รายงานวิจัยฉบับสมบูรณ์

การศึกษาการใช้ประโยชน์จากโปรตีนไอโซเลตจากปลาข้างเหลือง (*Selaroides leptolepis*) ในไส้กรอกหมูลดไขมันและทดแทนไขมันด้วยน้ำมันถั่วเหลือง

Study on utilization of protein isolate from yellow stripe trevally (*Selaroides leptolepis*) in fat-reduced and soybean oil replaced pork back-fat sausages

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มหาวิทยาลัยสงขลานครินทร์
ประจำปีงบประมาณ 2557–2558 รหัสโครงการ AGR580255c

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ส่วนที่ 2 เนื้อหา

1. ชื่อชุดโครงการ (ภาษาไทย): สมบัติเชิงหน้าที่และการใช้ประโยชน์ของโปรตีนไอโซเลตจากปลาข้างเหลือง
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2. ชื่อโครงการย่อย:

2.1 การปรับปรุงสมบัติเจลซูริมิและโปรตีนไอโซเลตจากปลาข้างเหลือง
2.2 การพัฒนาฟิล์มต้านจุลินทรีย์จากโปรตีนไอโซเลตจากปลาข้างเหลือง
2.3 การศึกษาการใช้ประโยชน์จากโปรตีนไอโซเลตจากปลาข้างเหลือง (*Selaroides leptolepis*) ในไส้กรอกหมูลดไขมันและทดแทนไขมันด้วยน้ำมันถั่วเหลือง

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บทคัดย่อ

ความตระหนักของผู้บริโภคเกี่ยวกับการรับประทานอาหารเพื่อสุขภาพที่เพิ่มขึ้นนั้นก่อให้เกิดความสนใจศึกษาเกี่ยวกับการลดปริมาณไขมันและกรดไขมันอิ่มตัวในผลิตภัณฑ์อาหาร การทดแทนไขมันสัตว์บางส่วนด้วยน้ำมันที่อุดมไปด้วยกรดไขมันไม่อิ่มตัวเชิงซ้อน (Polyunsaturated fatty acids, PUFA) เป็นวิธีการหนึ่งที่ช่วยปรับปรุงโภชนาการของผลิตภัณฑ์เนื้อได้จากการช่วยลดปริมาณคอเลสเตอรอลและกรดไขมันอิ่มตัว อย่างไรก็ตามการลดปริมาณไขมันรวมและการปรับเปลี่ยนแหล่งไขมันที่เติมลงในผลิตภัณฑ์เนื้อสับผสมต่างๆ นั้นอาจส่งผลกระทบต่อสมบัติของผลิตภัณฑ์ได้ งานวิจัยนี้เริ่มจากการศึกษาผลของการทดแทนไขมันหมูบางส่วนด้วยน้ำมันถั่วเหลือง (soybean oil, SBO) ที่มีผลต่อลักษณะของผลิตภัณฑ์ไส้กรอก โดยทำการเติม SBO แก่ตัวอย่างไส้กรอกในรูปแบบต่างๆ ได้แก่ SBO รูปแบบดั้งเดิมและรูปที่ผ่านการเตรียมเป็นอิมัลชันมาก่อน ที่ระดับการทดแทนไขมันหมูต่าง ๆ ได้แก่ ร้อยละ 25 35 และ 45 โดยน้ำหนักของไขมันหมู สำหรับ SBO ในรูปที่ผ่านการเตรียมเป็นอิมัลชันมาก่อน (pre-emulsified SBO, preSBO) นั้น จะถูกเตรียมขึ้นด้วยการใช้โปรตีนต่างๆ–ได้แก่ โปรตีนไอโซเลตจากปลา (fish protein isolate, FPI) โปรตีนจากถั่วเหลือง (soy protein isolate, SPI) และ โซเดียมเคซีเนต (Na-caseinate, Nc)–เป็นสารอิมัลซิไฟเออร์ ตัวอย่างไส้กรอกที่ไม่มีการเติม SBO ตัวอย่างที่มีการเติม SBO ในรูปแบบดั้งเดิม รูปแบบ preSBO ที่เสถียรด้วย FPI รูปแบบ preSBO ที่เสถียรด้วย SPI และ รูปแบบ preSBO ที่เสถียรด้วย Nc ให้หมายถึง Control NSBO FPI-preSBO SPI-preSBO และ Nc-preSBO ตามลำดับ ทำการตรวจสอบลักษณะของไส้กรอกโดยการวัดค่าสูญเสียจากการปรุงสุก (cooking loss) ค่าความสามารถในการจับน้ำ (water holding capacity, WHC) ลักษณะเนื้อสัมผัส ลักษณะโครงสร้างจุลภาค และค่าสี จากการทดลองนั้นไม่พบความแตกต่างของค่าสูญเสียจากการปรุงสุกระหว่างตัวอย่าง Control และ NSBO ที่ทุกระดับการทดแทนด้วย SBO ($p>0.05$) การเติม SBO ในรูปแบบ preSBO นั้นส่งผลต่อค่าสูญเสียจากการปรุงสุกของไส้กรอก โดยขึ้นอยู่กับชนิดของอิมัลซิไฟเออร์และระดับการทดแทนด้วย SBO โดยที่ FPI-preSBO แสดงค่าสูญเสียจากการปรุงสุกที่ต่ำกว่าตัวอย่าง Control ในขณะที่พบค่าสูญเสียจากการปรุงสุกที่มากกว่าสำหรับ SPI-preSBO และ Nc-preSBO บ่งชี้ให้เห็นว่าชนิดของอิมัลซิไฟเออร์ที่ใช้ในการเตรียม preSBO นั้นมีบทบาทที่สำคัญต่อความคงตัวของผลิตภัณฑ์ นอกจากนี้ FPI-preSBO ยังมีลักษณะเนื้อสัมผัสที่คงตัวกว่า และมีโครงสร้างจุลภาคที่มีความเรียบและเป็นเนื้อเดียวกัน การเพิ่มระดับการทดแทนด้วย SBO นั้นส่งผลลดค่า WHC ของผลิตภัณฑ์ไส้กรอก และการเพิ่มระดับการทดแทนด้วย SBO ถึงร้อยละ 45 นั้นส่งผลให้ค่าความแข็ง (hardness) ความเคี้ยวได้ (chewiness) และความเหนียว (gumminess) ลดลงอย่างมีนัยสำคัญ ($p<0.05$) อย่างไรก็ตามตัวอย่าง FPI-preSBO สามารถรักษาระดับ WHC ได้เทียบเคียงกับตัวอย่างควบคุมจนถึงระดับการทดแทนด้วย SBO ที่ร้อยละ 35 ($p>0.05$) ผลการทดลองเหล่านี้แสดงให้เห็นถึงความสามารถที่ดีกว่าของ FPI ในการรักษาความแข็งแรงของเมทริกซ์โปรตีนของตัวอย่างไส้กรอก

จากนั้นศึกษาผลของการลดปริมาณไขมันต่อลักษณะของไส้กรอก โดยกำหนดปริมาณไขมันรวมในตัวอย่างไส้กรอกเป็นร้อยละ 30 20 และ 10 ทำการทดแทนไขมันหมูด้วย SBO ในรูปแบบต่างๆ (ที่ระดับการทดแทนร้อยละ 25 โดยน้ำหนักของไขมันหมู) พบว่าการลดปริมาณไขมันรวมส่งผลลดความคงตัวของไส้กรอกอย่างชัดเจน อย่างไรก็ตามการเติม SBO ในรูปแบบ preSBO ที่เสถียรด้วย FPI นั้น สามารถช่วยพัฒนาความคงตัวของไส้กรอกได้ บ่งชี้จากค่าสูญเสียจากการปรุงสุกที่ต่ำกว่าและการรักษาลักษณะเนื้อสัมผัสได้ดีกว่า การลดปริมาณไขมันรวมยังส่งผลให้เกิดการสร้างโครงข่ายโปรตีนที่ลดลง โดยเฉพาะสำหรับตัวอย่าง Nc-preSBO โดยที่ลักษณะโครงสร้างจุลภาคของ Nc-preSBO นั้นมีความไม่เป็นเนื้อเดียวกันและมีรูพรุนขนาดใหญ่จำนวนมากปรากฏอยู่ด้วย ลักษณะเหล่านี้สัมพันธ์กับความคงตัวที่ด้อยกว่าของ Nc-preSBO ส่วนตัวอย่าง FPI-preSBO นั้นมีโครงสร้างจุลภาคที่แสดงให้เห็นถึงโครงข่ายโปรตีนที่แน่นและมีความเป็นเนื้อเดียวกันสูง ส่งผลสัมพันธ์กับความคงตัวที่ดีกว่าไส้กรอกสูตรอื่นๆ ประสิทธิภาพของ FPI ในการรักษาความคงตัวของเมทริกซ์ไส้กรอกนี้สันนิษฐานว่าเป็นผลมาจากระดับการเกิดอันตรกิริยาระหว่าง FPI กับโปรตีนเนื้อที่มากกว่า โดยการเตรียม FPI ด้วยวิธีการเพิ่มค่าความเป็นกรดด่าง (pH shift method) นั้น การเสียสภาพบางส่วนของ FPI สามารถเกิดขึ้นได้จากปฏิกิริยาไฮโดรไลซิสที่เร่งด้วยสภาวะเป็นด่างนั่นเอง

