รายงานวิจัยฉบับสมบูรณ์

โครงการ
การศึกษาคุณลักษณะ องค์ประกอบ และการเปลี่ยนแปลงระหว่างการเก็บรักษาปลาบึกเลี้ยงในจังหวัดเชียงราย

โดย

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บทคัดย่อ:

ปัจจุบันการเพาะเลี้ยงปลาบึกในบ่อดินเพื่อวัตถุประสงค์ทางการค้าประสบความสำเร็จเป็นอย่างดีในจังหวัดเชียงราย โดยคาดว่าปลาบึกจะกลายเป็นปลาน้ำจืดที่สำคัญทางเศรษฐกิจของประเทศอีกตัวหนึ่ง ซึ่งผู้เลี้ยงคาดหวังที่จะส่งออกเนื้อปลาบึกไปยังตลาดต่างประเทศโดยเฉพาะในกลุ่มประเทศอาเซียนและยุโรปซึ่งให้ความสนใจในการบริโภคเนื้อปลา อย่างไรก็ตามจากการสำรวจเอกสารพบว่ายังไม่มีข้อมูลเบื้องต้นรายงานให้ทราบเกี่ยวกับองค์ประกอบ คุณลักษณะของเนื้อปลา รวมไปถึงการเปลี่ยนแปลงลักษณะคุณภาพของเนื้อปลาภายหลังการจับและในระหว่างการเก็บรักษาโดยเฉพาะทางด้านชีวเคมี และด้านกายภาพ ด้วยเหตุนี้จึงเป็นที่มาของโครงการวิจัยดังกล่าว โดยมีเป้าหมายที่จะศึกษาวิจัยเพื่อให้ได้มาซึ่งข้อมูลเบื้องต้นต่าง ๆ เกี่ยวกับเนื้อปลาบึกในเชิงของการที่จะนำมาใช้เป็นวัตถุดิบอาหารสำหรับบริโภค

การศึกษาองค์ประกอบพื้นฐาน คุณสมบัติทางเคมีและกายภาพของเนื้อปลาบึกซึ่งแบ่งออกเป็นส่วนหลัง ส่วนท้อง และส่วนเนื้อดำ พบว่าค่าเฉลี่ยของโปรตีน ไขมัน และเถ้าของเนื้อส่วนต่าง ๆ มีค่า 16.88, 4.45 และ 1.24 กรัมต่อเนื้อ 100 กรัม ตามลำดับ ซึ่งพบโปรตีนมากที่สุดในเนื้อส่วนหลัง ในขณะที่พบว่าปริมาณไฮดรอกซีโปรลีนพบมากที่สุดในเนื้อส่วนท้อง (0.83 กรัมต่อกรัมเนื้อ) องค์ประกอบของไขมันแตกต่างกันไปตามส่วนของเนื้อ โดยพบว่าเนื้อส่วนหลังมีฟอสโฟไลปิดสูงสุด ในขณะที่ทั้งเนื้อท้องและเนื้อดำสามารถพบไตรกลีเซอร์ไรด์มากสุด ทุกส่วนของเนื้อปลาบึกประกอบด้วยกรดไขมันอิ่มตัวในปริมาณที่สูง ตามมาด้วยกรดไขมันอิ่มตัวชนิดโมโนและโพลี ความแข็งแรงและความเหนียวของเนื้อปลามีค่ามากในเนื้อส่วนหลังกว่าในเนื้อส่วนท้องและเนื้อดำ อย่างไรก็ตามพบว่าในเนื้อดำซึ่งเป็นแหล่งของไมโอโกลบินและเม็ดสีชนิดอื่น ๆ มีผลทำให้ค่าดัชนีความเป็นสีแดงสูงสุดในเนื้อส่วนนี้ และเมื่อจำแนกองค์ประกอบโปรตีนของกล้ามเนื้อปลาบึกโดยอาศัยความสามารถในการละลายสามารถแบ่งออกเป็น 3 กลุ่มหลักได้แก่ โปรตีไมโอไฟบริล โปรตีนซาร์โคพลาสมิก และโปรตีนที่สามารถละลายได้ในสารละลายด่าง

จากผลการวิจัยในส่วนแรกทำให้ทราบว่าเนื้อปลาบึกประกอบไปด้วยองค์ประกอบของ โปรตีน ไขมัน และ สารในกลุ่มเดียวกันค่อนข้างสูง ดังนั้นจึงได้ศึกษาเพื่อติดตามการเปลี่ยนแปลงที่เกิดขึ้นกับองค์ประกอบเหล่านั้นในระหว่างการเก็บรักษาที่อุณหภูมิตู้เย็น (4 องศาเซลเซียส) ในกล้ามเนื้อส่วนหลังและส่วนท้อง โดยเก็บรักษาเนื้อปลาบึกเป็นระยะเวลา 14 วัน พบว่าที่วันที่ 14 ของการเก็บรักษากิจกรรมของเอนไซม์ Ca^{2+}-ATPase ลดลงอย่างเห็นได้ชัดเมื่อเปรียบเทียบกับวันแรก ในขณะที่พบการลดลงเล็กน้อยสำหรับค่า surface hydrophobicity และ ค่าปริมาณ reactive sulfhydryls อย่างไรก็ตาม ค่า total volatile basic nitrogen และค่า

ปริมาณเปปไทด์ที่ละลายในสารละลายของกรด trichloroacetic ค่อย ๆ เพิ่มขึ้นเมื่อระยะเวลาในการเก็บรักษานานขึ้น โปรตีนไมโอซินเส้นหนักซึ่งวิเคราะห์โดย SDS-PAGE มีค่าลดลงเล็กน้อยเมื่อเพิ่มระยะเวลาในการเก็บรักษา ผลการติดตามการเปลี่ยนแปลงคุณลักษณะทางด้านกายภาพพบว่าค่าการสูญเสียปริมาณน้ำเนื่องจากแรงกดและความร้อนมีค่าสูงสุดในวันแรกของการเก็บรักษาและค่าดังกล่าวนี้จะลดลงตามระยะเวลาการเก็บที่นานขึ้น ค่าความแข็งแรงของกล้ามเนื้อพบว่าเนื้อท้องมีค่าดังกล่าวสูงกว่าในเนื้อส่วนหลัง และค่าความเหนียวจะมากที่สุดเมื่อเก็บรักษากล้ามเนื้อปลาบึกเป็นเวลา 2 วัน เมื่อนำกล้ามเนื้อที่เก็บรักษามาส่องดูโครงสร้างขนาดเล็กภายใต้กล้องอิเล็กตรอนแบบส่องกราดพบว่ามีช่องว่างขนาดใหญ่ระหว่างมัดกล้ามเนื้อเมื่อระยะเวลาในการเก็บรักษาเพิ่มขึ้น

การเสื่อมเสียของไขมัน การเกิด lipolysis และการเกิดออกซิเดชันของกล้ามเนื้อท้องและเนื้อส่วนหลังเพิ่มขึ้นเมื่อระยะเวลาในการเก็บรักษาเพิ่มขึ้น โดยพบว่าเมื่อระยะเวลาการเก็บยาวนานขึ้นการฟอร์มตัวของผลผลิตของปฏิกิริยาออกซิเดชั่นเพิ่มขึ้นซึ่งติดตามด้วยค่า conjugated diene และค่า TBARS ความเป็นกรด-ด่างของกล้ามเนื้อมีแนวโน้มที่จะเพิ่มขึ้นเมื่อระยะเวลาการเก็บรักษานานขึ้น การเพิ่มขึ้นของปริมาณกรดไขมันอิสระสามารถสังเกตได้ชัดเจนในระยะการเก็บรักษาช่วง 10 วันแรก การเก็บรักษาแบบแช่เย็นยังส่งผลต่อค่าดัชนีความเป็นสีแดงของกล้ามเนื้อปลาบึกอีกด้วยซึ่งสอดคล้องกับการเปลี่ยนแปลงปริมาณ metmyoglobin จากผลการทดลองทั้งหมดสามารถสรุปได้ว่าการเปลี่ยนแปลงของโปรตีน ไขมัน และสารประกอบที่เกี่ยวข้องส่งผลกระทบต่อลักษณะคุณภาพของกล้ามเนื้อปลาบึกในระหว่างการเก็บรักษาแบบแช่เย็น โดยเฉพาะการเปลี่ยนแปลงทางด้านชีวเคมีและกายภาพ

คำหลัก: ปลาบึก การเก็บรักษาแบบแช่เย็น องค์ประกอบทางเคมี การเปลี่ยนแปลงภายหลังการเก็บเกี่ยวกล้ามเนื้อปลา

Abstract

Project Code: **MRG5080295**

Project Title: **Characterization of farmed giant catfish compositions and post harvest changes during storage**

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Abstract:

Giant catfish has been successfully farmed in artificial pounds in Chiang Rai Province and it has become an economically important cultured freshwater fish in Thailand. The fish farmers hope to export them in the near future to Asian and European countries. However, no information regarding the basic information about the composition, the properties of meat cuts and also the changes after harvested in biochemical and physical properties has been reported. Thus, this study aims to full fill the leakage information about famed giant catfish in term of food raw material for human consumption.

Proximate composition, chemical and physical properties of dorsal, ventral and lateral line cuts of farmed giant catfish (*Pangasianodon gigas*) were determined. Protein, fat and ash content of the different cuts averaged 16.88, 4.45 and 1.24 g/100 g, respectively. Dorsal contains higher protein concentrations (19 g/100 g) than other two parts ($p<0.05$). Ventral showed the highest hydroxyproline content (0.83 mg/g). Differences in lipid composition and fatty acid profiles were found among different cuts with highest phospholipids in the dorsal and highest triglyceride in both ventral and lateral line ($p<0.05$). All the meat cuts contained high saturated fatty acid, followed by mono- and polyunsaturated fatty acid. High muscle hardness and toughness was found in the dorsal than that in the ventral ($p>0.05$). The highest content of myoglobin and total pigment in lateral line resulted in the highest redness index (a*/b*). Three major nitrogenous compositions classified based on solubility in giant catfish muscle were myofibrillar, sarcoplasmic and alkaline soluble proteins.

According to high protein and fat content for the fish, biochemical and physical changes on protein, lipid and related compounds (from dorsal and ventral cuts) were investigated during refrigerated storage (at 4°C) for 14 days. At day 14, Ca^{2+}-ATPase activity markedly decreased when compared to its value at day 1 (>90%), while a small decrease was observed for surface hydrophobicity and reactive sulfhydryls content. Total volatile basic nitrogen and trichloroacetic-soluble peptide content gradually increased when the storage period was extended. The myosin heavy chain decreased slightly on SDS-PAGE for both meat cuts with increased storage time.

Expressible drip and cooking loss were highest during the first day of storage and slightly decreased with storage time. Instrumental hardness was significantly higher in the ventral compared to the dorsal muscle, while the toughness was the highest at the second day of storage. The muscle bundles with scanning electron microscopy were less attached, resulting in the observed big gaps over increasing storage time.

Lipid deterioration, lipolysis, and lipid oxidation in both dorsal and ventral muscles increased as storage time increased. A progressive formation of primary lipid oxidation products monitored by the increase in conjugated dienes (CD) was observed ($p < 0.05$) and the increase in thiobarbituric reactive substances (TBARS) was noticeable throughout the storage ($p < 0.05$). The pH of the muscles tended to increase as storage time continued ($p < 0.05$). A gradual increase in free fatty acid (FFA) formation was found within the first 10 days of refrigerated storage ($p < 0.05$). Refrigerated storage also resulted in changes in redness index of both cuts. These changes were coincidental with the changes in metmyoglobin content. Results indicated that changes of proteins, lipid and related compounds in the cuts have detrimental effects on the quality attributes of farmed giant catfish muscles during refrigerated storage, particularly physical and biochemical properties.

