support, love and understanding. Without them, this work would not have been successful.

## TABLE OF CONTENTS

	<b>PAGE</b>
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	ix
List of Figures	x
 <b>CHAPTER</b>	
<b>I INTRODUCTION</b>	<b>1</b>
 <b>II THEORETICAL BACKGROUND AND LITERATURE REVIEW</b>	 <b>3</b>
 <b>III EXPERIMENTAL</b>	 <b>21</b>
 <b>IV ANALYSIS OF FISH SPOILAGE</b>	 <b>33</b>
4.1 Abstract	33
4.2 Introduction	34
4.3 Experimental	35
4.4 Results and Discussion	38
4.5 Conclusions	41
4.6 Acknowledgements	41
4.7 References	42

<b>CHAPTER</b>	<b>PAGE</b>
<b>V PH SENSOR INCOPORATED PP/ORGANOCLAY NANOCOMPOSITE</b>	<b>44</b>
5.1 Abstract	44
5.2 Introduction	44
5.3 Experimental	46
5.4 Results and Discussion	48
5.5 Conclusions	70
5.6 Acknowledgements	71
5.7 References	71
<b>VI THERMAL AND MECHANICAL PROPERTIES OF PP/ORGANOCLAY NANOCOMPOSITES</b>	<b>73</b>
6.1 Abstract	73
6.2 Introduction	73
6.3 Experimental	74
6.4 Results and Discussion	77
6.5 Conclusions	88
6.6 Acknowledgements	89
6.7 References	89
<b>VII CONCLUSION AND RECOMMENDATIONS</b>	<b>92</b>
<b>REFERENCES</b>	<b>95</b>
<b>APPENDICES</b>	<b>100</b>
<b>Appendix A</b>	<b>100</b>
<b>Appendix B</b>	<b>102</b>
<b>Appendix C</b>	<b>104</b>
<b>Appendix D</b>	<b>111</b>
<b>Appendix E</b>	<b>112</b>

<b>CHAPTER</b>	<b>PAGE</b>
<b>Appendix F</b>	<b>113</b>
<b>CURRICULUM VITAE</b>	<b>118</b>



**LIST OF TABLES**

<b>TABLE</b>		<b>PAGE</b>
<b>CHAPTER II</b>		
2.1	Examples of external and internal indicators and their working principle or reacting compounds to be used in intelligent packaging for quality control of packed food	4
<b>CHAPTER V</b>		
5.1	Effect of spin-coating speed on thickness of BCG type film	49
5.2	Weight of sensor disc and nanocomposite film	49
<b>CHAPTER VI</b>		
6.1	Thermal behavior of BTN and OBTN	79
6.2	Melting and crystallization behavior of PP and PP/organoclay nanocomposite films	80
6.3	Thermal behavior of PP and PP/organoclay nanocomposite films	84

## LIST OF FIGURES

FIGURE	PAGE
<b>CHAPTER II</b>	
2.1 Freshness indicator which gives information on the spoilage or lack of freshness of the product, in addition to temperature abuse or package leaks	5
2.2 Model of changes in total count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during chill storage of a fish product	8
2.3 Sensor responses (spin-coated at 1000 rpm) to ammonia concentration monitored by the optical scanner	9
2.4 Correlation of sensor response (1000 rpm) and changes in bacterial population of fresh cod kept at 20°C over time	10
2.5 Normalised data showing the correlation between sensor response and bacterial population (TVC and <i>Pseudomonas</i> spp.) in cod filet samples at 21°C. The error bars are SEM (standard error of the mean) values	11
2.6 Schematic representation of clay surface treatment	14
2.7 Flowchart of three processing techniques for clay-based polymer nanocomposites: <i>in-situ</i> polymerization (upper), solution exfoliation (middle) and melt intercalation (bottom)	16
2.8 Schemes of clay-based polymer composites, including conventional composite and nanocomposite with intercalated (I), exfoliated (II) or cluster (III) structure	17