ปลาข้างเหลือง (yellow stripe trevally; *Selaroides leptolepis*) ซึ่งเป็นปลาที่มีราคาถูก และพบได้มากในภาคใต้ของประเทศไทยนั้นสามารถนำมาใช้เป็นแหล่งผลิต FPI ที่มีสมบัติเชิงหน้าที่เพื่อใช้ในผลิตภัณฑ์เนื้อได้ จากการศึกษาการใช้ FPI เป็นอิมัลซิไฟเออร์เพื่อเตรียม preSBO นั้น พบว่า FPI แสดงความสามารถที่ดีในการช่วยรักษาความคงตัวของไส้กรอกที่ได้รับผลกระทบจากการลดปริมาณไขมันได้ดี โดย FPI แสดงประสิทธิภาพที่ดีกว่าโปรตีนที่ใช้ทางการค้าอื่นๆ คือ SPI และ Nc จากการศึกษาครั้งนี้พบว่าการใช้ FPI ที่ระดับความเข้มข้นร้อยละ 2 เป็นอิมัลซิไฟเออร์นั้นส่งผลให้ได้ไส้กรอกที่มีค่าสูญเสียจากการปรุงสุกต่ำ ลักษณะเนื้อสัมผัสที่คงตัว และมีโครงสร้างจุลภาคที่เรียบเนียนเป็นเนื้อเดียวกัน

Abstract

The increased concerning of consumer on healthy food consumption creates a growing need for reduction in fat content, and particularly lowering saturated fat content in food products. Partial replacement of animal fats with *n*-3 rich oils is a promising way to improve nutritive value of meat products by reducing cholesterol and saturated fat contents. By altering fat content and using different fat source, however, properties of comminuted meat products are affected. In this work, the effects of partial replacement of porcine fat with soybean oil (SBO) on characteristics of sausages were firstly studied. The SBO was introduced to the sausages alternatively in native or pre-emulsified forms at the oil substitution levels 25, 35, and 45 % by weight of porcine fat. The pre-emulsified SBO (preSBO) was prepared employing different non-meat proteins–*i.e.,* fish protein isolate (FPI), soy protein isolate (SPI), and Na-caseinate (Nc)–as emulsifiers. The sausages without SBO, with SBO adding in native,

FPI stabilized preSBO, SPI stabilized preSBO, and Nc stabilized preSBO were referred as the Control, NSBO, FPI-preSBO, SPI-preSBO, and Nc-preSBO, respectively. Characteristics of the sausages, including cooking loss, water holding capacity, texture attributes, microstructure, and color, were then determined. There was no significant difference on cooking loss between the Control and NSBO, irrespectively of SBO substitution levels ($p>0.05$). Introducing SBO in pre-emulsified form influenced cooking loss of the sausages in different manners depending on the employed emulsifier type and SBO substitution levels. The FPI-preSBO possessed lowered cooking loss than the Control ($p<0.05$), whereas higher cooking loss was found for the SPI-preSBO and Nc-preSBO, suggesting to an important role of non-meat proteins on stability of the emulsified meat matrix. Retained textural attributes, and smooth microstructure with high heterogeneity were also observed for the FPI-preSBO. Increase SBO substitution level affected to decrease WHC of the sausages. Moreover, lowered hardness, chewiness, and gumminess were observed when the sausages were formulated with SBO at 45 % ($p<0.05$). However, the FPI-preSBO could maintain a comparable WHC with the Control through 35 % SBO substitution level ($p>0.05$). These results suggested to better ability of the FPI to reinforce protein matrix strength of the sausages.

Next, characteristic of the sausages as affected by fat reduction was studied by varying total fat amount of the sausages as 30, 20, and 10 %. SBO in different forms was added to partially replace porcine fat (at 25 % by wt of porcine fat). Reduction of fat level markedly reduced stability of the sausages. However, incorporation of SBO, especially in a form of FPI stabilized preSBO, could successfully improve product stability as implied by lowered cooking loss and restored texture attributes. Less protein network formation was generally found with decreased total fat level, especially for the Nc-preSBO; The Nc-preSBO possessed microstructure with higher heterogeneity and presence of several large voids. This is related to less stability of the Nc-preSBO. For the FPI-preSBO, the protein network with more compact and higher homogeneity was present, which was correspond to their higher stability compared to the other sausage formulations. Effective ability of FPI to stabilize protein matrix of the sausages was postulated due to more pronounced interaction between the FPI and meat protein residues. By isolating via the pH shift method, partial denaturation of the FPI could be expected due to a hydrolysis reaction accelerated by alkaline environment.

A yellow stripe trevally (*Selaroides leptolepis*)–a low price fish species abundantly present in the Southern part of Thailand–could be used to prepare FPI with effective

functional property to be used in meat products. As employed as emulsifier to stabilize preSBO, the FPI possessed a good ability to retain sausage stability against fat reduction effect compared to the other commercially non-meat proteins–*i.e.,* SPI and Nc. In this work, partial substitution of porcine fat by preSBO could be accomplished by using the FPI at 2 % as emulsifier, by providing the sausages with lowered cooking loss, retained texture attributes, and microstructure with smoothness and high homogeneity.

1. Introduction

The increased concerning of consumer on healthy food consumption has created a growing need for reduction in fat, and particularly lowering saturated fat content in food products. Fat is important food component attributing desirable sensorial characteristics, but it is the most dense energy nutrient. Moreover, it has been well recognized that some chronic diseases, such as cardiovascular disorder, colon cancer, and obesity, are related with high saturated fat intake (Beecher, 1999; Youssef and Barbut, 2011b). Meat products always contains high amount of fat, saturated fatty acids, and cholesterol (Jiménez-Colmenero *et al.,* 2001), so production of meat goods by decreasing fat content and/or improving fat quality by partial replacing animal fat with unsaturated fatty acids has been growing recently (López-López *et al.,* 2010; Delgado-Pando *et al.*, 2010). To accomplish this purpose, some vegetable oils–*e.g.,* canola and olive oils, and fish oils–are attractive, because of their high polyunsaturated fatty acids (PUFAs) compared to animal fats (Eskin and McDonald, 1991; López-López *et al.,* 2011; Youssef and Barbut, 2011b). According health advantages of unsaturated fatty acids, *n*-3 fatty acids–*e.g.,* linolenic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)–provide positive effects on human health by prohibiting cancer and heart disease (Josquin *et al.,* 2012), whereas *n*-6 fatty acids–*e.g.,* linoleic and arachiconic acids–might affect to induce some health problems, *e.g.,* increase a risk in cancers and coronary heart disease (Enser et al., 2000). One of feasible ways to increase *n*-3 fatty acid in consumed food is using *n*-3 rich oils–*e.g.,* linseed, rape seed, soybean, and fish oils (Riemersma, 2001; Josquin *et al.,* 2012)–to partially replace animal fat.

To reformulate meat products by diminishing fat content and/or introducing different fat sources, however, it becomes necessary to retain qualities that are pertinent to consumer demand. In comminuted meat products–*e.g.,* sausage, meat patties, and bologna–fat plays important role on contributing desirable texture and flavor (El-Magoli *et al.*, 1996). In a processing of meat products by reduction fat content and/or *n*-3 rich oil substitution, stability and sensorial quality of the products might be affected, thereby influencing consumer acceptability (Akesowan, 2002; Miklos *et al.*, 2011; Youssef and Barbut, 2011b; Yang *et al.*, 2001). Yang *et al.,* (2001) suggested that reduction of fat content below 20 % led to unacceptable sensorial quality of meat products. To prepare emulsified meat products with desirable characteristics, fat introducing method is important. The pre-emulsification–defined as an emulsion preparing process before introducing oil ingredient to meat product–is a promising technique to produce comminuted meat products with a good stability (Jiménez-Colmenero, 2007; Delgado-Pando *et al.*, 2010). Through this means, oil droplets are stabilized by additional part of emulsifier applied in the pre-emulsifying step, so more meat proteins are allowed to function in gel matrix formation, thereby facilitating oil drop dispersion in protein matrix and leading to improve fat binding ability (Hoogenkamp, 1987; Jiménez-Colmenero, 2007; Delgado-Pando *et al.*, 2010). Improved quality of meat products via the pre-emulsification means was reported in olive oil substituted pork frankfurter (Bloukas *et al.,* 1997) and traditional Spanish fermented sausage (Muguerza *et al.*, 2001).

By introducing oil via the pre-emulsification method, non-meat proteins play important role on enhancement of product stability (Arrese *et al.,* 1991; Andrés *et al.*, 2006; Hsu and Sun, 2006; Jiang and Xiong, 2013). Herreroa *et al.*, (2008) observed improved texture characteristics in term of hardness and chewiness in heated meat sample when using soy protein isolate (SPI) as a meat binder. Comfort and Howell (2002) reported that replacement muscle proteins with low soluble wheat protein could develop smoothness of the processed meat product. Moreover, improvement of water holding capacity (WHC) and texture profile of the low salt meat product could be achieved using pre-heated whey protein isolate (Hongsprabhas and Barbut, 1999). Na-caseinate (Nc), whey proteins, and soy proteins are non-meat proteins widely employed in manufacturing of meat products (Omana *et al.*, 2012). Inferior functional property of SPI compared to other non-meat proteins was reported. Yang *et al.*, (2001) studying the effects of SPI and muscle protein isolate on quality of low-fat frankfurter (10 % fat) reported that even SPI and isolated muscle proteins could retain appreciable textural properties, higher purge loss and more discoloration were observed in SPI treated frankfurter compared to the muscle protein isolate added sample. Utilization of SPI, moreover, might result in "cereal-like" off-flavor (Matulis *et al.*, 1995), and cause discolorization when grinding process is operated at above 10 °C (Mittal and Usborne, 1985). Matulis *et al.*, (1995) also reported masking effect of SPI on flavor of frankfurter and expected since flavor compounds present in SPI.

Fish are typically low-cost resources that could provide proteins with desired functional properties. Emulsifying, fat holding, and gelling capacities of proteins isolated from various fishes such as tilapia (Rawdkuen *et al.*, 2009), arrowtooth flounder, herring (Sathivel *et al*,. 2004; Underland *et al.*, 2002), striped Bass (Tahergorabi *et al.*, 2012a), rainbow trout (Tahergorabi *et al.*, 2012b), and cod (Park *et al.*, 1997; Kristinsson and Hultin, 2003) have been reported. Yellow stripe trevally (*Selaroides leptolepis*)–a low price and abundantly present fish species in the Southern of Thailand–is an attractive fish species used to prepare protein. High content of several essential amino acids, including Lys (8.45 % of total amino acids, TAC), Leu (6.72 % of TAC), His (5.49 % of TAC), and Ile (4.31 % of TAC) of the yellow stripe trevally flesh was reported (Klompong *et al.*, 2009).