Keywords: giant catfish, refrigerated storage, chemical compositions, post harvest changes, fish muscle

Executive summary

โครงการ การศึกษาคุณลักษณะ องค์ประกอบ และการเปลี่ยนแปลงระหว่างการเก็บรักษาเนื้อปลาบึกเลี้ยงในจังหวัดเชียงราย มีวัตถุประสงค์เพื่อทราบองค์ประกอบทางเคมี คุณลักษณะทางกายภาพ และจุลินทรีย์ของเนื้อปลาบึกเลี้ยง รวมถึงการศึกษาและติดตามการเปลี่ยนแปลงองค์ประกอบดังกล่าวของเนื้อปลาบึกในระหว่างการเก็บรักษาแบบแช่เย็น ทั้งนี้เนื่องจากในปัจจุบันปลาบึกสามารถเพาะเลี้ยงในเชิงพาณิชย์ได้แล้วแทนการจับจากลุ่มน้ำโขงในอดีต ด้วยเอกลักษณ์ในรสชาติ คุณค่าทางอาหาร และความต้องการของผู้บริโภคทั้งตลาดภายในและต่างประเทศ ทำให้ปัจจุบันในจังหวัดเชียงรายมีเกษตรกรหันมาเลี้ยงปลาบึกกว่า 100 ราย โดยมีปริมาณการเลี้ยงตั้งแต่จำนวนต่ำกว่าสิบตัวถึงหมื่น ๆ ตัว และมีความหวังว่าจะสามารถส่งเนื้อปลาบึกออกไปจำหน่ายยังตลาดต่างประเทศได้ในอนาคตอันใกล้นี้ในรูปแบบของเนื้อปลาบึกแล่ หรือปลาบึกชิ้นแช่เย็นและแช่เยือกแข็ง อย่างไรข้อตามจากการสำรวจข้อมูลพบว่าการศึกษาวิจัยเรื่องปลาบึกในประเทศได้รับการสนับสนุนทุนวิจัยส่วนใหญ่ไปในด้านการผสมเทียม การเพาะเลี้ยงขยายพันธุ์ การศึกษาพ่อ-แม่พันธุ์ปลาบึก การเลี้ยงเพื่ออนุรักษ์ และการพัฒนาการเพาะเลี้ยงเป็นสัตว์น้ำเชิงพาณิชย์ เป็นหลัก ส่วนการศึกษาวิจัยถึงการนำเนื้อมาใช้เป็นอาหาร คุณค่าทางอาหาร การเพิ่มมูลค่าการแปรรูป การเก็บเกี่ยวเพื่อการบริโภค การเก็บรักษา การเปลี่ยนแปลงของคุณภาพภายหลังการเก็บเกี่ยวและการเก็บรักษา รวมถึงการดำเนินงานทางด้านการตลาดยังไม่มี ซึ่งการสนับสนุนการวิจัยในส่วนที่ขาดอยู่ดังกล่าวจะมีความสำคัญเชิงเศรษฐกิจที่สอดคล้องกับการสนับสนุนให้มีการนำปลาบึกมาเลี้ยงในเชิงพาณิชย์ โดยที่ปลาบึกอาจเป็นปลาเศรษฐกิจที่สำคัญของประเทศได้ในอนาคต

ในการศึกษาวิจัยภายใต้โครงการดังกล่าวแบ่งออกเป็นสองส่วนหลัก ๆ คือการศึกษาองค์ประกอบทางเคมี และลักษณะคุณภาพบางประการของเนื้อปลาบึก (ส่วนที่จะนำไปบริโภคได้) โดยมุ่งหาคุณลักษณะเฉพาะของเนื้อปลาชนิดนี้ต่อการยอมรับของผู้บริโภค ทั้งนี้ยังใช้เป็นข้อมูลสำหรับการที่จะนำวัตถุดิบเนื้อปลาบึกไปเก็บรักษาหรือแปรรูปเป็นผลิตภัณฑ์รูปแบบต่าง ๆ อีกด้วย โดยองค์ประกอบพื้นฐานที่ได้ทำการวิเคราะห์ได้แก โปรตีน ไขมัน เถ้า ความชื้น องค์ประกอบของกรดไขมัน เม็ดสี และปริมาณคอลลาเจน เป็นต้น สำหรับลักษณะทางกายภาพที่สำคัญต่อการยอมรับของผู้บริโภคที่ได้ศึกษาประกอบด้วย การวัดความแน่นเนื้อ ความแข็งของเนื้อจากแต่ละส่วน เช่น ส่วนหลัง ส่วนท้อง เป็นต้น การวัดค่าสีของเนื้อในรูปของ ค่าความสว่าง ค่าความเป็นสีแดง ความเป็นสีเหลือง ของเนื้อแต่ละส่วนเพื่อใช้เป็นข้อมูลประกอบการกำหนดเกณฑ์คุณภาพสำหรับเนื้อชนิดนี้ในอนาคต อย่างไรก็ตามในโครงการวิจัยที่ได้เสนอไป จัดให้มีการวิเคราะห์คุณภาพทางด้านจุลินทรีย์โดยเฉพาะปริมาณเชื้อที่รอดชีวิต ปริมาณยีสต์และรา ซึ่งในการทดลองดังกล่าวมีการศึกษาแต่ไม่ได้รายงานผลในผลงานวิจัยนี้ สำหรับงานส่วนที่สองที่ได้ทำการศึกษาวิจัยคือการติดตามการเปลี่ยนแปลงคุณภาพในแต่ละด้านของเนื้อปลาบึกแต่ละส่วนภายใต้การเก็บรักษาแบบแช่เย็นและแช่เยือกแข็ง อย่างไรก็ตามผลการวิจัยที่ได้ดำเนินการแล้วเสร็จในโครงการนี้คือผลของการเก็บรักษาแบบแช่เย็นเท่านั้น สำหรับผลการเก็บรักษาแบบแช่เยือกแข็งจะได้ดำเนินการในภายหน้า สำหรับผลการเก็บรักษาแบบแช่เย็นได้ทำการวิจัยกับเนื้อที่แบ่งออกเป็นส่วนหลัง และส่วนท้อง โดยเก็บไว้ภายใต้อุณหภูมิตู้แช่เย็น (4 องศาเซลเซียส) เป็นเวลานาน 14 วัน แล้วทำการสุ่มตัวอย่างเป็นระยะ ๆ ตลอดช่วงเวลาของการเก็บรักษาเพื่อติดตามการเปลี่ยนแปลงลักษณะคุณภาพของเนื้อปลาบึก โดยตรวจวัดการเปลี่ยนแปลงทางด้านเคมี อันได้แก่ การย่อย

สลาย/การเปลี่ยนแปลงของไขมันโดยวัดค่า conjugated diene, peroxide value, และค่า thiobarbituric acid-reactive substances การเปลี่ยนแปลงของโปรตีนโดยการติดตามรูปแบบของโปรตีนด้วย gel electrophoresis ปริมาณเปปไทด์ที่ละลายได้ในกรด กิจกรรมของเอนไซม์ ปริมาณหมู่ฟังก์ชันที่สำคัญในสารสกัดจากกล้ามเนื้อ เป็นต้น สำหรับการติดตามการเปลี่ยนแปลงทางด้านกายภาพ ได้แก่การวัดความแน่นเนื้อและความเหนียว การติดตามความสามารถในการอุ้มน้ำ การเปลี่ยนแปลงและปริมาณของเม็ดสี รวมถึงการเปลี่ยนแปลงของโครงสร้างขนาดเล็กของกล้ามเนื้อภายใต้กล้องจุลทรรศน์แบบอิเล็กตรอน สำหรับการเปลี่ยนแปลงคุณภาพทางด้านจุลินทรีย์ตลอดระยะเวลาการเก็บรักษาก็ตรวจวัดเช่นเดียวกันกับเนื้อปลาบึกเริ่มต้นแต่ไม่ได้รายงานไว้ในผลงานวิจัยในครั้งนี้

ผลงานจากการวิจัยที่เกิดขึ้นตลอดระยะเวลา 2 ปีของโครงการดังกล่าวสามารถใช้เป็นข้อมูลพื้นฐานสำหรับการอ้างถึงคุณค่าหรือคุณลักษณะทางคุณภาพของเนื้อปลาบึกที่จะนำมาบริโภคได้ นอกจากนั้นสามารถใช้เป็นข้อมูลเบื้องต้นในการจัดหาวิธีหรือแนวทางในการเก็บรักษา หรือแนวทางการนำไปใช้เป็นวัตถุดิบสำหรับการแปรรูปเป็นผลิตภัณฑ์ประเภทต่าง ๆ ต่อไป โดยอย่างน้อยผลการศึกษาเรื่ององค์ประกอบและลักษณะคุณภาพ และการเก็บรักษาแบบแช่เย็นก็สามารถเป็นข้อมูลให้ผู้ประกอบการแล่เนื้อปลาบึกเพื่อจำหน่ายสามารถทราบแนวทางการป้องกันเพื่อไม่ให้เกิดการเน่าเสียของเนื้อปลาบึกในระหว่างการวางจำหน่ายได้ สำหรับผลงานทางด้านวิชาการที่เกิดขึ้นได้แก่การตีพิมพ์เผยแพร่ผลงานวิจัยในวารสารวิชาการระดับนานาชาติจำนวน 3 ฉบับ ดังรายละเอียดดังนี้คือ เรื่อง Discoloration and lipid deterioration of farmed giant catfish (*Pangasianodon gigas)* muscle during refrigerated storage ในวารสาร Journal of Food Science ปี 2008, 73: 179-184. (impact factor **1.601**) ในปีแรกของการดำเนินงานวิจัย สำหรับปีที่สองได้แก่ผลงาน เรื่อง Chemical compositions and characteristics of farmed giant catfish (*Pangasianodon gigas*) muscle ในวารสาร LWT-Food Science and Technology ปี 2010, 43: 452-457. (impact factor **2.114**) และเรื่อง Assessment of protein changes in farmed giant catfish (*Pangasianodon gigas*) muscles during refrigerated storage ในวารสาร International Journal of Food Science & Technology ปี 2010, 45: 985-994. (impact factor **1.172**)

ข้อคิดเห็นที่เกิดขึ้นในระหว่างการดำเนินการวิจัยภายใต้โครงการดังกล่าวคือ ผู้ประกอบการต้องการสร้างเกณฑ์มาตรฐานเกี่ยวกับสินค้าตัวนี้ขึ้นเพื่อใช้ในการกำหนดราคาผลิตภัณฑ์ นอกจากนั้นยังต้องการวิธีการในการเก็บรักษาที่สามารถคงไว้คุณภาพให้ได้ใกล้เคียงกับวัตถุดิบสดมากที่สุด โดยในปัจจุบันที่ผู้ประกอบการดำเนินการอยู่คือการแช่เยือกแข็ง และการชำแหละสดเพื่อวางจำหน่ายในตู้เย็นให้หมดวันต่อวัน นอกจากนั้นแนวทางการใช้ประโยชน์จากเนื้อปลาบึกในอนาคตนอกจากการจำหน่ายในรูปของเนื้อปลาสดแล้ว การแปรรูปเป็นผลิตภัณฑ์ต่าง ๆ ก็ยังเป็นที่ต้องการของผู้ประกอบการรายย่อยและเกษตรกรรายใหญ่อยู่ ซึ่งถือเป็นโจทย์วิจัยที่ฝากไว้ให้นักวิจัยได้ทำการศึกษาค้นคว้าต่อไป

สัญญาเลขที่ MRG5080295

โครงการ: การศึกษาคุณลักษณะ องค์ประกอบ และการเปลี่ยนแปลงระหว่างการเก็บรักษาปลาบึกเลี้ยงในจังหวัดเชียงราย

รายงานสรุปการเงินในรอบ 24 เดือน

ชื่อหัวหน้าโครงการวิจัยผู้รับทุน ผศ.ดร. สาโรจน์ รอดคืน

รายงานในช่วงตั้งแต่วันที่ 2 กรกฎาคม 2550 ถึงวันที่ 1 กรกฎาคม 2552

รายจ่าย

หมวด (ตามสัญญา)	รายจ่ายสะสมจากรายงานครั้งก่อน	ค่าใช้จ่ายงวดปัจจุบัน	รวมรายจ่ายสะสมจนถึงงวดปัจจุบัน	งบประมาณรวมทั้งโครงการ	คงเหลือ (หรือเกิน)
1. ค่าตอบแทน	60,000	30,000	90,000	120,000	30,000
2. ค่าจ้าง					
3. ค่าวัสดุ	60,494.12	77,385.70	137,879.82	130,000	-7,879.82
4. ค่าใช้สอย	31,050.50	71,822.20	102,872.70	110,000	7,127.30
5. ค่าครุภัณฑ์					
6.					
รวม	**151,544.62**	**179,207.90**	**330,752.52**	**360,000**	**29,247.48**