<b>FIGURE</b>	<b>PAGE</b>
<b>CHAPTER III</b>	
3.1 Chemical structure of Stepantex™ SP-90	20
3.2 Experimental design for pH measurement of TVB-N	27
3.3 Experimental design for determination of TVB-N	29
3.4 Experimental design for fish spoilage monitoring	32
<b>CHAPTER IV</b>	
4.1 Experimental design for pH measurement of TVB-N	35
4.2 Experimental design for determination of TVB-N	37
4.3 Changes in pH of barramundi filets during storage at 25°C	38
4.4 Changes of TVB-N of barramundi filets during storage at ambient temperature	39
4.5 Changes in APC of barramundi during storage at ambient temperature	41
<b>CHAPTER V</b>	
5.1 Experimental design for fish spoilage monitoring	47
5.2 Changes in Hunter color values for BCG indicator response to increasing ammonia concentration after standing at 12 hr during storage at room temperature: (A) 1,000 rpm, (B) 2,000 rpm and (C) 3,000 rpm	51
5.3 Changes in Hunter color values for BCG indicator response to increasing ammonia concentration after standing at 12 hr during storage at room temperature: (A) 1%wt BCG, (B) 3%wt BCG and (C) 5%wt BCG	53

<b>FIGURE</b>	<b>PAGE</b>
5.4 Changes in Hunter color values for BCG indicator response to increasing ammonia concentration after standing at 12 hr during storage at room temperature: (A) PP, (B) PP/Surlyn, (C) 1%wt clay, (D) 3%wt clay, and (E) 5%wt clay	55
5.5 Changes in TCD values of the indicator films to increasing ammonia concentration after standing at 12 hr during storage at ambient temperature: (A) Vary thickness, (B) Vary %wt BCG, and (C) Vary %wt clay	57
5.6 Color change of smart packaging using spin coating speed 1000 rpm, 3%wt BCG, and 5%wt Clay with 20g of fish meat at ambient temperature	58
5.7 Changes in Hunter color values of the BCG indicator films in barramundi fish during storage at ambient temperature: (A) 1,000 rpm, (B) 2,000 rpm and (C) 3,000 rpm.	60
5.8 Changes in Hunter color values of the BCG indicator films in barramundi fish during storage at ambient temperature: (A) 1%wt BCG, (B) 3%wt BCG and (C) 5%wt BCG	61
5.9 Changes in Hunter color values of the BCG indicator films in barramundi fish during storage at ambient temperature: (A) PP, (B) PP/Surlyn, (C) 1%wt clay, (D) 3%wt clay, and (E) 5%wt clay	64
5.10 Changes in TCD values of the indicator films in the barramundi fish during storage at ambient temperature	65
5.11 Changes in Hunter color values of the BCG indicator films response to increasing TVB-N during storage at ambient temperature: (A) Fish meat 20 g, (B) Fish meat 40 g, and (C) Fish meat 60 g	67
5.12 Changes in TCD values of the indicator films response to increasing TVB-N during storage at ambient temperature	67

<b>FIGURE</b>	<b>PAGE</b>
5.13 Correlation of change in microbial populations and TVB-N of fish meat, allowed to spoil at room temperature and sensor response of 5%wt BCG spin-coated at 1000 rpm onto 5%clay nanocomposite films, of meat samples of 20 g during storage time at room temperature: (A) curve of aerobic plate count (APC), TVB-N, and TCD values with time and (B) curve of aerobic plate count (APC) and TCD values with TVB-N	69
5.14 The leakage of the indicator dyes incorporated into the nanocomposite pH sensor film	70
<b>CHAPTER VI</b>	
6.1 FTIR spectra of (a) Na-BTN and (b) OBTN	78
6.2 The XRD patterns: (a) BTN and (b) OBTN	79
6.3 DSC thermograms of PP/organoclay nanocomposites film (A) Melting temperature, and (B) Crystallization temperature	81
6.4 DTG curves of PP/organo-bentonite nanocomposite films	83
6.5 TG curves of PP/organo-bentonite nanocomposite films	83
6.6 The WAXD patterns of pure PP and PP/organoclay composites varied the compositions	84
6.7 Young's modulus of PP and PP/organoclay composites with various organoclay loadings	85
6.8 Tensile strength of PP and PP/organoclay composites with various organoclay loadings	86
6.9 %Elongation at break of PP and PP/organoclay composites with various organoclay loadings	86
6.10 SEM images of nanocomposite films at 10000 magnification; (A) 1%wt organoclay, (B) 3%wt organoclay, (C) 5%wt organoclay	87

<b>FIGURE</b>	<b>PAGE</b>
6.10 Oxygen transitions rate of PP and PP/OBTN nanocomposite films at different amount of clay content	88