The present work aimed to elucidate emulsifying activity of fish protein isolate (FPI) in emulsified meat model, in order to maximize utilization of FPI in food industry. The FPI was prepared from a yellow stripe trevally, regarded due to its abundant presence, low price, and nutritive value of essential amino acids. Pork sausages were used as a studied model, and their characteristics as affected by partial substitution of porcine fat with SBO and fat reduction were observed. SBO was incorporated to the sausages in alternative forms–*i.e.*, native and pre-emulsified forms. The FPI was employed as emulsifier to prepare preSBO and its emulsifying ability was observed in comparing with the commercially available non-meat proteins–*i.e.*, SPI and Nc.

2. Objectives

- To prepare and characterize properties of fish protein isolate (FPI) prepared from yellow stripe trevally (*Selaroides leptolepis*) using a pH-shift extraction method
- To investigate emulsifying ability of the FPI
- To study the effects of partial replacement of porcine fat with soybean oil (SBO) in alternative forms–*i.e.*, native and pre-emulsified forms–on stability and characteristics of sausages
- To determine emulsifying capacity of the FPI to be used as a non-meat protein ingredient for sausage preparation
- To reformulate nutritive pork sausages by substitution of porcine fat using SBO and reducing total fat content

3. Literature review

3.1 Preparation and physicochemical properties of fish protein isolate (FPI)

Study on protein isolation from various fish species, *e.g.*, short-bodied mackerel (*Rastrelliger brachysoma*) (Chaijan *et al.*, 2010), Pacific whiting (*Merluccius productus*) (Kim *et al.*, 2003), Atlantic croaker (*Micropogon undulatus*) (Perez-Mateos *et al.*, 2004), Atlantic menhaden (*Brevoortia tyrannus*) (Perez-Mateos *et al.*, 2007), suggested that recovery method crucially affected on physicochemical and functional properties of the derived proteins. Conventionally, pre-washing treatment is always conducted for fish flesh before protein extraction, because it is a potential way to remove soluble sarcoplasmic proteins, lipid, blood, and other water soluble foreign materials. Upon the pre-washing process, therefore, myofibrillar protein could be successfully concentrated (Chaijan *et al.*, 2006, 2010; Park *et al.*, 1997), leading to improve functional and sensory properties of the received protein (Rawdkuen *et al.*, 2009). The pre-washing treatment, however, led to lower recovered protein yield (Rawdkuen *et al.*, 2009). To tackle this drawback, a pH-shift process, conducted by solubilizing muscle protein in alkaline pH condition, subsequently with separating water soluble proteins and unwanted materials via centrifugation, before recovering the muscle protein by isoelectric point precipitation, has been introduced (Rawdkuen *et al.*, 2009; Chaijan *et al.*, 2010; Kristinsson *et al.*, 2005; Underland *et al.*, 2002). By using the pH shift method, recovery efficiency could be developed. Protein extraction yields of *ca.* 68 and 72 % were received from tilapia muscle when conventional and the pH-shift processes were performed, respectively (Rawdkuen *et al.*, 2009). Better efficiency of the pH-shift than the conventional means was also reported for protein recovering from the muscles of channel catfish (Kristinsson *et al.*, 2005) and Pacific whiting (Choi and Park, 2002). Not only a higher recovery efficiency, the pH-shift extraction could isolate protein with better functional properties compared to conventional procedure (Chaijan *et al.*, 2010; Kristinsson *et al.*, 2005; Underland *et al.*, 2002; Rawdkuen *et al.*, 2009).

Regarding to the structure of proteins isolated through the pH-shift process, some extension of conformational change was evidenced as suggested by increased tryptophan fluorescence intensity (Chaijan *et al.*, 2010). As a result of the pH-shift method, degradation of myosin heavy chain to smaller peptides was also confirmed by electrophoretograms for the tilapia protein isolate (Rawdkuen *et al.*, 2009) and rockfish protein isolate (Yongsawatdigul and Park 2004). The conformational change of protein in these cases was postulated due to a hydrolysis process induced by alkaline condition. It has been well recognized that unfolding of proteins affected to enhance hydrophobic interaction between protein molecules that might lead to enhance emulsifying properties of proteins (Kim *et al.*, 2005; Knudsen *et al.*, 2008). During emulsion formation, protein molecules tended to rearrange their conformation by exposing buried hydrophobic amino acid residues to interact with oil phase through "*surface denaturation*" process, resulting in a formation of adsorbed protein layers around dispersed drops (Norde, 1998). With a partial unfolding, moreover, interaction between adsorbed protein molecules could be enhanced leading to strengthen interfacial protein films to effectively retard aggregation of dispersed drop (Kim *et al.*, 2002). Better gelling ability of the protein isolated by the pH-shift method was reported as indicated by higher gel strength of the gel compared to the gel of protein recovered by conventional treatment (Chaijan *et al.*, 2010). This was expected since a partial denaturation of the protein molecules induced by alkaline condition (Chaijan *et al.*, 2010).

Comparing to a conventional method, the pH-shift process could isolate protein with lower contents of foreign undesirable materials–*e.g.*, lipids and blood–thereby resulting in better functional properties of the derived proteins (Chaijan *et al.*, 2006, 2010; Rawdkuen *et al.*, 2009). Chaijan *et al.*, (2010) reported that the proteins isolated from short-bodied mackerel using conventional and the pH-shift process had the remained lipid content of *ca.* 2.4 % and 0.3 %, respectively. This is in accordant with the study of Batista *et al.*, (2007); By conducting conventional and the pH-shift extraction methods, the percentage of lipid removing from sardine muscle protein isolate were *ca.* 51 % and 65 %, respectively. Four times higher in lipid reduction by alkaline-aided process was also confirmed in protein isolated from Atlantic croaker (Kristinsson and Liong, 2006), tilapia (Rawdkuen *et al.*, 2009), and channel catfish (Kristinsson *et al.*, 2005). Naturally, lipids are present as a membrane lipid and storage lipid by co-aggregating with proteins. In alkaline environment, proteins are well solubilized, so lipids residues could be easily separated by gravitational or centrifugal force (Kristinsson *et al.*, 2005; Hultin, 2003). Presence of lipid affected to interrupt gel formation and led to weaken gel strength, because of its low water holding capacity (WHC) and inability to form gel (Chaijan *et al.*, 2010). This adverse effect was also observed by a presence of sarcoplasmic proteins–*e.g.*, myoglobin (Hultin and Kelleher, 2000; Yongsawatdigul and Park, 2004). The remained myoglobin in protein isolated from tilapia was *ca.* 39.5 % and

18.5 %, when conventional and the pH-shift processes were conducted, respectively (Rawdkuen *et al.*, 2009). Better efficiency for myoglobin removing by alkaline condition was also observed in protein isolation from sardine and mackerel muscles (Chaijan *et al.*, 2006). Alkaline pH could effectively get rid of myoglobin, postulated due to higher degree of muscle protein degradation in alkaline condition that affected to facilitate myoglobin migration from degraded protein molecules (Rawdkuen *et al.*, 2009). Alkaline extraction, moreover, was presumed to be a potent method to remove pigments from fish muscle (Rawdkuen *et al.*, 2009).

3.2 *The matrix and stability of sausage*

Sausage is a gel-type meat product mainly consisting of protein and fat. According to micrograph of sausage matrix observed by transmission electron microscope, fat globules coated by interfacial protein layers are embedded in a continuous protein matrix (Su *et al.*, 2000). Jones (1982) suggested that free myosin mainly accounted for interfacial adsorbed film forming around dispersed fat droplets by orienting heavy meromyosin head toward hydrophobic phase, and protruding light meromyosin tail into hydrophilic area. Formation of interfacial protein layers around oil drops is a major mechanism to stabilize fat globules within a meat batter (Wu *et al.*, 2009). Interactions between protein molecules after adsorption to oil drop surfaces played a crucial role to thicken and strengthen the interfacial protein layer. In sausage, therefore, protein is an important ingredient to form gel matrix contributing water entrapment and immobilization of suspended fat globules (Ziegler and Acton, 1984). In comminuted meat products, gel matrix has relevant functions on both product stability and textural characteristic which is a main factor governing sensorial attributions (Jones and Mandigo, 1982; Ross, 2009; Su *et al.*, 2000).

Typically, fat contributes desirable flavor, texture, and mouth-feel in meat products (Yilmaz, 2005). In sausage matrix, fat is present in emulsified droplets which are stabilized by interfacial layers of myofibrillar protein (Jones and Mandigo, 1982; Su *et al.*, 2000) and/or physical entrapment within a gel protein matrix via specific protein-protein interactions (Lee *et al.*, 1981; Su *et al.*, 2000). Fat in sausage is partially present within its intact cell membrane, while the rest can be squeezed out of the cell structure (Youssef and Barbut, 2010). Different fats with dissimilar physicochemical characteristics led to different stability characteristics of fat globules in comminuted meat products (Youssef and Barbut, 2010). Broken or damage of fat globules dramatically led to destabilize meat batter as suggested by increased cooking loss (Tinbergen and Olsman, 1979).