จำนวนเงินที่ได้รับและจำนวนเงินคงเหลือ

จำนวนเงินที่ได้รับ

งวดที่ 1	180,000	บาท	เมื่อ 10/9/50
งวดที่ 2	150,000	บาท	เมื่อ 20/8/51
ดอกเบี้ยรวม 6 ครั้ง	1,781.50	บาท	เมื่อ 30/6/53
ฯลฯ			
	รวม 331,781.50		**บาท** ∂

ค่าใช้จ่าย

งวดที่ 1 เป็นเงิน	101,837.30	บาท
งวดที่ 2 เป็นเงิน	49,707.32	บาท
งวดที่ 3 เป็นเงิน	179,207.90	บาท
ฯลฯ		
	รวม 330,752.52	**บาท** ❷

จำนวนเงินคงเหลือ ∂ - ❷ 1,028.98 **บาท**

(ผศ.ดร. สาโรจน์ รอดคืน)
หัวหน้าโครงการวิจัยผู้รับทุน

(น.ส พัชรินทร์ คำมะวงค์)
เจ้าหน้าที่การเงินโครงการ

Biochemical and Physicochemical Changes of Farmed Giant Catfish Muscle during Refrigerated Storage

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Abstract

After death of fish, several biochemical changes are triggered, resulted in the deterioration of muscle which causes the loss of quality. Low temperature storage is one of the primary preservation methods to delay the deteriorative processes. The objectives of this study were to determine the quality changes of farmed giant catfish muscle during storage. The flesh was excised into dorsal and ventral and cut into pieces, packaged in polyethylene bags, and stored at 4°C for 14 days. During storage, the pieces of fish were randomly taken at days 0, 2, 4, 7, 10, and 14 for biochemical and physicochemical analysis. No changes in Ca^{2+}-ATPase activity with a gradual decrease in hydrophobicity and reactive sulfhydryl content in both dorsal and ventral was found during the first 10 days of storage. A slight increase in TVB-N and TCA-soluble peptide content with a concomitant decrease in expressible water and cooking loss was observed throughout the storage period. Shear force and toughness of both muscles was highest at day 2 of storage and then decreased up to 14 days. Fresh fish had the well organized structure of myofibrils. After 7 days of storage, the myofibrils were less attached as indicated by the noticeable gaping between bundles.

Keywords: biochemical, changes, giant catfish muscle, physicochemical, refrigerated storage

Outputs

1. Rawdkuen, S., Jongjareonrak, A., Benjakul, S., Chaijan, M. *Discoloration and lipid deterioration of farmed giant catfish (Pangasianodon gigase) muscle during refrigerated storage*. Journal of Food Science. 2008, 73: 179-184.
2. Chaijan, M., Jongjareonrak, A., Phatcharat, S., Benjakul, S., Rawdkuen, S. *Chemical compositions and fresh quality attributes of farmed giant catfish (Pangasianodon gigas) muscle*. LWT-Food Science and Technology. *Submitted.*

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Effect of Frozen Storage on the Quality of Farmed Giant Catfish Fillets

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ABSTACT

Frozen storage is a widely used procedure to preserve foods. However, deterioration of these foods is easy to take place. Protein denaturation, lipid oxidation and also some microbiological spoilage are drawbacks associated with storage, which cause the quality changes of the products. So, the effects of frozen storage on the quality of farmed giant catfish muscles were investigated. Flesh cut of farmed giant catfish (*Pangasianodon gigas*) obtained from Jarun farm in Chiang Rai, Thailand was prepared. Fillets were stored at -18°C for 0 to 90 days. During frozen storage, the fillet was taken for measurements of physical (texture, colour, drip loss, and SEM), chemical (TCA-soluble peptides, SDS-PAGE, TBARS, and conjugated diene) and microbiological qualities (Total viable count: TVC, yeast, and mold). There was no significant effect of frozen storage on the hardness and toughness, drip loss and cooking loss ($P>0.05$). However, changes of colour attributes (L^*, a^*, and b^*) were observed ($P<0.05$). Loosen of myofibril structure in the samples were markedly observed with increased storage time. TCA-soluble peptides content increased continuously with a coincidental increase in the myofibrillar proteins (myosin heavy chain and actin) degradation. TBARS and conjugated diene increased as the storage time increased ($P<0.05$). The microbial counts increased from the initial value of 50 to 375 CFU/g sample, ND to 160 CFU/g sample, and ND to 45 CFU/g sample for TVC, yeast, and mold, respectively. As the results, some quality attributes of the fillets significantly affected as frozen storage time continued.

Keywords: frozen storage, giant catfish, quality changes, fish fillet, deterioration

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Discoloration and Lipid Deterioration of Farmed Giant Catfish (*Pangasianodon gigas*) Muscle during Refrigerated Storage

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ABSTRACT: Discoloration and lipid deterioration of farmed giant catfish (*Pangasianodon gigas*) muscle during 14 d refrigerated storage were investigated. Lipid deterioration, lipolysis, and lipid oxidation in both dorsal and ventral muscles increased as storage time increased. A progressive formation of primary lipid oxidation products monitored by the increase in conjugated dienes (CD) was observed ($P < 0.05$) and the increase in thiobarbituric reactive substances (TBARS), an index of secondary lipid oxidation products, was noticeable throughout the storage ($P < 0.05$). The pH of both dorsal and ventral muscles tended to increase as storage time continued ($P < 0.05$). A gradual increase in free fatty acid (FFA) formation was found within the first 10 d of refrigerated storage ($P < 0.05$), suggesting hydrolysis induced by lipases and phospholipases. However, a sharp decrease in FFA content was observed at the end of storage. Refrigerated storage also resulted in changes in redness index of both dorsal and ventral muscles. These changes were coincidental with the changes in metmyoglobin content. Therefore, the discoloration and lipid changes in giant catfish muscle during refrigerated storage depended on the muscle type and might be related to the difference in composition between dorsal and ventral muscles.

Keywords: discoloration, farmed giant catfish, lipid oxidation, lipolysis, muscle, refrigerated storage

Introduction

Chilling is a means to preserve fish before processing or consumption. The growth of microorganisms and chemical reactions are slowed when fish is stored at low temperature (Benjakul and others 1997; Pacheco-Aguilar and others 2000). However, lipid deterioration easily takes place and limits the shelf life of fish during storage (McDonald and Hultin 1987; Cho and others 1989). Both lipolysis and lipid oxidation in fish muscle are associated with quality deterioration (Pacheco-Aguilar and others 2000). Hydrolysis, induced by lipases and phospholipases, produces free fatty acids that undergo further oxidation to produce low-molecular-weight compounds that are responsible for the rancid off-flavor and taste of fish and fish products (Toyomizu and others 1981). The lipid components of postmortem fish muscle tissue are prone to oxidation because fatty acids of fish lipids are much more unsaturated than those of mammals and birds (Foegeding and others 1996). The basic mechanisms of lipid oxidation can be characterized by 3 distinctive steps: initiation, propagation, and termination reactions (Nawar 1996; Undeland 2001). This phenomenon can be influenced by both intrinsic and extrinsic factors such as the fatty acid composition, the concentration of prooxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, and oxygen consumption reaction (Undeland 2001; Andreo and others 2003). Metmyoglobin formation can induce the discoloration of fish muscle (Haard 1992). This reaction is positively correlated with lipid oxidation (Chan and others 1997; Lee and others 2003). Chaijan and others (2005) reported that the release of nonheme iron from myoglobin in sardine and mackerel during iced storage might be associated with the induced oxidation process in the muscle.

The giant catfish (*Pangasianodon gigas*) is the world's largest scale-less freshwater fish. This species can reach about 3 m in length and can weigh more than 300 kg (Roberts and Vidthayanon 1991). The giant catfish is an endemic species of the main channel and tributaries of the Mekong River of Thailand, Laos, Cambodia, and Vietnam (Jondeung and others 2007). Nowadays, the giant catfish is successfully bred in captivity, and has shown one of the most rapid growth rates of any freshwater fish in Thailand (Roberts and Vidthayanon 1991). The majority of cultured giant catfish is sold raw for cooking in restaurants. After harvesting and being sold, fish are normally refrigerated at 4 °C to keep the quality. Roberts (2001) reported that product quality of farmed fish depends on a wide range of factors, including the nature of the fish stock, slaughter techniques, and methods of storage, processing, and transport. However, no information regarding the changes in lipid and discoloration in muscle of giant catfish cultured in Thailand during refrigerated storage has been reported. Thus, this study aimed to investigate the changes of lipid components, lipolysis and lipid oxidation and discoloration in dorsal and ventral muscle of farmed giant catfish (*Pangasianodon gigas*) meat during extended storage at 4 °C.

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Materials and Methods

Chemicals

Palmitic acid, cupric acetate, and pyridine were purchased from Sigma (St. Louis, Mo., U.S.A.). Trichloroacetic acid, anhydrous sodium sulfate, and isooctane were obtained from Merck

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(Darmstadt, Germany). Chloroform was purchased from BDH (Poole, U.K.). 2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane were procured from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

Sample preparation

Giant catfish (age 5 y with an average weight of 25 to 30 kg; $n = 5$) were obtained from a farm in Phan, Chiang Rai, and transported alive to the Food Technology Laboratory, Mae Fah Luang Univ., Chiang Rai, Thailand. Upon arrival, fish were exsanguinated and filleted. The fillets were packaged in polyethylene bags, kept on ice with a meat/ice ratio of 1:2 (w/w), and transported to the Dept. of Food Technology, Prince of Songkla Univ., Hat Yai, Songkhla within 24 h. The fish samples were manually excised into dorsal and ventral muscles and cut into 0.5 kg pieces, packaged in polyethylene bags individually, and stored at 4 °C for 14 d. During storage, 3 pieces of fish were randomly taken at days 0, 2, 4, 7, 10, and 14 for analyses. The flesh was chopped to uniformity and used for analyses. The muscles were kept on ice during preparation and analysis.

pH determination

The pH of fish muscle was measured as described by Benjakul and others (1997). Fish muscle was homogenized using an IKA Labortechnik homogenizer (Selangor, Malaysia) with 10 volumes of deionized water (w/v), and the pH was measured using a pH meter (Cyberscan 500, Singapore).

Determination of redness index

Colorimetric values of the mince were obtained in triplicate by using a portable Hunterlab Miniscan/EX instrument (10° standard observers, illuminant D65, Hunter Assoc. Laboratory; Va., U.S.A.). The instrument was calibrated to a white and black standard. The tristimulus L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) measurement mode was used as it relates to the human eye response to color. The redness index (a^*/b^*) of fish mince was calculated as described by Chen and others (1997).

Measurement of metmyoglobin content

The analysis of metmyoglobin formation was performed relatively as described by Lee and others (1999). A chopped fillet sample (2 g) was weighed into a 50-mL polypropylene centrifuge tube and 20 mL of cold 40 mM phosphate buffer, pH 6.8, was added. The mixture was homogenized with an IKA Labortechnik homogenizer (Selangor, Malaysia) at 13500 rpm for 10 s. The homogenate was centrifuged at 3000 × *g* for 30 min at 4 °C, using a Sorvall RC 26 Plus (Sorvall, Norwalk, Conn., U.S.A.). The supernatant was filtered with Whatman nr 1 filter paper. The supernatant was subjected to absorbance measurement at 630 and 525 nm using a 40 mM phosphate buffer, pH 6.8 as blank. The ratio of A_{630} to A_{525} was calculated according to Hansen and Sereika (1969). A high A_{630}/A_{525} ratio indicates a high relative proportion of metmyoglobin.

Measurement of thiobarbituric acid reactive substances (TBARS)

TBARS assay was performed as described by Buege and Aust (1978). Ground sample (0.5 g) was homogenized with 2.5 mL of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl, using an IKA Labortechnik homogenizer. The mixture was heated in a boiling water bath (95 to 100 °C) for 10 min to develop a pink color, cooled with running tap water, and centrifuged at 3600 × *g* at 25 °C for 20 min using a RC-5B plus centrifuge (Sorvall). The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malonaldehyde/kg sample.

Lipid extraction

Lipid was extracted by the Bligh and Dyer (1959) method. Sample (25 g) was homogenized with 200 mL of a chloroform:methanol:distilled water mixture (50:100:50) at a speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogenizer. The homogenate was treated with 50 mL of chloroform and homogenized at 9500 rpm for 1 min. Then, 25 mL of distilled water were added and homogenized again for 30 s. The homogenate was centrifuged at 1000 × *g* at 4 °C for 15 min using a RC-5B plus centrifuge (Sorvall), and transferred into a separating flask. The chloroform phase was drained off into the 125 mL Erlenmeyer flask containing about 2 to 5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman nr 4 filter paper. The solvent was evaporated at 25 °C using an EYELA rotary evaporator N-100 (Tokyo, Japan), and the residual solvent was removed by flushing nitrogen.