The stability of meat batter depends on many factors, including water and fat holding capacity; amount of meat proteins, water, fat, salt, non-meat additives; and a production condition–*e.g.*, mechanical and heat treatments (Youssef and Barbut, 2010). Generally, increased meat protein content affected to enhance water binding capacity and formation of dense protein network, thereby affecting to restrict fat and fluid migration out of the matrix (Youssef and Barbut, 2010). In some cases, however, excessive muscle protein amount might affect to inferior product stability, because of increasing in thickness and interfacial protein film rigidity, when excess soluble proteins were available (Youssef and Barbut, 2009; 2010). A thick and rigid interfacial protein film restricted expanding of fat globules during heating and would result in fractured interfacial membrane, thereby allowing fat separation from emulsion breakdown (Jones and Mandigo, 1982). In reduced-fat meat products, excessive protein content above a certain level led to inferior product qualities. When protein content of low-fat beef patties was increased up to 15 %, harden texture with high moisture and irregular shape of fat globules was observed (Youssef *et al.*, 2011). Inconsistent morphology of fat globules suggested to an occurring of drop coalescence, which subsequently led to capillary formation within the gel matrix. With an excessive protein concentration, the interaction between protein molecules was enhanced, leading to facilitate aggregation of the proteins, subsequently resulting in a capillary formation in gelled matrix. Presence of capillary in the matrix structure allowed water and fat to migrate out of the gel easily, resulting in a high cooking loss and inferior textural characteristic of meat products (Youssef *et al.*, 2011).

Gelation of salt soluble protein (SSP) plays a crucial role on characteristics of numerous meat products. Sun *et al.,* (2012), investigating influence of peanut protein isolate (PPI) on gel formation behavior of chicken meat breast and thigh SSP, reported that that PPI could greatly promote gelation and enhance sustainment of the SSP gel. This behavior further resulted in improved WHC of the gel matrix. The improved functionalities of SSP using PPI could be supposed since capability of PPI to act as a gel binder in continuous network of SSP via both chemical–*i.e.*, hydrogen bond formation– and physical–*i.e.*, molecular entanglements–factors (Edwards *et al.*, 1987). Nonetheless, using too high concentration–*i.e.,* 3.5 and 4 % of PPI in breast and thigh SSP, respectively–might led to decrease gel strength, because of self association between SSP and PPI. Heat treatment is generally conducted in processed meat production. In sausage making, heating in the range of *ca.* 68–75 °C is typically applied (Youssef and Barbut, 2011a), which might lead to denaturation of the composited proteins. Denaturation of myosin tail was the most critical factor affecting to WHC in processed meat products (Deng *et al.*, 2002). Improvement of myofibrillar protein stabilization against thermal process was observed using protein isolate, as suggested by increasing in enthalpy of denaturation of chicken thigh protein composition when PPI was applied (Sun *et al.*, 2012). Considering from scanning electron microscope images, the improved microstructure–*i.e.*, more compact, finer, and homogeneous gel network–of heat induced gel by PPI adding was confirmed, presumably since cross linking between PPI and SSP via an enhanced hydrophobic interactions after heat exposing (Sun *et al.*, 2012).

3.3 "Health-friendly" emulsified meat products by fat reducing and n-3 rich oil inclusion

3.3.1 Fat reducing

Fat plays important role on sensory attributes of processed meat products, including flavor and texture characteristics. Therefore, inferior qualities–*e.g.*, lowered WHC and less appreciable texture, were always possessed in low-fat meat products (Keeton, 1994). Generally, more water amount was applied to substitute fat in reduced-fat meat products, leading to higher fluid loss after cooking and soften texture of the products (Wu *et al.*, 2009; Youssef and Barbut, 2011b). Degree of fluid loss always proportionally increased with fat diminishing level (Hughes *et al.*, 1997), which might be probably due to insufficient amount of meat protein to hold all added water (Youssef and Barbut, 2011b). Non-muscle proteins–*e.g.*, whey protein (WP) and soy protein isolate (SPI)–are widely employed as additive to improve quality of fat-reduced meat products by improving product stability, water and fat retention (Bloukas *et al.*, 1997; Hsu and Sun, 2006; Andrés *et al.*, 2006). Improved water and fat retention capacity allowed better texture characteristics of comminuted meat batters (Wallingford and Labuza, 1983), especially for fat-reduced formula.

WP is surface active globular proteins, and its several functional properties–*i.e.*, gelation property, water and fat binding capacity, and emulsifying ability–in fat reduced meat products were proven (El-Magoli *et al.*, 1996; Sun *et al.*, 2007). El-Magoli *et al.*, (1997) found improved water retention and lowered shrinkage of low-fat beef patties by adding WP especially at increased concentration, which was supposed due to ability of WP to reduce interfacial tension and enhance fat-protein interactions (Lucca and Tepper, 1994). Using WP and SPI as a non-meat protein ingredient could reduce fat loss and cooking loss of meat batters when animal fat was partially replaced with canola oil, resulting in improved textural parameters–*i.e.,* springiness and chewiness–(Youssef and Barbut, 2011b). This is supposed since the ability of WP and SPI to interact with water and fill in the interstitial space within meat protein matrix (Youssef and Barbut, 2011b). Dexter *et al.,* (1993) successfully reduced cooking loss and got better viscosity of low-fat (12 %) turkey bologna by incorporating 2 % of SPI. This is in accordant with the report of Lin and Mei (2000) showing that SPI could improve WHC of low-fat (15 %) pork batter during heating. This effect was postulated due to 2 reasons–*i)* capacity of SPI to protect heat denaturation of muscle proteins during cooking and *ii)* formation of more stable complex of the denatured meat proteins induced by heat treatment that enhance water and fat entrapment in the batter matrix (Lin and Mei, 2000). Akesowan (2008) reported that moisture content and cooking yield of fat-reduced pork sausage (10 % fat) could be increased by incorporating SPI (2 %) leading to improved firmness and juiciness of the product. This effect was explained by ability of SPI to retain moisture via the enhanced protein-protein and protein-water interactions in the gel matrix (Gregg *et al.*, 1993). Diminish in purge loss during heating and refrigerated storage of pork sausage (Akesowan, 2008) and bologna (Chin *et al.*, 2000) could also be accomplished by adding SPI. Yang *et al.,* (2001) could produce low-fat (10 %) frankfurter with comparable textural acceptability to high-fat (22 %) formula using SPI (4 %). Improved quality of comminuted meat products by SPI was also reported in buffalo meat emulsion sausages (Ahmad *et al.*, 2010), beef batters (Youssef and Barbut, 2011b), pork and beef frankfurthers (Hughes *et al.,* 1998; Atughonu *et al.,* 1998).

According to functional properties of protein isolated from animal sources to be used as non-meat proteins in fat-reduced meat products, Yang *et al.*, (2001) reported the efficiency of muscle protein isolates to improve stability of low-fat (10 %) frankfurter; There was no difference on textural attributes–*i.e.*, firmness, springiness, and cohesiveness–and purge loss during storage between high fat (22 %) and muscle protein isolate treated low-fat frankfurters. Poultry protein isolate could significantly reduce purge loss of low-fat turkey patties during storage in a retail display case, which was expected since WHC of the protein isolate that affected to lower free water availability in the matrix and led to a formation of dense gel structure (Omana *et al.*, 2012).

3.3.2 *n-3 rich oil inclusion*

Substitution of animal fat with *n*-3 rich oils is a feasible way to improve nutritive value of meat products (Josquin *et al.*, 2012; Pelser *et al.*, 2007). Josquin *et al.*, (2012) reported that partial replacement of animal fat with fish oil could improve nutritional value of dry fermented sausages by decreasing *ca.* 3–8 % of the content of saturated fatty acids (SFAs), and increasing 2–8 % of the content of polyunsaturated fatty acids (PUFAs). Moreover, the ratio of *n*-6/*n*-3 fatty acids of the control samples was decreased from 8.73 to 1.99 and 0.92, when animal fat was substituted by fish oil at the level of 15 % and 30 %, respectively (Josquin *et al.*, 2012). It should be noted that high ratio of *n*-6/*n*-3 fatty acids is related to an occurrence of some chronic diseases, such as cancers and coronary heart disorder (Enser, 2000). Lower ratio of *n*-6/*n*-3 fatty acids indicates better product quality in a view of health aspect, and the ratio less than 4 was recommended by nutritional authorities (Wood *et al.*, 2004). Diminished *n*-6/*n*-3 fatty acids ratio of Dutch-style fermented sausage was also reported when flaxseed oil was used to substitute porcine fat, especially at the increased degree of oil inclusion (Pelser *et al.*, 2007).

Characteristics of comminuted meat products could be affected when animal fats were partially replaced with *n*-3 rich oils. Lowered fluid loss of the frankfurter was observed, when canola oil was employed to replace beef fat; Cook loss of the frankfurters formulated with beef fat and canola oil was *ca.* 4 and 2 %, respectively (Youssef and Barbut, 2011b). This effect was expected since 2 reasons–*i)* better WHC of monounsaturated fatty acids abundantly present in canola oil (St. John *et al.*, 1986), and *ii)* a smaller sized canola oil droplets compared to the animal fat globules that provided larger interfacial areas to be covered by more amount of proteins (Youssef and Barbut, 2009). Improved textural characteristics were also investigated in the sausages (Park et al., 1997; Youssef and Barbut, 2011b; Paneras *et al.*, 1998), beef patties (Youssef *et al.*, 2011a), pork meat emulsion (Choi *et al.*, 2009), and pork frankfurters (Paneras *et al.*, 1998), when animal fats were substituted with PUFAs rich oils. Microstructure with larger amounts of smaller sized and closely packed fat globules was observed for beef patties when canola oil was employed to substitute beef fat (Youssef *et al.*, 2011a). To get desirable textural quality, size and size distribution pattern of fat globules distributing in protein gel matrix were suggested to be relevant factors; Fat globules should be comparable in size and small enough to be covered by a sufficient amount of proteins to enhance interaction of myofibrillar proteins, thereby providing homogeneous and dense gel network (Xiong *et al.*, 1992; Youssef and Barbut, 2009; Youssef and Barbut, 2010; Youssef *et al.*, 2011a).