Measurement of conjugated diene (CD)

CD was measured according to the method of Frankel and Huang (1996). Oil sample (0.1 g) was dissolved in 5.0 mL of isooctane and the absorbance was measured at 234 nm using a UV-1601 spectrophotometer (Shimadzu, Japan).

Measurement of free fatty acid (FFA)

FFA content was determined according to the method of Lowry and Tinsley (1976). The lipid sample (0.1 g) was treated with 5 mL of isooctane and swirled vigorously to dissolve the sample. The mixture was then treated with 1 mL of 5% (w/v) cupric acetate–pyridine reagent, prepared by dissolving reagent-grade cupric acetate, filtering, and adjusting the pH to 6.0 to 6.2 using pyridine. The mixture was shaken vigorously for 90 s using a Vortex-Genie2 mixer (Bohemia, N.Y., U.S.A.) and allowed to stand for 10 to 20 s. The upper layer was subjected to absorbance measurement at 715 nm, using a UV-1601 spectrophotometer (Shimadzu). A standard curve was prepared using palmitic acid in isooctane at concentrations ranging from 0 to 50 μmol/5 mL. FFA content was expressed as g FFA/100 g lipid.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test ($P < 0.05$; Steel and Torrie 1980). Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Changes in pH

Changes in pH of dorsal and ventral muscles of giant catfish during refrigerated storage for 14 d are shown in Figure 1. The initial pH value of dorsal and ventral muscle was 6.33 and 6.35, respectively. The ultimate pH value of fish muscle is around 6.2 to 6.6 (Foegeding and others 1996). Huss (1988) suggested that the initial postmortem pH of fish varies with species, catching ground, and season. Chaijan and others (2004) reported that the postmortem pHs of sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle were approximately 6.53 to 6.58 and 6.24 to 6.27, respectively. In addition, the postmortem pH value of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) was 6.40 (Chomnawang and others 2007). The results showed fluctuation in pH for both dorsal and ventral muscles. The pH of ventral muscle increased up to day 4 of refrigerated storage, continuously decreased up to day 10, and gradually increased until the end of storage period (day 14) ($P < 0.05$). The decline in pH is influenced by the glycolytic activity and the hydrolysis of ATP (Foegeding and others 1996). After death, the anaerobic glycolysis continues to regenerate some ATP with the end product, lactate, accumulating. ATP can be further hydrolyzed and produced hydrogen atoms (Foegeding and others 1996). On the other hand, the increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes. Benjakul and others (2002) reported that the decomposition of nitrogenous compounds caused an increase in pH in fish flesh. For the dorsal muscle, the pH decreased after 2 d of storage and reached the highest value at day 4. Thereafter, it decreased up to day 10 and tended to increase until the end of storage period (day 14). The differences in pH changes between the 2 muscle types were probably due to the differences in glycogen contents and buffering capacity of muscle. Foegeding and others (1996) reported that some amino acids and dipeptides such as serine and carnosine, respectively, function as buffering agents in the muscle cell. The storage glycogen was presumably greater in dorsal muscle when compared to ventral muscle as shown by the decrease in pH of dorsal muscle after 2 d of storage. Glycolysis might still occur at that time and generate the lactic acid and other protons and hence cause the decrease in pH. Furthermore, the activity of enzymes converting glycogen into lactic acid might be different between 2 muscles. Lactic acid, generated in anoxic conditions from glycogen, is the principal factor in lowering the postmortem pH in the fish muscles (Sikorski and others 1990). The increase in pH at day 4 of storage might contribute to the slowdown of glycolysis caused by the depletion of glycogen and by the generation of volatile bases such as ammonia and trimethylamine in the muscle. Thereafter, the gradual decrease in pH until day 10 was observed. This was probably due to the greater rate of ATP degradation, which contributed to the decrease in pH. At the end of storage, the degradation of muscle proteins with the growth of some microorganisms might produce the volatile bases to a greater extent. As a consequence, the content of alkaline compounds was higher than that of protons generated by ATP hydrolysis, the increase in pH was observed.

Changes in CD

The formation of primary lipid oxidation products as well as the formation of CD of giant catfish oil extracted from both dorsal and ventral muscles during refrigerated storage up to 14 d is shown in Figure 2. In general, after peroxides are formed, the nonconjugated double bonds (C=C-C-C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C-C=C) (Gunstone and Norris 1983). This is accompanied by increased UV absorption at 234 nm (Zuta and others 2007). The increase in absorbance at 234 nm is an indicator of autooxidation and is reported to increase with uptake of oxygen and formation of peroxides during the early stages of oxidation (Weber and others 2008). The changes in CD of dorsal and ventral muscles occurred in the same manner (Figure 2). The maximum value of CD in both dorsal and ventral muscles was found at day 14 of storage ($P < 0.05$). For dorsal muscle, the CD sharply decreased at the first 2 d of storage and gradually increased throughout the storage period ($P < 0.05$). For ventral muscle, it decreased in the first 4 d of storage and continuously increased until the end of storage time. The formation and decomposition rates of CD participated in the changes in CD value (Chaijan and others 2006). If these rates are equal, no changes in CD are observed (Chaijan and others 2006). The decrease in CD in the 1st period was probably due to the higher rate of decomposition of primary lipid oxidation products to yield the secondary products. The increase in CD in the later stage might contribute to the excessive oxidation of lipid. The rate of CD formation could be greater than the decomposition rate, leading to increase in CD accumulated in the lipid fraction. Perez-Alonso and others (2003) reported no changes in CD in dorsal muscle of Atlantic pomfret within the first 9 d of chilled storage, followed by a gradual increase up to 19 d of storage. For sardine (*S. gibbosa*) muscle, no difference in CD was found within the first 12 d of iced storage and a slight increase in CD was observed at the end of storage (day 15) (Chaijan and others 2006).

Changes in TBARS

TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar 1996). The TBARS value of both dorsal and ventral muscles of giant catfish during refrigerated storage tended to increase throughout the storage ($P < 0.05$; Figure 3). The initial value of TBARS of dorsal and ventral muscles was 0.61 and 0.75 mg/kg meat, respectively, suggesting that lipid oxidation occurred during postmortem handling to some extent. The TBARS value increased from day 0 to day 7, gradually decreased at day 10, and increased at the end of storage (day 14). The increase in TBARS indicated the formation of secondary lipid oxidation products, especially

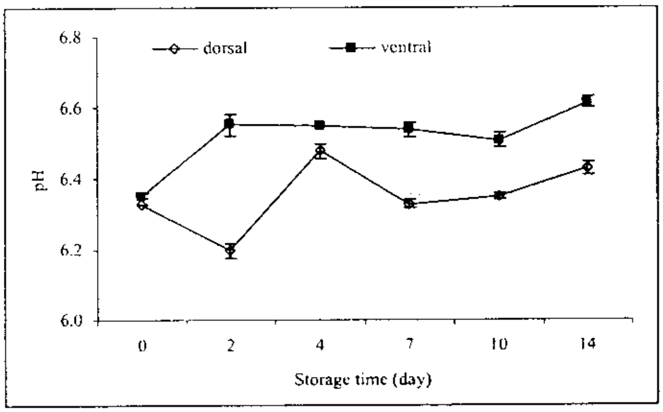

Figure 1 – Changes in pH of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

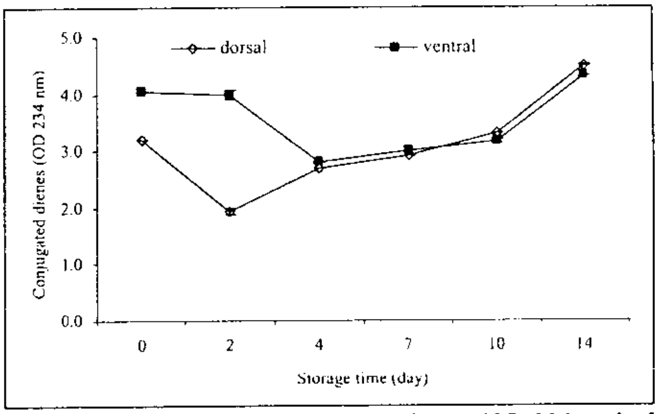

Figure 2 – Changes in conjugated dienes (OD 234 nm) of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

aldehydes (Kolakowska 2002). Hydroperoxides break down in several steps, yielding a wide variety of decomposition products, including aldehydes (Nawar 1996). The oxidation of lipid in both dorsal and ventral muscles occurred progressively at the first 7 d. The decrease in TBARS at day 10 was probably due to the decomposition of aldehydes or the interaction of aldehydes with muscle proteins. Esterbauer and others (1991) reported that lipid oxidation generates a wide range of secondary aldehyde products, including n-alkanals, trans-2-alkenals, 4-hydroxy-trans-2-alkenals, and malonaldehyde. Secondary products from lipid oxidation, especially aldehydes, can induce myofibrillar proteins crosslinking, resulting in structural and functional changes in these proteins (Li and King 1999; Tironi and others 2002). However, it is most likely that lipid oxidation took place at a higher rate at the end of the storage period. The marked increase in TBARS was probably due to the destruction of hydroperoxides into the secondary oxidation products, especially aldehydes, in the later stages of lipid oxidation. This was probably due to the greater degradation of muscle proteins, which released prooxidants as well as the nonheme iron from the excessively degraded muscles with increasing storage time. Chaijan and others (2005) reported that the nonheme iron content in sardine and mackerel muscle tended to increase throughout iced storage for 15 d. In accordance with Decker and Hultin (1990), the soluble hemin concentration in mackerel muscle increased within 13 d of iced storage. The increase in prooxidants with the disappearance of the natural antioxidants at the end of storage period caused the oxidation to the higher extent when compared to the decomposition rate of oxidation products. Hence, the increase in TBARS was observed. This phenomenon occurred in both dorsal and ventral muscles and resulted in the deterioration and unacceptability of the giant catfish meat.

Changes in FFA

Hydrolysis of glycerol-fatty acid esters is one important change that occurs in fish muscle lipids postmortem with the release of FFAs. This is catalyzed by lipases and phospholipases (Pacheco-Aguilar and others 2000). Changes in FFA content of giant catfish oil extracted from fresh and aged dorsal and ventral muscles are shown in Figure 4. The free fatty acid content of both dorsal and ventral muscles tended to increase up to day 10 of refrigerated storage and decreased until the end of storage period ($P < 0.05$). The greater hydrolysis of triglyceride and/or phospholipids occurred in dorsal muscle when compared to ventral muscle. It can be postulated that the activity of lipases and phospholipases in dorsal muscle was greater than that in ventral muscle. However, extracellular lipase, produced by certain microorganisms such as *Pseudomonas fragi*, also contributed to the lipolytic breakdown of fish lipids (Nayak and others 2003). The decrease in FFA found at day 14 might be related to the increased lipid oxidation as shown by the increased TBARS (Figure 3). The released FFA especially unsaturated fatty acid might be further oxidized and caused the decrease in FFA content. Furthermore, the decrease in FFA content could be attributable to the growth of some microorganisms that can use the FFA as an energy source.