3.4 Introducing of oils to meat products through pre-emulsification technique

Size and size distribution pattern of fat globules embedded in protein gel matrix greatly affect to stability and quality of emulsified meat products. Controlling size and monodispersity of fat globules is a promising strategy to produce emulsified meat products with good stability and characteristic. To accomplish this goal, pre-emulsification process defined as a prior preparation of fat/oil in emulsified form before introducing to meat product manufacture as fat ingredient, is a feasible technique (Jiménez-Colmenero, 2007; Delgado-Pando *et al.*, 2010). The pre-emulsification technique could improve moisture and fat holding ability of protein gel matrix (Delgado-Pando *et al.*, 2010). It has been revealed that reduced cook loss and improved texture was successfully got for fat-reduced meat products, when oil was introduced through the pre-emulsification means (Youssef and Barbut, 2011b; Su *et al.*, 2000; Wu *et al.*, 2009; Josquin *et al.*, 2012). In low-fat pork frankfurter, better stability of protein gel was observed when canola oil was added in pre-emulsified form compared to its native form (Youssef and Barbut, 2011b). Improved WHC of cooked pork muscle protein gels from 28 % to 44 % could be accomplished by incorporating peanut oil through the pre-emulsification process (Wu *et al.*, 2009). Na-caseinate (Nc) with a powerful emulsifying activity could significantly reinforce gel strength when using to stabilize pre-emulsified oil and incorporated to fat-reduced beef frankfurter (Youssef and Barbut, 2011b) and bologna (Bishop *et al.*, 1993). By using pea protein isolate as emulsifier, pre-emulsified vegetable oil incorporating could successfully develop thermal stability, WHC and fat retention capacity of fat-reduced frankfurter during heating process (Su *et al.*, 2000). When oil was introduced via the pre-emulsification process, more amount of soluble meat proteins were available for fat and water binding, because part of fat and water were already stabilized by the non-meat proteins used as emulsifier (Su *et al.*, 2000). According to textural attributes of reduced-fat meat products, oil incorporating through the pre-emulsification means could provide meat products with firmer texture compared to the counterparts added with oil via a conventional method (Youssef and Barbut, 2011b; Cáceres *et al.*, 2008). This effect was explained by a presence of larger protein amount on pre-emulsified oil drops since their small size, thereby allowing more protein-protein interaction and leading to a formation of dense gel matrix (Youssef and Barbut, 2011b). Protein portion adding in the pre-emulsification step, moreover, might interact with myofibrillar protein, subsequently affected to strengthen gel matrix (Xiong *et al.*, 1992). Thus, incorporating fat/oil through the pre-emulsification means might accomplish a dense and homogeneous protein gel matrix formation, leading to improved stability, physicochemical properties, and sensory attributes of the comminuted meat products.

To incorporate oil to comminuted meat products through the pre-emulsification technique, non-meat proteins employed as emulsifier has an important role. Cooking loss of the frankfurters formulated with pre-emulsified canola oil emulsions (17.5 % fat) using SPI and whey protein isolate (WPI) as emulsifier was less than 1 %, whereas twice times higher in cooking loss was observed when Nc was employed as emulsifier (Youssef and Barbut, 2011b). Although Nc is a potent emulsifier used in regular/high fat meat products (Youssef and Barbut, 2011b; Cáceres *et al.*, 2008), its less efficiency to hold water in reduced-fat frankfurter was reported, which was supposed due to inability of Nc to form gel network at the used cooking temperature (Youssef and Barbut, 2011b). Using Tween 80 to pre-emulsify canola oil and incorporate to beef patties led to significantly higher moisture and fat loss, especially when protein in the patties was increased to 9–15 % (Youssef *et al.*, 2011a). Tween 80 is a non-ionic surfactant with higher hydrophilic-lipophilic (HLB) value than the muscle proteins, so it could more preferentially interact with oil than the muscle protein, thereby affecting to reduce interactions between protein and oil and leading to lowered amount of adsorbed muscle proteins on oil drop surfaces (Van Eerd, 1971). The microstructure with high heterogeneity and many capillaries was observed for the meat batter when Tween 80 was used in the product, leading to facilitate fluid migration out of the gel matrix (Gordon and Barbut, 1991). The emulsifier employed in the pre-emulsification step crucially affected to interfacial adsorbed protein layers, as well as continuous protein matrix, thereby influencing on stability and characteristic of emulsified meat products.

4. Methodology

4.1 Preparation and characterization of FPI

4.1.1 Fish sample preparation

Yellow stripe trevally (*Selaroides leptolepis*) with the size of *ca.* 20 fishes per kg was purchased from a local market and taken to Prince of Songkla University within 1 h by placing in ice with the ratio of fish:ice of 1:2 (w/w). Upon arrival, fishes were washed, and meat was separated. The meat was minced and stored in ice until use.

4.1.2 FPI preparation

FPI was prepared via the pH-shift extraction method according the procedure of Hultin and Kelleher (2000) as modified by Rawdkuen *et al.*, (2009). The minced meat was homogenized with cold distilled water (4 °C), before adjusting pH to 11.2. The insoluble materials were separated by centrifugation, and muscle protein was recovered by precipitating at pH 5.5. Then, the isolated protein was adjusted to pH 7, freeze dried, and kept at -18 °C until further analyze. The FPI preparation flow chart is illustrated in Fig. 1.

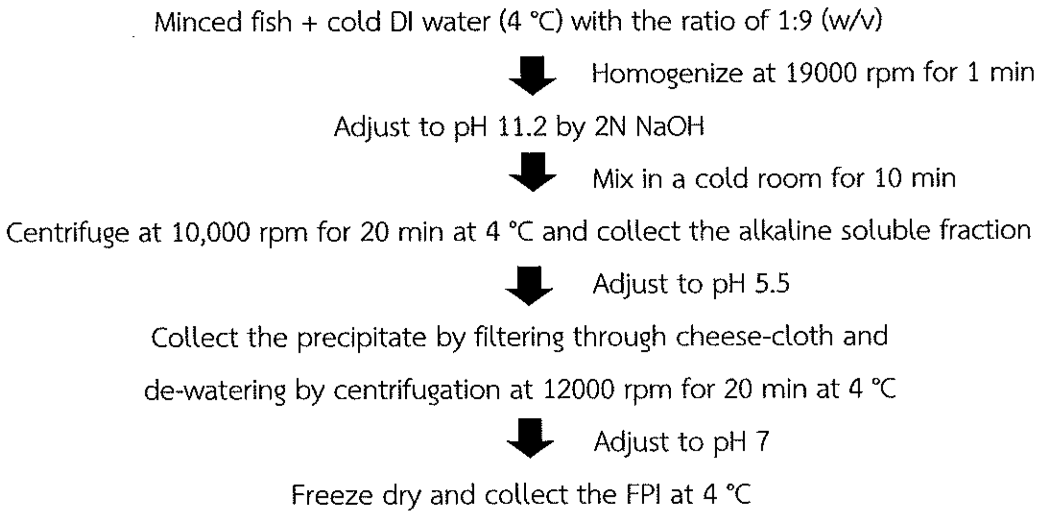


Fig. 1 FPI preparation by pH-shift method

Properties of the FPI were then investigated by measuring:

- Protein recovery: followed the method of Kim *et al.*, (2003)
- Protein solubility: by Bradford technique (Bradford, 1976).
- Protein pattern: by SDS-polyacrylamide gel electrophoresis (SDS PAGE) (Laemmli, 1970)

4.1.3 Emulsifying ability of FPI

Emulsifying capacity of FPI was determined by comparing with SPI and Nc at different protein concentrations. Emulsions were prepared by dissolving the selected proteins–*i.e.,* FPI, SPI, or Nc–in 10 mM phosphate buffer (pH 7) at designated concentrations–*i.e.,* 2, 4, and 6 % w/v. The protein solution was then homogenized with SBO at 19,000 rpm for 5 min to receive the emulsions with 0.5 oil volume fraction. The final concentrations of the used emulsifiers were 1, 2, and 3 %. The emulsion was stored overnight at 4 °C, before analyses. Table 1 shows the formulation of the emulsions of the present work.

Table 1 Formulations of the SBO emulsions stabilized by FPI, SPI, and Nc

Sample	SBO (ml)	Water (ml)	FPI (g)	SPI (g)	Nc (g)
FPI1	50	48	2	-	-
FPI2	50	46	4	-	-
FPI3	50	44	6	-	-
SPI1	50	48	-	2	-
SPI2	50	46	-	4	-
SPI3	50	44	-	6	-
Nc1	50	48	-	-	2
Nc2	50	46	-	-	4
Nc3	50	44	-	-	6

- pH: the emulsions was mixed with water at the ratio of 1:10, before determining their pH (Jiménez-Colmenero *et al.,* 2010)
- Thermal stability: by determining percentage of oil releasing after heating at 70 °C for 30 min as per the method of Jiménez-Colmenero *et al.*, (1995)
- Oil droplet size and size distribution pattern of oil droplets: using laser diffraction particle size analyzer (ZetaPALS, Brookhaven Instrument, Holtsville, NY, USA)

4.2 Study on properties of pork sausages as affected by SBO substitution via pre-emulsification technique using FPI as emulsifier

Effect of partial replacement of porcine fat using SBO in different forms–*i.e.,* native and pre-emulsified forms–on characteristics of pork sausages were studied.

4.2.1 Meat sample preparation

Lean shoulder meat and pork back-fat were purchased from a local market (Hatyai, Songkhla). All visible connective tissue and fat were trimmed from the meat. Then, the chemical compositions of the porcine meat and fat were determined (AOAC, 1995). After that, the meat and fat were comminuted separately to receive a homogeneous mass, vacuum-packed separately, and frozen (–18 °C) in a polyethylene bags (750 g/pack) for less than 1 month before use. The frozen meat and fat were thawed at 4 °C overnight prior to use to prepare the sausages.

4.2.2 Preparation of pre-emulsified SBO (preSBO)

On the day before sausage production, preSBO was prepared followed the method of Youssef and Barbut (2011c) with a slight modification. Firstly, the selected protein–*i.e.,* FPI, SPI, or Nc–was dissolved in 10 mM phosphate buffer (pH 7) at a designated concentration–*i.e.,* 2, 4, and 6 % w/v. The protein solution was then homogenized with SBO at 19,000 rpm for 5 min at an oil volume fraction of 0.5. The emulsion was stored overnight at 4 °C before use.