Metmyoglobin formation

Changes in metmyoglobin content of giant catfish dorsal and ventral muscles during refrigerated storage are presented in Figure 5. Metmyoglobin content in ventral muscle gradually decreased up to 10 d and increased at the end of storage ($P < 0.05$). The decrease in metmyoglobin content can be classified into 2 mechanisms, reduction and oxidation. The enzyme metmyoglobin reductase and some reducing agents such as NAD^+ or FAD^+ remaining in the muscle might be activated during this storage period, resulting in decreased metmyglobin content. It has been known that metmyoglobin reductase is a component of red blood cells and can be found in fish muscle (Al-Shaibani and others 1977). In muscle tissue, reducing substances like NAD^+ or FAD^+ are endogenously produced, and they are responsible for the constant reduction of the brown-gray metmyoglobin or the purple myoglobin

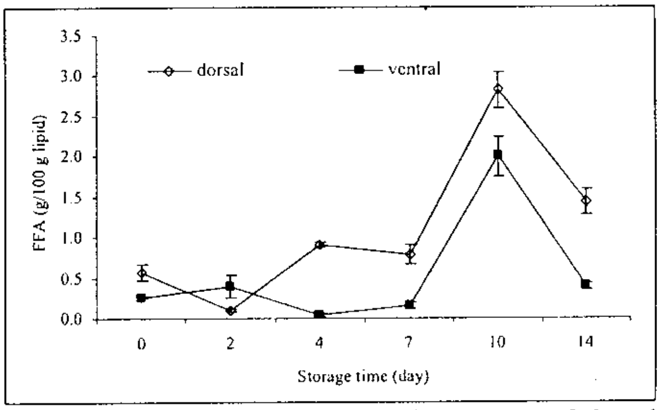

Figure 4—Changes in free fatty acid contents of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

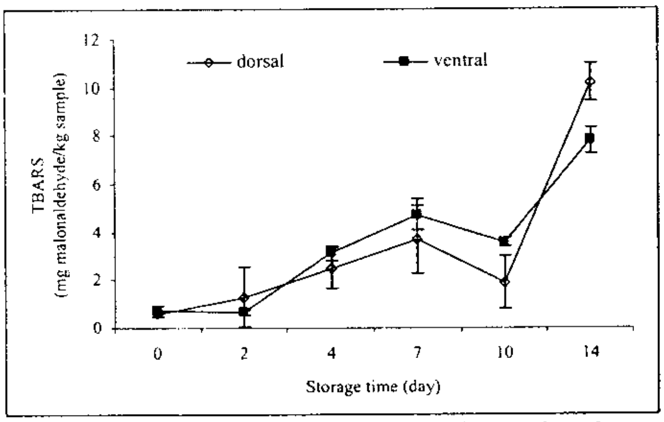

Figure 3—Changes in TBARS values of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

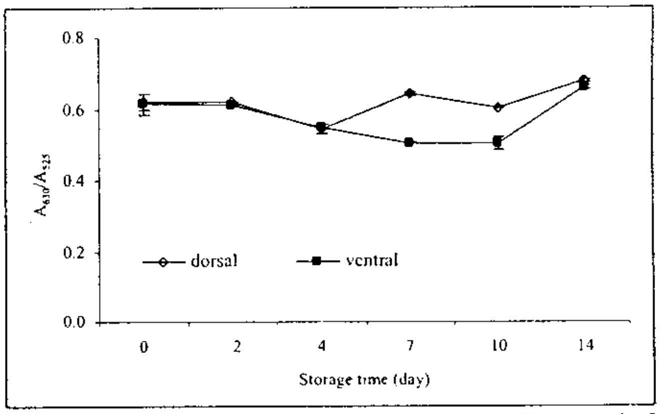

Figure 5—Changes in metmyoglobin content (A_{630}/A_{525}) of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

(Eder 1996). The metmyoglobin reducing capacity in postrigor muscle depended upon the availability of substrate, cofactors (for example, pyridine nucleotide) and associated enzymic activities (for example, dehydrogenase) (Giddings 1974). The generation of cytosolic NADH was essential for metmyoglobin reduction (Watts and others 1966). However, certain microorganisms, especially psychrophilic bacteria, might grow on the samples and possibly reduced metmyoglobin to some extent. Faustman and others (1990) reported that in the presence of high levels of bacterial contamination such as fluorescent pseudomonas, meat may display red color, which appeared to coincide with an increase in pH (Figure 1). On the other hand, some metmyoglobin can be further oxidized to yield the perferrylmyoglobin and/or ferrylmyoglobin. Hence, the decrease in metmyoglobin was also found. The reaction between hydrogen peroxide, a potential by-product of myoglobin oxidation, and metmyoglobin resulted in the formation of a red pigment, ferrylmyoglobin (Baron and Andersen 2002). Davies (1990, 1991) reported that interaction between metmyoglobin and hydrogen peroxide is a complex mechanism, resulting in the generation of 2 distinct hypervalent myoglobin species, perferrylmyoglobin ($^{\cdot}$MbFe(IV) = O) and ferrylmyoglobin (MbFe(IV) = O). These hypervalent myoglobin species served as prooxidants in lipid oxidation (Baron and Andersen 2002). This result was in agreement with the increase in TBARS (Figure 3), which was used as an index of oxidation. Metmyoglobin in ventral muscle increased at day 14. This was probably due to the inactivation of metmyoglobin reductase and the degradation of reducing substances in muscle. Then, the myoglobin underwent oxidation to a greater extent. The inactivation of enzymes, which maintain the reduced state of hemoproteins, was also presumed to increase the formation of metmyoglobin (Benjakul and Bauer 2001).

For dorsal muscle, the metmyoglobin decreased up to day 4 of storage and tended to increase throughout the storage period ($P < 0.05$; Figure 5). The difference in changes in metmyoglobin content between the 2 muscle types is possibly due to the difference in muscle composition. Dorsal muscle might contain lower metmyoglobin reductase and other reducing agents when compared to ventral muscle. Al-Shaibani and others (1977) reported that metmyoglobin reductase is localized in both red blood cells and muscle. Since some blood is retained in the muscle, especially ventral muscle, residual activity of this enzyme could be present and resulted in the retardation of color deterioration. Distribution and localization of myoglobin in both muscles might also be different, leading to the different susceptibility of myoglobin to oxidation.

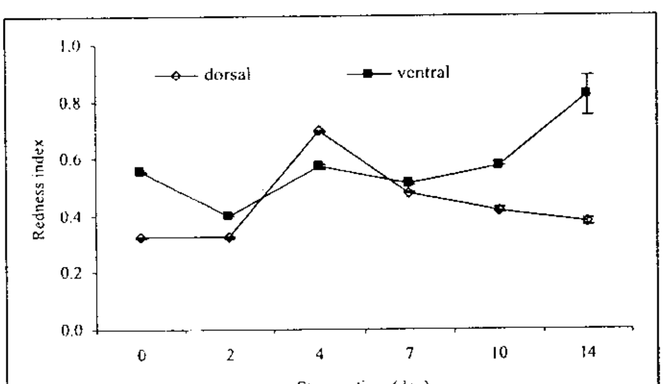

Figure 6—Changes in redness index of dorsal and ventral muscles of giant catfish during refrigerated storage. Bars indicate standard deviations from triplicate determinations.

Changes in redness index

Changes in redness index (a^*/b^*) of giant catfish dorsal and ventral muscles during refrigerated storage occurred in a different manner (Figure 6). This ratio was used as an index of apparent change in redness (Chen and others 1997) and used to evaluate the discoloration in tuna meat during storage (Lee and others 2003). The redness index of ventral muscle decreased at the first 2 d of storage and tended to increase throughout the storage period ($P < 0.05$). The increased redness index or a red shift was coincidental with the decrease in metmyoglobin content in ventral muscle (Figure 5). For the dorsal muscle, redness index increased up to day 4 and continuously decreased until the end of storage ($P < 0.05$). This result was also in accordance with the formation of metmyoglobin (Figure 5). The difference in color changes in dorsal and ventral muscle might be due to the difference in composition between these 2 muscles.

Conclusions

Giant catfish lipids were susceptible to hydrolysis and oxidation during refrigerated storage. Primary lipid oxidation products, conjugated dienes, were generated and underwent some changes to yield secondary oxidation products, especially aldehydes. An increase in lipolysis as evidenced by free fatty acid formation was observed throughout the storage period. Those lipid changes showed a detrimental effect on giant catfish meat quality and might be associated with the discoloration of muscle. The results could possibly be used for the appropriate postharvest management of cold-stored giant catfish meat for human consumption and the possibility of utilizing giant catfish meat for value-added products.

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(BME) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium dodecyl sulphate (SDS), N, N, N′, N′- tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium chloride, Sodium dithionite, Trichloroacetic acid (TCA) and Tris (hydroxymethyl)–aminomethane were obtained from Merck (Damstadt, Germany).

2.1.2. Fish preparation

Giant catfish (age ~5 years with an average weight of 25–30 kg) was obtained from Charun farm in Phan District, Chiang Rai province, placed in plastic box and transported to the food technology laboratory, Mae Fah Luang University. Upon the arrival, fish were exsanguinated and filleted. The fillets were placed in ice at a meat/ice ratio of 1:3 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 8 h. The fillets were kept on ice during preparation for analysis and until subjected to analysis.

2.2. Proximate compositions and chemical property analyses

2.2.1. Proximate compositions

Three part of fish muscles (dorsal, ventral and lateral line; Fig. 1) were subjected to analyse for moisture, ash, fat and protein contents according to the method of AOAC (2000). The values (from 3 replications) were expressed as g/100 g sample (wet weight basis). Crude protein ($N = 6.25$) was determined according to the Kjeldahl method. Moisture content was determined by oven drying at 105 ± 2 °C to constant weight and ash by heating in a muffle furnace at 550 °C to constant weight. Crude fat content was determined by the Soxhlet extraction system.

2.2.2. pH determination

The pH of fish muscle was measured after the fish muscle was homogenised using an IKA Labortechnik homogeniser (Selangor, Malaysia) with 10 volumes of deionised water (w/v). The pH of the prepared sample was measured using a pH meter (Cyberscan 500, Singapore).

2.2.3. Hydroxyproline content

The hydroxyproline content of muscle was analysed according to the method of Bergman and Loxley (1963) with a slight modification. The sample was hydrolysed with 6 mol/L HCl in a screw cap tube at 110 °C for 24 h in an oil bath (model B-490, BUCHI, Flawil,

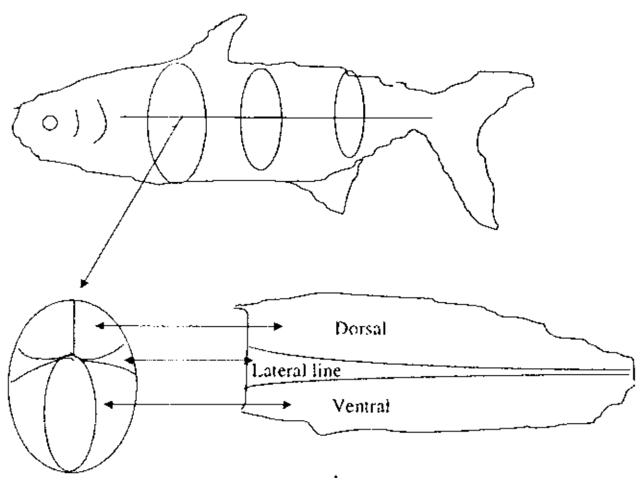

Fig. 1. Diagram showing the dorsal, ventral and lateral line regions from where the meat was excised for study.

Switzerland). The hydrolysed sample was clarified with activated carbon and filtered through Whatman No. 4 filter paper. The filtrate was then neutralised with 10 mol/L NaOH to obtain a pH of 6.0–6.5. The neutralised sample (0.1 mL) was transferred into a test tube and isopropanol (0.2 mL) was added and mixed well; 0.1 mL of oxidant solution (mixture of 7 g/100 mL chlororamine T and 0.1 mol/L acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v)) was added and mixed thoroughly. A 1.3 mL of Ehrlich's reagent solution (mixture of solution A; 2 g of p-dimethylamino-benzaldehyde in 3 mL of 60 mL/100 mL perchloric acid) and isopropanol at a ratio of 3:13 (v/v) were added and mixed. The mixture was heated at 60 °C for 25 min in a water bath (Memmert, Schwabach, Germany) and then cooled for 2–3 min using running water. The solution was diluted to 5 mL with isopropanol. Absorbance of the mixture was measured at the wavelength of 558 nm. Hydroxyproline standard solution, with concentration ranging from 0 to 60 ppm, was also included. Hydroxyproline content was calculated and expressed as mg/g of sample.

2.2.4. Myoglobin content

The myoglobin content was determined by direct spectrophotometric measurement, as described by Chaijan, Benjakul, Visessanguan, and Faustman (2004). A chopped sample of flesh (2 g) was weighed into a 50-mL polypropylene centrifuge tube and 20 mL of cold 40 mmol/L phosphate buffer, pH 6.8 were added. The mixture was homogenised at 13,500 rpm for 10 s, followed by centrifuging at 3000 × g for 30 min at 4 °C, using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The supernatant was filtered with Whatman No. 1 filter paper. The supernatant (2.5 mL) was treated with 0.2 mL of 1 g/100 mL sodium dithionite (Merck, Damstadt, Germany) to reduce the myoglobin. The absorbance was read at 555 nm using a UV-1601 spectrophotometer (Shimadzu, Japan). Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 and a molecular weight of 16,110. The myoglobin content was expressed as mg/g sample.

2.2.5. Total pigment

The total pigment content was determined according to the method of Chaijan et al. (2004). Flesh (2 g) was mixed with 9 mL of acid acetone (90 mL/100 mL acetone, 8 mL/100 mL deionised water and 2 mL/100 mL HCl). The mixture was macerated with a glass rod and allowed to stand for 1 h at room temperature. The extract was filtered with a Whatman No. 42 filter paper, and the absorbance was read at 640 nm against an acid acetone blank. The total pigments were calculated as hematin using the following formula:

$$\text{Total pigment content}(\text{mg/kg}) = A_{640} \times 680.$$

2.3. Lipid determination

2.3.1. Lipid extraction

Lipid was extracted by the method of Bligh and Dyer (1959). Sample (25 g) was homogenised with 200 mL of a chloroform:methanol:distilled water mixture (50:100:50) at the speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was treated with 50 mL of chloroform and homogenised at 9500 rpm for 1 min. Then, 25 mL of distilled water were added and homogenised again for 30 s. The homogenate was centrifuged at 4026 × g at 4 °C for 15 min using a RC-5 B plus centrifuge (Sorvall, Norwalk, CT, USA), and transferred into a separating flask. The chloroform phase was drained off into a 125-mL Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman No. 4 filter paper. The solvent was evaporated at 25 °C, using an EYELA rotary evaporator

N-100 (Tokyo, Japan), and the residual solvent was removed by flushing with nitrogen.

2.3.2. Lipid composition

Lipid classes were determined using a thin layer chromatography/flame ionisation detection analyser (IATROSCAN–TLC/FID Analyser, IATRON Laboratories, Inc., Tokyo, Japan). One μl of lipid sample (0.25 mg/mL) was spotted onto the scanned quartz rod (silica powder coated Chrmatorod-S III, IATRON Laboratories, Inc., Tokyo, Japan) and separated using a mixture of benzene:chloroform:acetic acid (50:20:0.7) for 35 min. The developed sample was dried in an oven at 105 °C for 5 min and immediately scanned with the TLC–FID analyser with a scanning speed of 30 s/scan. The analytical conditions were: H_2, flow rate of 160 mL/min; air, flow rate of 2000 mL/min. Retention times of lipid standards were used to identify chromatographic peaks of the samples. Each lipid was calculated, based on peak area ratio and expressed as mg lipid/kg meat.

2.3.3. Fatty acid profile

Fatty acid profile was determined as fatty acid methyl esters (FAMEs). The FAMEs were prepared according to the method of AOAC (2000). The prepared methyl ester was injected to the gas chromatography (Shimadzu, Kyoto, Japan) equipped with the flame ionisation detector (FID) at a split ratio of 1:20. A fused silica capillary column (30 m × 0.25 mm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250 °C and detector temperature of 270 °C. The oven was programmed from 170 °C to 225 °C at a rate of 1 °C/min (no initial or final hold). Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g oil.

2.4. Physical attributes

2.4.1. Textural analyses

Texture was analysed by a texture analyser, model TA-XT2 (SMS, Stable Micro Systems; Surrey, England), equipped with a Warner–Bratzler blade (60° knife edge blade) according to the method of Espe et al. (2004). The cylindrical longitudinal muscle samples of the fillet were cut out with a borer of 11 mm in diameter. The blade was pressed down at a constant speed of 2 mm/s through the sample, cutting the muscle fiber transversely. Maximum shear force (*N*) and work needed to cut the sample (area under the curve during shearing: *N*s) were recorded from six measurements for each cut.

2.4.2. Colour

Fillet colour was measured with a portable Hunterlab instrument (10° standard observers, illuminant D65, Hunter Associates Laboratory; Virginia, USA) according to the method of Chaijan et al. (2004). The instrument was calibrated to a white and black standard. The tristimulus L^*, a^* and b^* measurement mode were used. L^* represents lightness ($L^* = 0$, black; and $L^* = 100$, white), + a^* represents the intensity in red and + b^* represents the intensity in yellow. Colour was measured at six locations on each fillet, and the mean value of the six measurements was used. The redness index (a^*/b^*) of meat was calculated.

2.5. Muscle protein fractionation

Non-protein nitrogenous constituents, sarcoplasmic protein, myofibrillar protein, alkali-soluble protein and stromal proteins in different cuts were fractionated according to the method of. Hashimoto, Watabe, Kono, and Shiro (1979). Nitrogen content in each fraction was measured by the Kjeldahl method (AOAC, 2000). Protein patterns of different fractions were determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), with 10 mL/100 mL running gel and 4 mL/100 mL stacking gel, as described by Laemmli (1970).

2.6. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests ($p < 0.05$). Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, Ill., USA).

3. Results and discussion

3.1. Proximate composition

Proximate composition of farm raised giant catfish muscle is presented in Table 1. Different parts of muscle exhibited the different compositions. Water was the major constituent in all parts of muscle. The highest value was found in ventral portion (81.67 g/100 g). Protein, fat and ash content were found in the ranges of 14–19, 0.54–8.60 and, 1.11–1.47 g/100 g, respectively in these three parts. When comparing the meat cuts, dorsal muscle can be classified into a lean meat because it showed the highest content of protein (~19 g/100 g) and the lowest content of fat (~0.54 g/100 g). Meat from lateral line possesses 2.04 and 15.93-fold fat content when compared to those from ventral and dorsal, respectively.

Wide variation in moisture content between species was observed in raw freshwater fish, ranging from 65 to 80 g/100 g (Puwastien et al., 1999). Crude protein content in fish flesh varies depending on the species, the nutritional condition, the type of fish, the state of nutrition, and the productive cycle of animal as well as the parts of the organism (Sikorski et al., 1990). Puwastien et al. (1999) also reported that the protein content ranged from 17 to 20 g/100 g for raw freshwater fish. Chaijan et al. (2004) found that dark muscle of mackerel and sardine contained the lower protein content, compared with the ordinary and whole muscles. Generally, the lipid content of cultured fish muscle is higher than that of wild fish which may be caused by lack of exercise, overfeeding and high energy diets (Rodrguez et al., 2004). Nakamura, Ando, Seoka, Kawasaki, and Tsukamasa (2006) reported that the lipid content of dorsal was significantly lower than that of the ventral part. They also reported that the lipid content in the wild type of Pacific bluefin tuna was lower than that of the cultured. Dark muscles generally contain about 2–5 times more lipids than the ordinary muscle (Sikorski et al., 1990). Ash content in the ranges of 0.6–1.8 g/100 g was reported from cultured and wild Pacific bluefin tuna and

Table 1
Proximate composition, hydroxyproline content, pigment content and pH of farmed giant catfish from different cuts.

Composition	Meat cut*		
	Dorsal	Ventral	Lateral line
Moisture[a]	78.88 ± 0.17 b**	81.67 ± 0.00 c	75.51 ± 0.99 a
Protein[a]	19.00 ± 0.03 c	14.36 ± 0.03 a	17.27 ± 0.44 b
Fat[a]	0.54 ± 0.14 a	4.21 ± 0.26 b	8.60 ± 0.14 c
Ash[a]	1.47 ± 0.12 b	1.13 ± 0.03 a	1.11 ± 0.15 a
Hydroxyproline[b]	0.73 ± 0.01 a	0.83 ± 0.01 c	0.75 ± 0.01 b
Myoglobin content [b]	3.47 ± 0.04 a	3.55 ± 0.24 b	7.83 ± 0.07 c
Total pigment[c]	2.73 ± 0.00 a	6.13 ± 0.01 b	17.03 ± 0.03 c
pH	6.33 ± 0.00 b	6.35 ± 0.01 c	6.26 ± 0.01 a

*Values are given as mean ± S.D from triplicate determinations.
**Different letters in the same row indicate significant differences ($p < 0.05$).
[a] g/100 g (wet weight).
[b] mg/g sample.
[c] mg/kg.

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Original article

Assessment of protein changes in farmed giant catfish (*Pangasianodon gigas*) muscles during refrigerated storage

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Summary Farmed giant catfish (*Pangasianodon gigas*) muscles (dorsal and ventral sites) were stored in a refrigerator (at 4 °C) for 14 days to determine the effect of refrigerated storage on biochemical and physical changes. The analyses were carried out at 0, 2, 4, 7, 10 and 14 days of storage. At day 14, Ca^{2+} -ATPase activity markedly decreased when compared to its value at day 1 (>90%), while a small decrease was observed for surface hydrophobicity and reactive sulfhydryls content. Total volatile basic nitrogen and trichloroacetic-soluble peptide content gradually increased when the storage period was extended. The myosin heavy chain decreased slightly on SDS-PAGE for both meat cuts with increased storage time. Expressible drip and cooking loss were highest during the first day of storage and slightly decreased with storage time. Instrumental hardness was significantly higher in the ventral compared to the dorsal muscle, while the toughness was the highest at the second day of storage. The muscle bundles with scanning electron microscopy were less attached, resulting in the observed big gaps over increasing storage time. Results indicated that changes of proteins have detrimental effects on the quality attributes of farmed giant catfish muscles during refrigerated storage, particularly physical and biochemical properties.

Keywords Farmed giant catfish, muscle sites, protein changes, refrigerated storage.

Introduction

Immediately after death, several biochemical and enzymatic changes are triggered in fish muscle. This is especially the case if improperly handled. Among post-harvest changes, the degradation of fish muscle caused by endogenous proteases is a primary cause of quality loss during cold storage or post-harvest handling (Haard, 1994). The fish muscle degradation as well as microbial induced activity cause the loss of nutritive value, acceptability and protein functionality. In general, the functional properties of fish myofibrillar proteins are important for determining and predicting the final quality of fishery products (Roura & Crupkin, 1995).

The most important factor for increasing shelf life is the product temperature, since fish are much more perishable than other muscle foods, the temperature is even more important. Chilling is a means of preserving fish before processing or consumption. When fish is stored at low temperature, both enzymatic and chemical reactions are slowed down. Biochemical and physicochemical changes have been widely used as measurable parameters for determining muscle food spoilage (Benjakul *et al.*, 1997; Rawdkuen *et al.*, 2008). Myofibrillar ATPase activities, trimethylamine, total volatile bases and individual nucleotides have been used to monitor post-mortem changes during iced or frozen storage. Autolytic degradation products have also been used as indices for fish proteins deterioration. Texture, water loss and muscle microstructure can also be used to predict the fish freshness (Torrissen *et al.*, 2000).

The giant catfish (*Pangasianodon gigas*) is the world's largest freshwater catfish. It grows to about 3 m in length and to a weight of more than 300 kg. Recently, giant catfish have been successfully farmed in artificial ponds in Chiang Rai Province in the northern part of Thailand. The farmed giant catfish are significantly smaller (25–30 kg) than that normally found in natural habitats (>150 kg). Giant catfish are in high consumer demand due to their quality attributes such as texture, colour and nutritive values. It has become an economically important cultured freshwater fish in Thailand.

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The majority of farmed giant catfish is sold raw for cooking in restaurants. The fish farmers hope to export them in the near future, especially to Asian and European countries. Thus, many fish farmers in Chiang Rai and the surrounding area have gradually expanded their production. Proximate compositions and some of the chemical and physical properties of its fish muscles have been investigated (Chaijan *et al.*, 2010). Measurements of quality parameters show variations along the fish fillets from head to tail. The bulk of the muscle consists of anaerobic white fibres, while the red fibres are found in a thin strip lying beneath the lateral line. These muscle fibres vary not only in colour but also in innervations, blood supply, myoglobin content, fibre size and abundance of mitochondria (Johnston *et al.*, 1972). Aursand *et al.* (1994) reported that fat, pigments and collagen are distributed differently throughout salmon fillets. Lipid contents of the ventral ordinary muscle and muscle of the skin side of wild bluefin tuna are higher than those of the dorsal ordinary muscle and muscles of the central part (Fudge *et al.*, 2001). The compositional characteristics, especially the protein and lipid contents, are needed to facilitate the utilisation and marketing of farm raised giant catfish products.

No information regarding the changes in biochemical and physical properties in the giant catfish muscle during refrigerated storage has yet been reported. This study aims to investigate the quality changes (biochemical and physical) of the meat cuts from the dorsal and ventral parts of farmed giant catfish during storage at 4 °C.