4.2.3 Preparation and characterization of sausages

Sausages were prepared according to the method of Youssef and Barbut (2011c) with a slight modification. The control sausages contained 30 % of porcine fat, whereas SBO was used to partially substitute pork fat at various degrees–*i.e.*, 25, 35, and 45 % by wt. of porcine fat–for the studied samples. Protein level of all sausage formulations was controlled at 15 %. To control the protein content of all sausages, amounts of proteins of the used emulsifiers–*i.e.*, for FPI (84.7 %), SPI (93.6 %), and Nc (97.0 %), respectively–were subtracted from the added amount of muscle protein. To control the same water content in all formulations, moisture content of the sausage with the highest water level (the sausages formulated with preSBO at oil substitution level of 45 %) was used as a fix level, and additional water was added for the other samples. Formulations of the sausages are shown in Table 2.

Table 2 Formulations of the sausages (1 batch) at different SBO substitution levels

SBO substitution level (%)	sample	emulsifiers	Meat (g)	Back-fat (g)	SBO		Water (g)
					Native	preSBO	
0	Control	-	248.0	180.1	-	-	149.6
25	NSBO	-	294.8	135.1	45.0	-	120.4
	FPI-preSBO	1% FPI	288.4	135.1	-	90.1	80.0
		2% FPI	282.1	135.1	-	90.1	84.6
		3% FPI	275.8	135.1	-	90.1	89.2
	SPI-preSBO	1% SPI	287.3	135.1	-	90.1	80.8
		2% SPI	279.8	135.1	-	90.1	86.3
		3% SPI	272.3	135.1	-	90.1	91.7
	Nc-preSBO	1% Nc	287.3	135.1	-	90.1	80.8
		2% Nc	279.8	135.1	-	90.1	86.2
		3% Nc	272.3	135.1	-	90.1	91.7
35	NSBO	-	297.1	117.1	63.1	-	120.7
	FPI-preSBO	1% FPI	288.2	117.1	-	126.2	64.1
		2% FPI	279.3	117.1	-	126.2	70.6
		3% FPI	270.4	117.1	-	126.2	77.1
	SPI-preSBO	1% SPI	286.6	117.1	-	126.2	65.3
		2% SPI	276.1	117.1	-	126.2	72.9
		3% SPI	265.6	117.1	-	126.2	80.1
	Nc-preSBO	1% Nc	286.6	117.1	-	126.2	65.3
		2% Nc	276.1	117.1	-	126.2	72.9
		3% Nc	265.6	117.1	-	126.2	80.6
45	NSBO	-	299.4	99.1	81.1	-	120.9
	FPI-preSBO	1% FPI	288.0	99.1	-	162.2	48.2
		2% FPI	276.5	99.1	-	162.2	56.6
		3% FPI	265.1	99.1	-	162.2	64.9
	SPI-preSBO	1% SPI	285.9	99.1	-	162.2	49.7
		2% SPI	272.5	99.1	-	162.2	59.5
		3% SPI	258.9	99.1	-	162.2	69.4
	Nc-preSBO	1% Nc	258.9	99.1	-	162.2	49.7
		2% Nc	272.5	99.1	-	162.2	59.5
		3% Nc	258.9	99.1	-	162.2	69.4

All formulations consisted of NaCl (7.5 g), sucrose (2.5 g), sodiumtripolyphosphate (1 g), seasoning powder (0.5 g), sodium nitrite (0.05 g), and monosodium glutamate (0.4 g).

Control: the sausages without SBO; NSBO: the sausages added with SBO in native form; FPI-preSBO, SPI-preSBO, and Nc-preSBO: the sausages added with preSBO using FPI, SPI, and Nc as emulsifiers, respectively

The additive was mixed on the day of use by dissolving in the added water and chilled at 4 °C. The minced meat was chopped at low speed for 30 sec, followed by adding with a half of back-fat (or SBO in alternative forms) and the additive mixture, before further mixing for 1 min. The mixture was then added with the rest of the additive and porcine fat (or SBO in alternative forms), further chopped for 1 min, and allowed to stand for 1.5 min for protein extraction, before further chopping for 2 min. The temperature was controlled to lower than 12 °C throughout the preparation. After that, the batter was stuffed in a cellulose casing (15 mm in diameter), heated at 75 ± 2 °C for 30 min in a water bath, and then cooled in cold water until core temperature of the sausages was lowered than 10 °C. The sausages were placed in a polyethylene bag, packed without vacuum, and stored at 4 °C until analysis. In each formulation, 30 sausages were prepared in each batch, and 2 batches were prepared separately.

Physicochemical properties, texture profile, and microstructure of the sausages were investigated.

- pH: according to the method of Lin and Mei (2000)
- cooking loss: according to the method of Youssef and Barbut (2010)
- water holding capacity (WHC): as per the method of McClord *et al.*, (1998)
- color measurement: color of the fresh cut cross-section sample (10 pieces) was determined through CIE system and reported as Hunter L^* (lightness), a^* (redness), and b^* (yellowness) (Wiegand and Waloszek, 2003)
- microstructure: morphology of the sausages was investigated using a scanning electron microscope (FEI Quanta 400, FEI Europe BV, Eindhoven, the Netherlands) followed the method of Jiang and Xiong (2013) with a slight modification. A cubic sample ($2.5 \times 2.5 \times 2.5$ mm^3) was fixed by glutaraldehyde (2 %) for 24 h at 4 °C. Then, dehydrating was conducted by the following series of ethanol solutions with concentrations of 50, 70, 80, and 90 % once each for 10 min, and twice in absolute ethanol for 10 min. The samples were then subjected to a critical point dryer (Balzer model CPD 030, Liechtenstein, Switzerland) using CO_2 as transition fluid. The dried samples were sputter-coated with gold, and observed at an accelerating voltage of 15 kV.
- Texture profile analysis (TPA): Five cylindical shaped samples (length 250 mm) were prepared from the middle of sausages and subjected to TPA using a texture analyzer (TA-XT2 Stable Micro System, Surrey, UK). The samples were compressed to 50 % of their original height at a cross speed of 5 mm/s by a cylindrical plunger (diameter 5 mm) for twice cycles. Texture attributes–*i.e.*, hardness (N/cm^2; the maximum force during the first compression cycle), cohesiveness (the extension which the sample could be deformed before rupture),

chewiness (Ns; the work required to chew a solid sample to a steady state of swallowing), springiness (cm; the height that sample recovers during the time that elapses between the end of first compression and the begin of second compression), and gumminess (N/c^{m2}; the force needed to disintegrated a semisolid sample to a steady state of swallowing)–were then identified (Bourne 1978).

From the collected data, oil introducing technique, SBO substitution level, and effective FPI (or SPI and NSC) concentration providing the sausages with a good stability and desirable characteristic were selected to formulate pork sausage in the next study.

4.3 Study on properties of pork sausages as affected by reducing fat content

Next, the characteristics of the pork sausage as affected by reducing total fat content and partial replacement of porcine fat using pre-SBO was studied. The sausages were prepared with the method as described in the previous part. Protein content was controlled at 15 %. Table 3 shows the sausage formulations with various fat reducing degrees. According from the previous part, the SBO substitution level was selected at 25 % by wt. of porcine fat.

Table 3 Formulations of the sausages (1 batch) with various degrees of fat reduction. Porcine fat was partially replaced with SBO in alternative forms at 25 % by wt. of porcine fat.

Total fat content (%)	sample	Meat (g)	Back-fat (g)	SBO		Water (g)
				Native	preSBO	
30	Control	248.0	180.1	-	-	149.6
	NSBO	294.8	135.1	45.0	-	120.4
	FPI-preSBO	282.1	135.4	-	90.0	84.6
	SPI-preSBO	287.3	135.1	-	90.0	80.8
	Nc-preSBO	287.3	135.1	-	90.0	84.6
20	Control	296.7	120.1	-	-	135.3
	NSBO	300.6	90.1	25.0	-	121.1
	FPI-preSBO	296.4	90.1	-	50.0	105.0
	SPI-preSBO	296.8	90.1	-	50.0	104.8
	Nc-preSBO	293.6	90.1	-	50.0	106.8
10	Control	304.4	60.1	-	-	121.0
	NSBO	306.3	45.0	12.5	-	121.8
	FPI-preSBO	304.2	45.0	-	25.0	110.8
	SPI-preSBO	304.4	45.0	-	25.0	110.7
	Nc-preSBO	302.2	45.0	-	25.0	112.3

All formulations consisted of NaCl (7.5 g), sucrose (2.5 g), sodiumtripolyphosphate (1 g), seasoning powder (0.5 g), sodium nitrite (0.05 g), and monosodium glutamate (0.4 g).

** The concentrations of FPI (2 %), SPI (1 %), and Nc (1%) were used to prepare preSBO*

Physicochemical properties, TPA, and microstructure of the sausage samples were determined as aforementioned in part 4.2.3.

4.4 Statistical analysis

All experiments were run in triplicate and the data were reported as means ± SD (*n*=3). Two-way analysis of variance (ANOVA) was used, and mean comparison was performed using Duncan multiple range test by the SPSS statistic program (Version 10.0; SPSS Inc., Chicago, IL, USA).

5. Results and Discussion

5.1 Preparation and characterization of fish protein isolate (FPI)

FPI was prepared from the yellow stripe trevally (*Selaroides leptolepis*) via the pH–shift method. Chemical composition of the fish flesh was firstly determined: The fish meat consisted of protein (21.3 ± 0.5 %), fat (1.6 ± 0.1 %), moisture (75.2 ± 0.3 %), and ash (1.2 ± 0.1 %). To calculate efficiency of protein recovery, weights of recovered fish minces and initial fish meats were present in Table 4. Protein recovery efficiency was 63.68 ± 1.49 %. Higher efficiency of the alkaline–aided extraction (71.5 %) to isolate protein from minced tilapia than did the conventional method (67.9 %) was reported (Rawdkuen *et al.*, 2009). This tendency was postulated since the maintained sarcoplasmic proteins in muscle for the alkaline–aided extraction, whereas an extensive washing of conventional method affected to enhance sarcoplasmic protein solubility, thereby lowering the recovered protein amount (Rawdkuen *et al.*, 2009).