Materials and methods

Chemicals

Bovine serum albumin (BSA) was obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). L-tyrosine, 25% glutaldehyde, adenosine-tripolyphosphate (ATP), 8-Anilino-1-naphthalenesulfonic acid (ANS), dithio-bis-nitrobenzoic acid (DTNB) and β-mercaptoethanol (BME) were all purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium dodecyl sulphate (SDS), *N,N,N',N'*-tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA). Urea, ethylenediaminetetraacetic acid (EDTA), calcium chloride, trichloroacetic acid (TCA), Tris-maleate and tris (hydroxymethyl)–aminomethane were procured from Merck (Damstadt, Germany).

Sample preparation and storage

Giant catfish (age of ~5 years with an average weight of 25–30 kg) were obtained from the Charun farm in Phan District, Chiang Rai, and transported alive to the Food Technology Laboratory, Mae Fah Luang University, Chiang Rai, Thailand. Samples of four farmed giant catfish were used in two experiments (two fish for each experiment). Upon arrival, fish were slaughtered by immersing in a box of ice and then gill cutting. The internal organs were removed at the same time with bloodletting from the fish belly. After washing the fish was then filleted and skinned. The fillets were packaged in polyethylene bags and then placed in ice with a meat/ice ratio of 1:3 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 8 h. The flesh was manually excised into dorsal and ventral muscles (dark muscle was discarded) and cut into ~0.5 kg pieces (~5.0 × 10.0 × 2.5 cm). They were packaged in polyethylene bags, and stored at 4 °C for 14 days. During storage, three pieces of each muscle type (for each replication) were randomly taken at days 0, 2, 4, 7, 10, and 14 for analysis.

Determination of biochemical changes

Natural actomyosin preparation

Natural actomyosin (NAM) was prepared according to the method described by Benjakul *et al.* (1997). The sample (8 g) was homogenised at a speed of 11 000 rpm for 2 min with a homogenizer (IKA® Work (Asia) Sdn Bhd, Selangor, Malaysia) in 80 mL chilled 0.6 M KCl, pH 7.0 for 4 min. The extract was centrifuged at 9000 × g for 30 min at 0 °C by using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). Three volumes of chilled de-ionised water were added to precipitate the actomyosin. The actomyosin was collected by centrifugation as mentioned above and the pellet was dissolved in an equal volume of chilled 1.2 M KCl, pH 7.0 in an iced bath and stirred for 30 min. Un-dissolved material was removed by centrifugation as mentioned above.

ATPase activity assay

ATPase activity of NAM was determined according to the method of Benjakul *et al.* (1997). NAM was diluted to 2.5–4 mg mL^{-1} with 0.6 M KCl, pH 7.0. One millilitre of the diluted solution was added to 0.6 mL of 0.5 M Tris-maleate, pH 7.0. About 10 mM $CaCl_2$ was then added o the mixture for the Ca^{2+} -ATPase activity assay to a total volume of 9.5 mL. To the assay solution, 0.5 mL of 20 mM ATP was added to initiate the reaction. The reaction was conducted for exactly 10 min at 25 °C and terminated by adding 5 mL of chilled TCA (15%, w/v). The reaction mixture was centrifuged at 3500 × g for 5 min and the inorganic phosphate liberated in the supernatant was measured. Specific activity was expressed as μmol inorganic phosphate (Pi) released mg^{-1} protein min^{-1}. A blank solution was prepared by adding chilled TCA prior to the addition of ATP.

Surface hydrophobicity
Surface hydrophobicity was determined as described by Benjakul *et al.* (1997), using ANS as a probe. NAM dissolved in a 10 mM phosphate buffer (pH 6.0 containing 0.6 M NaCl) was diluted to 0.1%, 0.2%, 0.3%, and 0.5% (w/v) protein, using the same buffer. Twenty microlitre of 8 mM ANS in 0.1 M phosphate buffer (pH 7.0) were added to the diluted protein solution (2 mL). The fluorescence intensity of ANS-conjugates was measured using a RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

Reactive sulfhydryls
Total sulfhydryl content was determined using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), according to the method modified by Benjakul *et al.* (1997). To 1.0 mL of NAM solution (4.0 mg mL^{-1}), 9 mL of 0.2 M Tris-HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added. DTNB (0.4 mL of 0.1%) was added to 4 mL of the mixture and incubated at 40 °C for 25 min. Absorbance at 412 nm was then measured using a UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was prepared by replacing the sample with 0.6 M KCl. Sulfhydryl content was calculated using the extinction coefficient of 13 600 M^{-1} cm^{-1}.

Total volatile basic nitrogen content (TVB-N)
TVB-N content was determined using the Conway micro-diffusion assay as described by Ng (1987a). A sample (2 g) was added to 8 mL of 4% TCA (w/v) and homogenised at a speed of 11 000 rpm for 2 min. The homogenate was centrifuged at 3000 × *g* for 15 min using the centrifuge at room temperature. The supernatant obtained was placed in the outer ring of the Conway apparatus. The inner ring solution (1% boric acid with bromocresol green and methyl red indicator) was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 was mixed with the sample extract. The Conway unit was closed and incubated at 37 °C for 60 min. The inner ring solution was then titrated with 0.02 N HCl until the green colour turned to pink.

TVB-N (expressed in mg/100 g sample) = $[14\ (N)\ (A\text{-}B)\ (V)(100)]/M$

where N = Normality of titrated hydrochloric acid solution; A, B = volume of titrated hydrochloric acid solution in the sample and blank, respectively; V = total volume of prepared sample used; M = weight of sample in g.

Measurement of autolysis
The autolytic degradation products were measured by the method described in Morrissey *et al.* (1993). Fish muscle (3 g) was homogenised in 27 mL of 5% (w/v) TCA. The homogenate was kept on ice for 60 min and centrifuged at 5000 × *g* for 10 min. The soluble peptide content in the supernatant was measured according to the Lowry method (Lowry *et al.*, 1951) using tyrosine as a standard, and expressed as mmol tyrosine g^{-1} muscle.

Electrophoresis
SDS-PAGE was carried out according to the method of Laemmli (1970), using a 10% running gel and 4% stacking gel. A muscle sample (3 g) was mixed with 27 mL of 5% (w/v) SDS solution. The mixture was homogenised at a speed of 11 000 rpm for 2 min and then incubated in a water bath (85 °C) for 1 h to dissolve the proteins. It was then centrifuged at 8000 × *g* for 5 min to remove undissolved debris. Twenty microgram of the solubilised sample was loaded into the gel and then subjected to electrophoresis at a constant current of 15 mA per gel using Mini Protean II (Bio-Rad Laboratories, Richmond, CA, USA). After separation, proteins were fixed and stained for 3 h in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol and 10% glacial acetic acid. Gels were destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

Determination of physical changes

Expressible drip
The expressible drip was determined according to the method of Ng (1987b). The fish sample (2 g) was placed between two filter papers (Whatman paper No.1, Maidstone, UK) on the top and three filter papers at the bottom. The samples were then pressed with a standard weight of 5 kg for 2 min. The sample was removed from the paper and the pressed sample was weighed. The expressible drip was calculated based on the difference in weight before and after pressing.

Cooking loss
The fish sample (~10 g) was steamed for 2 min and then cooled at room temperature. The cooked sample was surface-dried with a filter paper, and reweighed using an analytical balance (ML204, Mettler-Toledo International Inc., Bangkok, Thailand). Cooking loss was calculated by the difference in raw weight and cooked weight.

Shear force
The texture was analysed using a TAxT2 texture analyzer (Stable Micro Systems, Surrey, UK), equipped with a Warner–Bratzler blade in accordance with the method of Espe *et al.* (2004). Seven rectangular shaped samples (4 × 2 × 2 cm) were prepared. Each sample was cut perpendicular to the longitudinal orientation of the muscle fibres. The blade was pressed down at a constant

speed of 2 mm s^{-1} through the sample. Maximum shear force (N) and total force (N_s) were recorded. They are the maximum resistance (toughness or breaking point) and total forces needed to cut the sample, respectively.

Microstructure

Muscle samples were fixed with 2.5% glutaraldehyde in 0.2 M potassium phosphate buffer, pH 7.2 for 2 h at room temperature. Fixed specimens were dehydrated in graded ethanol solutions with serial concentrations of 50%, 70%, 80%, 90% and 100% (v/v) and critical point dried (Balzers mod. CPD 030, Blazers Process Systems, Vaduz, Liechtenstein), using CO_2 as a transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzers mod. SCD 004) and examined on a JSM 5200 scanning electron microscope (JEOL, Ltd, Akishima, Japan) with a magnification of 10 000 × at an acceleration voltage of 10 kV.

Statistical analysis

Completely randomised design and one-way analysis of variance (ANOVA) were used. The data obtained was subjected to statistical analysis using the SPSS program for windows (SPSS version 10.0, SPSS Inc., Chicago, IL, USA). Duncan's multiple-range test was used to compare the difference between means. The accepted level of significance for all comparisons was $P < 0.05$. Experiments were conducted in duplicate and the analysis was run as three or seven measurements.

Results and discussion

Biochemical changes of refrigerated giant catfish muscles

ATPase activity

Ca^{2+}-ATPase activities were monitored in both dorsal and ventral cuts of farmed giant catfish muscle over 14 days of refrigerated storage (Fig. 1a). The activity is a measure of the muscle tissue's ability to hydrolyse adenosine triphosphate (ATP) in the presence of Ca^{2+} ions. The myofibrillar ATPase is located in the myosin head region (Chan *et al.*, 1995). Thus, it has been used as an indicator of myosin integrity. From the results, no significant decrease in Ca^{2+}-ATPase activity of NAM from farmed giant catfish was observed in up to 10 days of storage ($P > 0.05$). During the 10 days of storage, higher Ca^{2+}-ATPase activities were found in the dorsal than in the ventral part. Abrupt decreases in Ca^{2+}-ATPase activities were observed when the storage time was more than 10 days. The results indicated that myosin underwent some changes in native conformation during refrigerated storage. The loss in Ca^{2+}-ATPase was possibly due to the proteolysis of the myosin molecule (Benjakul *et al.*, 2003). Hemung & Yongsawatdigul (2005) reported that decreased Ca^{2+}-ATPase activity indicates conformational changes of the myosin head. Moreover, denaturation of myosin was possibly caused by the oxidation of SH groups or disulfide interchanges as well as changes in surface hydrophobicity during iced storage (Benjakul *et al.*, 1997).

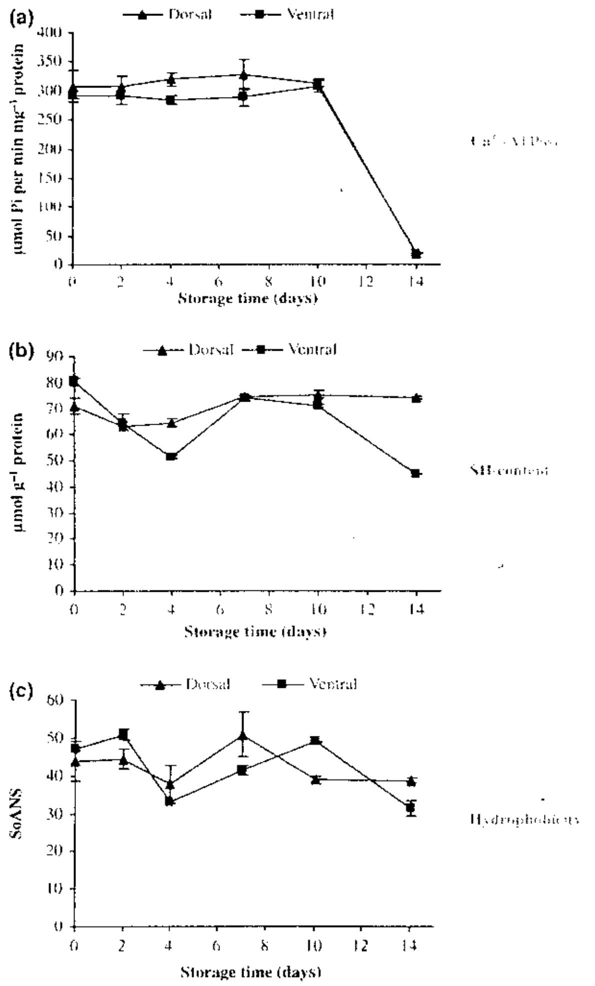

Figure 1 Changes of Ca^{2+}-ATPase activity (a), surface hydrophobicity (b) and reactive sulfhydryls (c) of farmed giant catfish meat cuts during 14 days refrigerated storage. Bar indicates standard deviation from triplicate determinations.

Reactive sulfhydryls

The sulfhydryl content of NAM from both meat cuts decreased during refrigerated storage for up to 14 days (Fig. 1b). A sharp decrease in sulfhydryl content was observed in the first week of storage, especially during the first 4 days. At week 2, the sulfhydryl content was essentially constant in the dorsal cut, while a marked decrease was observed in the ventral part compared to that found in the fresh sample. The differences in sulfhydryl content among meat cuts during refrigerated storage were postulated to be due to the differences in susceptibility of myofibrillar proteins to sulfhydryl oxidation. The unfolding of myosin and actin resulted in an exposure of free SH groups, which subsequently underwent disulfide interchanges (Hemung & Yongsawatdigul, 2005). The sharp decrease in sulfhydryl content was coincidental with the decrease in Ca^{2+}-ATPase. It was presumed that conformational changes of myosin, especially in the head region occurred rapidly in the first 2–3 days and also after 10 days of storage. Moreover, the masking of sulfhydryl groups by protein aggregates was also presumed to lead to the decrease in free sulfhydryl groups. Sulfhydryl groups located in the head portion (SH-1 and SH-2) play an essential role in ATPase activity (Kielley & Bradley, 1956). Furthermore, sulfhydryl groups localised in light meromyosin (SHa) also contributed to oxidation (Sompongse *et al.*, 1996).

Surface hydrophobicity

The changes in surface hydrophobicity were observed throughout the storage for up to 14 days (Fig. 1c). In general, surface hydrophobicity of NAM from farmed giant catfish tended to decrease during 14 days of storage. Compared with the starting materials, at day 4, the surface hydrophobicity of NAM from the meat cuts decreased by 14% and 30% for the dorsal and ventral cuts, respectively. For the dorsal cuts, it was found that surface hydrophobicity increased for up to 7 days and then decreased up to day 14. The ventral cut, which had a higher initial surface hydrophobicity increased when stored for up to 10 days with a subsequent decrease until day 14. Paredi & Crupkin (2007) reported that surface hydrophobicity of actomyosin from pre-spawned flounder linearly increased up to day 6 of storage and thereafter remained unchanged. Increased surface hydrophobicity and decreased sulfhydryl groups indicated that denaturation possibly occurred via hydrophobic interaction and disulfide formation. During extended refrigerated storage, the proteins underwent conformational changes, in which the hydrophobic portions were exposed. As a result, hydrophobic interaction might take place, leading to the aggregation and loss in solubility. During denaturation, hydrophobic and hydrogen bonds buried inside the protein molecules become exposed (Benjakul *et al.*, 1997). As a consequence, conformational changes in the coiled or helical sections of the peptide chain occur and reform in a manner different from those in the native structure. Thus, refrigerated storage directly affected the conformational changes in protein molecules, leading to the loss in functionality.

Total volatile basic nitrogen

TVB-N analyses have been traditionally used as quality indicators in fisheries products stored in ice. The TVB-N content quantifies a wide range of basic volatile compounds (ammonia, methylamine, dimethylamine, trimethylamine, etc.), that can be produced as a result of microbiological activity during low temperature storage or that can arise from the thermal breakdown of endogenous compounds during processing (Benjakul *et al.*, 1997). In the present study, the amount of TVB-N in farmed giant catfish muscles (dorsal and ventral sites) increased significantly with time (Table 1). Initial TVB-N content on day 0 was 6.2 and 7.8 mg TVB-N 100 g^{-1} sample for ventral and dorsal, respectively. Chomnawang *et al.* (2007) reported that the TVB-N content of the hybrid catfish samples on day 0 was in the range of 15.7–16.9 mg TVB-N 100 g^{-1} and exceeded 30 mg TVB-N 100 g^{-1} flesh after day 9 of storage. The initial TVB-N content of 10 mg TVB-N 100 g^{-1} in a lizardfish sample suggested that it possibly underwent some deterioration (Benjakul *et al.*, 2003). In this work, TVB-N increased linearly with time to a final value of 13 and 14 mg TVB-N 100 g^{-1} on day 14 for dorsal and ventral parts, respectively. TVB-N of 25 mg TVB-N 100 g^{-1} was suggested to be a limit level for sardines (Marrakchi *et al.* 1990), while a TVB-N value of

Table 1 Changes of total volatile basic nitrogen and TCA-soluble peptide content of farmed giant catfish meat cuts during refrigerated storage for 14 days

	Dorsal cut		Ventral cut	
Storage (days)	TVB-N (mg TVB-N 100 g^{-1})	TCA-soluble peptide (mmol Tyr g $sample^{-1}$)	TVB-N (mg TVB-N 100 g^{-1})	TCA-soluble peptide (mmol Tyr g $sample^{-1}$)
0	$7.8 \pm 0.05^{ab**}$	0.73 ± 0.03^{b}	6.2 ± 0.00^{b}	0.68 ± 0.05^{a}
2	7.9 ± 0.16^{b}	0.75 ± 0.07^{a}	5.1 ± 0.22^{a}	0.75 ± 0.04^{ab}
4	7.6 ± 0.16^{a}	0.80 ± 0.01^{a}	6.5 ± 0.00^{b}	0.80 ± 0.03^{b}
7	7.8 ± 0.01^{ab}	0.88 ± 0.01^{b}	6.5 ± 0.00^{b}	0.92 ± 0.23^{c}
10	9.3 ± 0.03^{c}	1.22 ± 0.07^{c}	8.8 ± 0.66^{c}	1.33 ± 0.05^{d}
14	13.3 ± 0.07^{d}	1.45 ± 0.06^{d}	14.1 ± 0.66^{d}	2.13 ± 0.10^{e}

Values are given as mean ± SD from triplicate determinations. Different superscripts in the same column indicate significant differences ($P < 0.05$).

small protein bands obtained in muscle extracts were a result of myosin hydrolysis induced by the activation of endogenous enzymes. The actin band still showed high band intensity after 14 days of refrigerated storage. This result is consistent with Benjakul *et al.* (2003) who reported that MHC was more prone to proteolytic degradation than other muscle proteins, such as AC, TN and TM. The results of this experiment indicated less proteolysis for up to 14 days of storage.

Physicochemical changes of refrigerated giant catfish muscle

Expressible drip

The expressible drip of refrigerated farmed giant catfish muscles decreased with storage time and amounted to more than 5% after 14 days of storage (Fig. 3a). Decreases of about 30% of expressible drip were found during storage of both dorsal and ventral parts compared with day 0. During the long time of refrigerated storage, a fraction of water evaporates and the result seems to be that the water is more poorly retained by the muscle structure. Dalgaard *et al.* (1993) reported that the drip loss of ice chilled vacuum packed cod fillets to be 4.7% at the time of sensory rejection (14 days). The expressible drip was less in the ventral than in the dorsal part. High expressible drip in the refrigerated samples might be due to increased proteolysis, resulting in a looser structure and thereby affecting the ability to retain water. The reason for the difference between dorsal compared to ventral samples might be that the arrangement of the muscle composition of both cuts results in different abilities of the tissues to retain water (Chaijan *et al.*, 2010). In contrast to these results, Simpson & Haard (1987) found that free drip increased during storage of Atlantic cod at 0 or −3 °C, and the increase was largest for partially frozen samples.

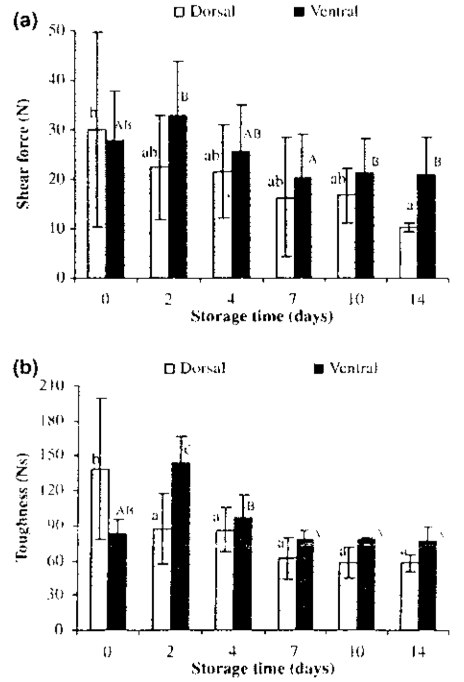

Figure 4 Changes of shear force (a) and toughness (b) of meat cuts from farmed giant catfish during 14 days refrigerated storage. Bar indicates standard deviation from five determinations. Different alphabets in the same muscle type indicate significant differences ($P < 0.05$).

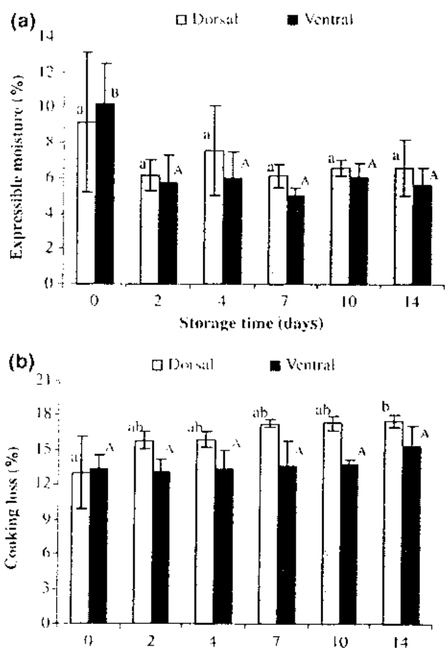

Figure 3 Changes of expressible moisture (a) and cooking loss (b) of meat cuts from farmed giant catfish during 14 days refrigerated storage. Bar indicates standard deviation from three determinations. Different alphabets in the same muscle type indicate significant differences ($P < 0.05$).

Cooking loss

The cooking loss of the dorsal and ventral parts of farmed giant catfish is shown in Fig. 3b. Cooking loss slightly increased as the storage time increased up to 14 days. The cooking loss was significantly higher in the dorsal part compared to ventral part of farmed giant catfish muscles (Fig. 5b). No significant differences of cooking loss was found in the ventral part ($P > 0.05$). Initially, the cooking loss of the dorsal part was ~13%. The value increased to 17% after 2 weeks of refrigerated storage. Meanwhile, the cooking loss in the ventral part

between samples. This result was possibly caused by the lack of homogeneity of the sample.

Large structural differences have been observed between maturing and post-spawned cod with the latter having large gaps between the myofibrillar units (Ofstad *et al.*, 1996). The texture of the fish muscle depends on numerous intrinsic biological factors related to the density of the muscle fibres, as well as the fat and collagen content of the fish (Sigurgisladottir *et al.*, 1999; Olafsdottir *et al.*, 2004). Previous research has shown that fish become less firm with longer chilling periods (Alasalvar *et al.*, 2001). The results suggested that denaturation (aggregation and/or hydrolysis) of muscle proteins during the storage period was negligible with only a minimal effect on muscle texture. Texture loss during the storage of fish samples has been commonly reported, which may be associated with low muscle pH or due to the involvement of several proteolytic enzymes (Torrissen *et al.*, 2000).

Microstructure

Microstructures of both transverse sections (Fig. 5a) and longitudinal sections (Fig. 5b) of farmed giant catfish muscles are illustrated in Fig. 5. The starting muscle (day 0) had a well organised structure of the muscle bundle. After 1 week of refrigerated storage, the muscle bundles were less attached and there was a loss of muscle interaction. After a period of time (day 7–14), muscle bundles became larger and showed gaps between them. Both meat cuts showed the same microstructure patterns during refrigerated storage. Nip & Moy (1988) reported that during the refrigerated storage of the fish, the muscle fibres of the anterior-most sections degraded gradually. Degradation of muscle tissue started from the perimysium, endomysium, the Z-line, and the H-zones. For the longitudinal sections (Fig. 5b), similar microstructures of meat cuts were found. Dense bundle structures were noticeable in fresh cuts, while loose bundle structures were found in refrigerated stored meat cuts.

Conclusions

Greater changes in both biochemical (ATPase, TVB-N, and protein hydrolysis) and technological aspects (expressible drip, cooking loss, shear force, and microstructure) of farmed giant catfish muscles (dorsal and ventral sites) were found at day 14 of storage. However, storage at refrigeration temperature (~4 °C) for 14 days leads to a loss of some quality parameters, which did not affect the appearance of the sample.

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