Table 4 Weights of initial fish meats and recovered fish mince for FPI preparation

Replicate	Weight (g)		Protein recovery efficiency (%)
	Initial fish meat	Recovered fish mince	
1	180.49	112.33	62.25
2	210.52	137.34	65.23
3	190.39	121.03	63.57
average ± standard deviation			63.68 ± 1.49

**The moisture content used for calculation was 75.23 %.*

The protein content of FPI was 84.74 ± 0.78 %.

Protein pattern of the FPI was observed by SDS-PAGE analysis (Fig. 2). Dense protein bands with molecular weight of *ca.* 35–50 kDa were observed for the FPI, which were supposed to be actin myofibrillar protein (Klompong *et al.*, 2009). It was reported that actin could be recovered more potentially at high pH range (25.8 % at pH 11) compared to low pH condition (16.9 % at pH 2.5) (Kristinsson and Ingadottir, 2006). In the present work, the band of myosin heavy chain with molecular weight of *ca.* 200 kDa (Klompong *et al.*, 2009) was not present. By using acid and alkaline solubilisation processes, degradation of myosin heavy chain could be induced, resulting in a protein band with lower molecular weight (Yongsawatdigul and Park, 2004).

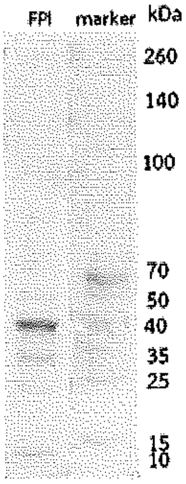


Fig. 2 SDS PAGE patterns of FPI

Next, solubility of FPI was observed as a function of pH, and the result was depicted in Fig. 3. Solubility of proteins obviously depended on pH level. The lowest protein solubility was observed at pH 5.5 supposed to be the isoelectric point (IEP) of the FPI (Kim *et al.*, 2003). The lowest solubility at pH around IEP of proteins could be attributed to a low electrostatic repulsion between charged amino acid residues, resulting in a closer contact of the proteins and thereby promoting hydrophobic interactions (protein–protein interaction) and aggregation of the protein molecules (Kristinsson and Ingadottir, 2006). At pH away from IEP, proteins became electrical charge, leading to promote solubility of the proteins via electrostatic repelling and hydration forces between the charged residues (Damodaran, 1996). In this work, greater protein solubility was observed in alkaline pH than those observed in acidic condition. This tendency was in agreement with other studies: Maximum solubility of the protein isolated from Pacific whiting (*Merluccius productus*) (Kim *et al.*, 2003) and Tilapia (*Oreochromis niloticus*) (Kristinsson and Ingadottir, 2006) was observed at pH 12. To recover proteins from Tilapia, alkaline aided–extraction (cold extraction at 4 °C using 2 N Na_2CO_3 at pH 11.2) provided higher protein recovery efficiency compared to a conventional method (cold extraction at 4 °C using distilled water) (Rawdkuen *et al.*, 2009). However, lower solubility (*ca.* 0.57 mg/g) was observed for the proteins recovered by the former methods, which was attributed to muscle protein denaturation induced by alkaline environment (Rawdkuen *et al.*, 2009).

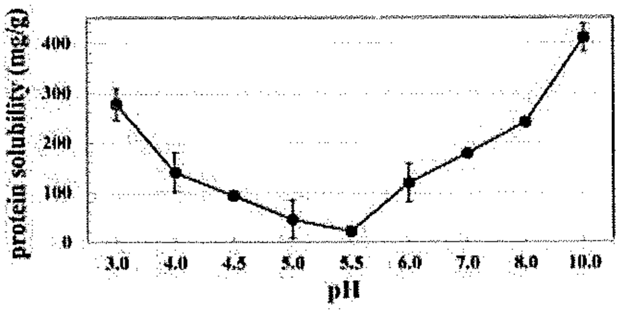


Fig. 3 Solubility of FPI at various pH levels

5.2 Study on properties of pork sausages as affected by SBO substitution via pre-emulsification technique using FPI as emulsifier

5.2.1 Preparation of porcine meat

All visible connective tissues and fat were firstly removed from porcine meat. Then, chemical compositions of the porcine meat and fat were determined, and the result was shown in Table 5.

Table 5 Chemical compositions (%) of porcine meat and back-fat

Sample	Chemical compositions			
	protein	crude fat	moisture	ash
Lean meat	24.03±1.23	1.34±0.10	72.91±0.37	1.15±0.01
Back-fat	3.08±0.02	83.27±1.17	10.85±0.63	0.16±0.01

5.2.2 Emulsifying activity of FPI used to prepare pre-emulsified SBO (preSBO)

Emulsifying activity of FPI was determined by comparing with the non meat proteins commercially used in meat manufacture–*i.e.*, soy protein isolate (SPI) and Na-caseinate (Nc). SBO emulsions were prepared with oil volume fraction of 0.5, and stabilized by the selected proteins at different concentrations–*i.e.*, 1, 2, and 3 %, wt/v of the total emulsion, before determining their characteristics involving mean diameter of oil droplets, pH, and heat stability. Table 6 shows the properties of the emulsion samples.

Table 6 Characteristics of the SBO emulsions stabilized by FPI, SPI, and Nc at various concentrations

Emulsifier	Concentration (%)	*Oil droplet size* (μm)	pH	Cook loss (%)
FPI	1	5.27±0.27Aa	10.05±0.07Aab	1.01 ± 0.27Ca
	2	4.99±0.40Aab	10.25±0.05Aa	0.79 ± 0.01Cab
	3	4.63±0.35Ab	10.33±0.02Aa	0.48 ± 0.01Cb
SPI	1	4.24±0.26Ba	8.43±0.02Ba	1.72 ± 0.01Bab
	2	2.76±0.40Bb	8.50±0.06Ba	2.11 ± 0.15Ba
	3	2.78±0.34Bb	8.47±0.04Ba	2.11 ± 0.42Ba
Nc	1	3.75±0.28Ca	7.94±0.01Ca	3.46 ± 0.10Aab
	2	2.84±0.19Cb	7.97±0.05Ca	3.95 ± 0.24Aa
	3	2.28±0.15Cc	7.92±0.02Ca	3.60 ± 0.27Aa

Means ± standard deviations (n=3) were shown. In each column, capital letters indicate significant difference between means at a same emulsifier concentration ($p<0.05$). Small letters indicate significant difference between means at a same emulsifier type ($p<0.05$).

**The pH of the emulsions stabilized by FPI was higher than the others, because FPI was firstly dissolved in alkaline environment to enhance its solubility.*

Bigger oil droplet size was found for the emulsions stabilized by FPI than the counterparts stabilized by SPI and Nc ($p<0.05$). Increased emulsifier concentration generally provided the emulsions with smaller oil droplets. Nonetheless, increase emulsifier concentration affected to broader size distribution patterns of the emulsions (see Fig. 4). The emulsions stabilized by FPI exhibited the highest heat stability as suggested by the lowest cook loss of the emulsions compared to the samples stabilized by SPI and Nc ($p<0.05$). By using pea protein isolate (PPI), improvement on thermal stability of myofibrillar proteins could be achieved, as indicated by increasing in denaturation enthalpy of chicken thigh proteins when PPI was added (Sun *et al.*, 2012). Considering from scanning electron microscope images, moreover, better microstructure characteristic–*i.e.*, more compact, finer, and homogeneous gel network–of heat induced gel could be observed when PPI was incorporated, presumably since cross linking between PPI and the muscle proteins via hydrophobic interactions induced by heating (Sun *et al.*, 2012). Increase FPI concentration to 3 % led to lowered cook loss of the emulsions ($p<0.05$), whereas different cook loss of the emulsions incorporated with SPI and Nc at different concentrations was not noticeable (*p*>0.05). By adding PPI at too high concentration (3.5 and 4 % of PPI adding to the gels of chicken breast and thigh proteins, respectively), decrease gel strength of the muscle proteins could be observed. This phenomenon was expected since self association between muscle proteins and PPI that affected to restrict stabilizing effect of PPI (Sun *et al.*, 2012).

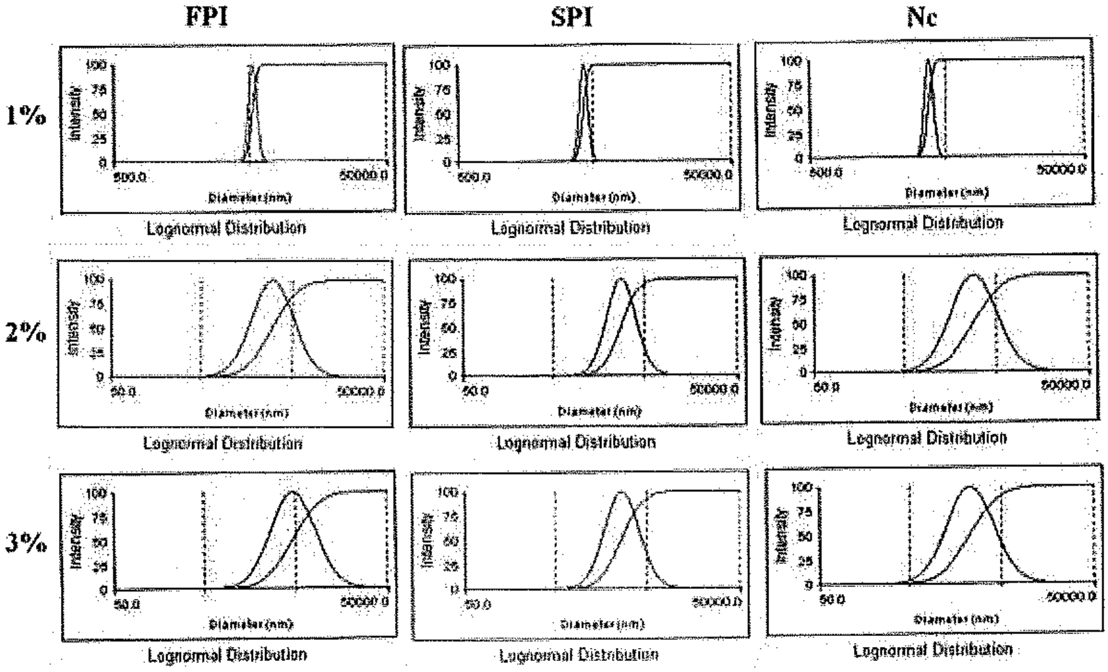


Fig. 4 Size distribution patterns of oil droplets of the emulsions stabilized by FPI, SPI, and Nc at different concentrations (1, 2, and 3 %).

In the present work, Nc possessed the best emulsifying capability compared to other non-meat proteins as suggested by the lowest oil droplet size of the emulsions stabilized by Nc compared to those stabilized with SPI and FPI (*p*<0.05). Nc was regarded to be a potent emulsifier used in regular/high fat meat products (Youssef and Barbut, 2011c; Cáceres *et al.*, 2008). Nonetheless, the highest cook loss of the Nc stabilized emulsions was found (*p*<0.05). Inferior water holding capacity of the reduced-fat frankfurter added with Nc was observed, which was postulated due to inability of Nc to form gel network at the used cooking temperature (Youssef and Barbut, 2011c). Cooking loss of the frankfurters containing pre-emulsified canola oil emulsions (17.5 % fat) stabilized by SPI and whey protein isolate (WPI) was less than 1 %, whereas twice times higher in cooking loss was observed when Nc was employed as emulsifier (Youssef and Barbut, 2011c).

Although the inferior emulsifying ability of FPI compared to SPI and Nc was implied in the present work, better thermal tolerance of the FPI based emulsions was observed. By incorporating PPI stabilized pre-emulsified vegetable oil to fat-reduced frankfurter, improvement on thermal stability, water holding capacity, and fat retention during heating process was observed (Su *et al.*, 2000). In emulsified meat matrix, gelling ability and water retention capacity of non-meat proteins had a greater role rather than emulsifying capacity in determining stability of the product (Su *et al.*, 2000). Effect of FPI to be used as non–meat protein on characteristics of sausages was studied in the following parts by comparing with SPI and Nc.

5.2.3 Effects of SBO adding via pre-emulsification technique on characteristics of sausages

The characteristic of pork sausages as affected by partial replacement of back-fat using SBO was studied. Control sausages consisted of 30 % porcine fat. For the studied samples, the porcine fat was partially substituted with SBO at different degrees–*i.e.*, 25, 35, and 45 % by wt of porcine fat. SBO was incorporated to the sausages in alternative forms–*i.e.,* native form and pre-emulsified form using FPI, SPI, and Nc as emulsifiers at varying concentrations–*i.e.*, 1, 2, and 3 %. Characteristics of the sausages were examined measuring cooking loss, water holding capacity (WHC), pH and color.

Alteration of fat source and fat introduction method affected to properties of sausages. Fig. 5 shows cooking loss of the sausage samples. There was no significant difference on cooking loss between Control (the sausages without SBO) and NSBO, irrespectively of SBO inclusion levels ($p>0.05$). Introducing SBO in pre-emulsified form influenced cooking loss of the sausages in different manners depending on the employed emulsifier type and SBO substitution levels. As compared to the Control, higher cooking loss was observed for SPI-preSBO and Nc-preSBO ($p<0.05$), but FPI-preSBO possessed lowered cooking loss ($p<0.05$). This result suggested to the improved protein matrix strength of the sausages by incorporating FPI. In emulsified meat products, proteins plays important role on a formation of gel network contributing to entrap water and dispersed fat globules (Ziegler and Acton, 1984).

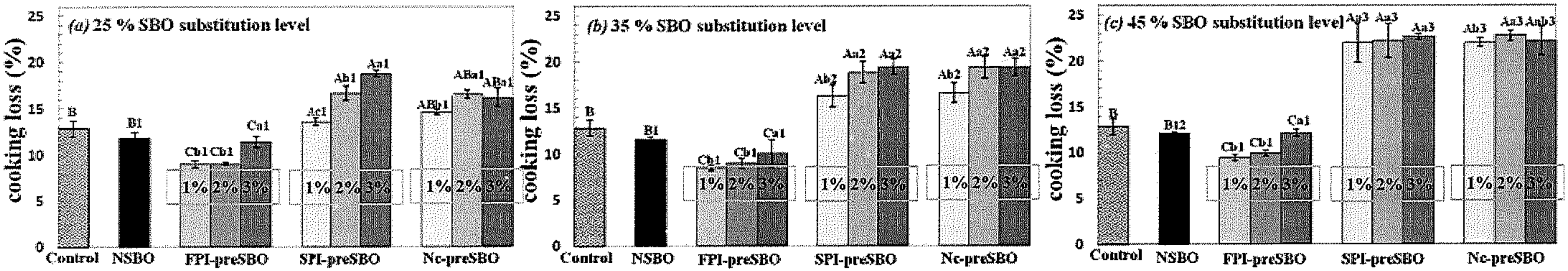


Fig. 5 Cooking loss of the sausages as affected by partial replacement of porcine fat with SBO at *(a)* 25 %, *(b)* 35 %, and *(c)* 45 % by weight of porcine fat. SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized pre-emulsified SBO, SPI stabilized pre-emulsified SBO, and Nc stabilized pre-emulsified SBO, which were referred to Control, NSBO, FPI-preSBO, SPI-preSBO, Nc-preSBO, respectively. FPI, SPI, and Nc were used at different levels–*i.e.*, 1 %, 2 %, and 3 %.

Means ± standard deviations (*n*=3) were shown.

In each subfigure, capital letters indicate significant difference between means at a same emulsifier concentration ($p<0.05$). Small letters indicate significant difference between means at a same SBO introducing form ($p<0.05$).

Different numeric letters indicate significant difference between means at a same emulsifier concentration and SBO introducing form ($p<0.05$).

Regarding to the effect of emulsifier concentration, higher cooking loss was observed when FPI content was increased to 3 % ($p<0.05$), whereas using SPI and Nc at 2 % led to increase cooking loss of the sausages at 35 and 45 % SBO substitution levels ($p<0.05$). By adding pea protein isolate at too high concentration (peas protein isolate concentrations of 3.5 and 4 % for the gels of chicken breast and thigh proteins, respectively), decreasing in gel strength was reported, which was expected due to self association between muscle proteins and the isolated proteins, thereby reducing the stabilizing effect of the isolated proteins (Sun *et al.*, 2012). Considering on effect of SBO substitution level, the SPI-preSBO and Nc-preSBO showed increased cooking loss thoroughly with increasing oil substitution level ($p<0.05$). Nonetheless, increased cooking loss with level of SBO was not noticeable for the NSBO and FPI-preSBO ($p>0.05$).

Fig. 6 illustrates WHC of the sausage samples. Partial replacement of porcine fat with SBO generally affected to diminish WHC of the sausages, especially at the increased SBO substitution level. There was no significant difference on WHC of the NSBO and FPI-preSBO (SPI-preSBO and Nc-preSBO) at all observed SBO adding levels ($p>0.05$). At 25 and 35 % SBO substitution levels, the FPI-preSBO could maintain a comparable WHC with the Control ($p>0.05$), suggesting to better effective ability of FPI to retain matrix strength than did Nc and SPI. This tendency was in agreement with the result of cooking loss as shown in Fig. 5. Emulsifiers employed for pre-emulsified oil preparation played a crucial role on stability of comminuted meat product. Although effective emulsifying ability of Nc was reported, decrease in the protein gel strength was observed when pre-emulsified canola oil stabilized by Nc was incorporated (Cáceres *et al.*, 2008; Youssef and Barbut, 2011b). This phenomenon was attributed to inferior gelation activity of Nc at elevated temperature (Youssef and Barbut, 2011b). Higher purge loss of low-fat frankfurter incorporated with SPI than did muscle protein isolate was reported (Yang *et al.*, 2001).

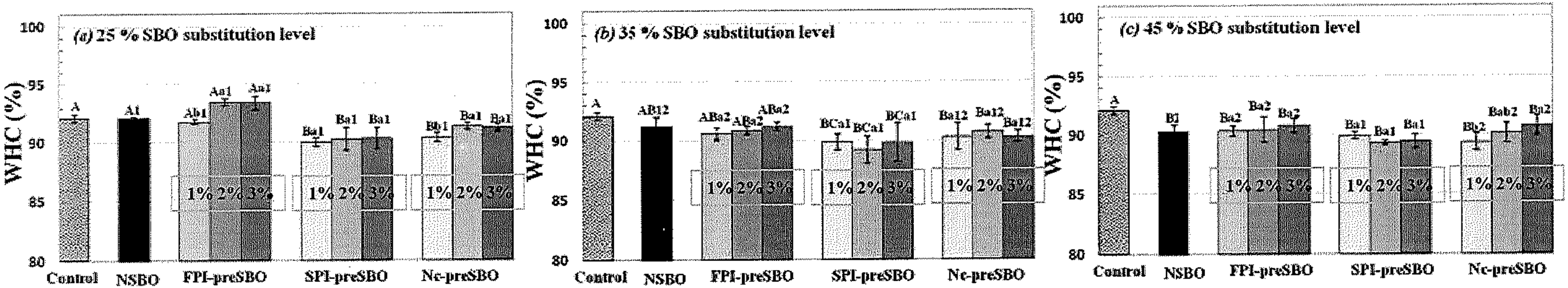


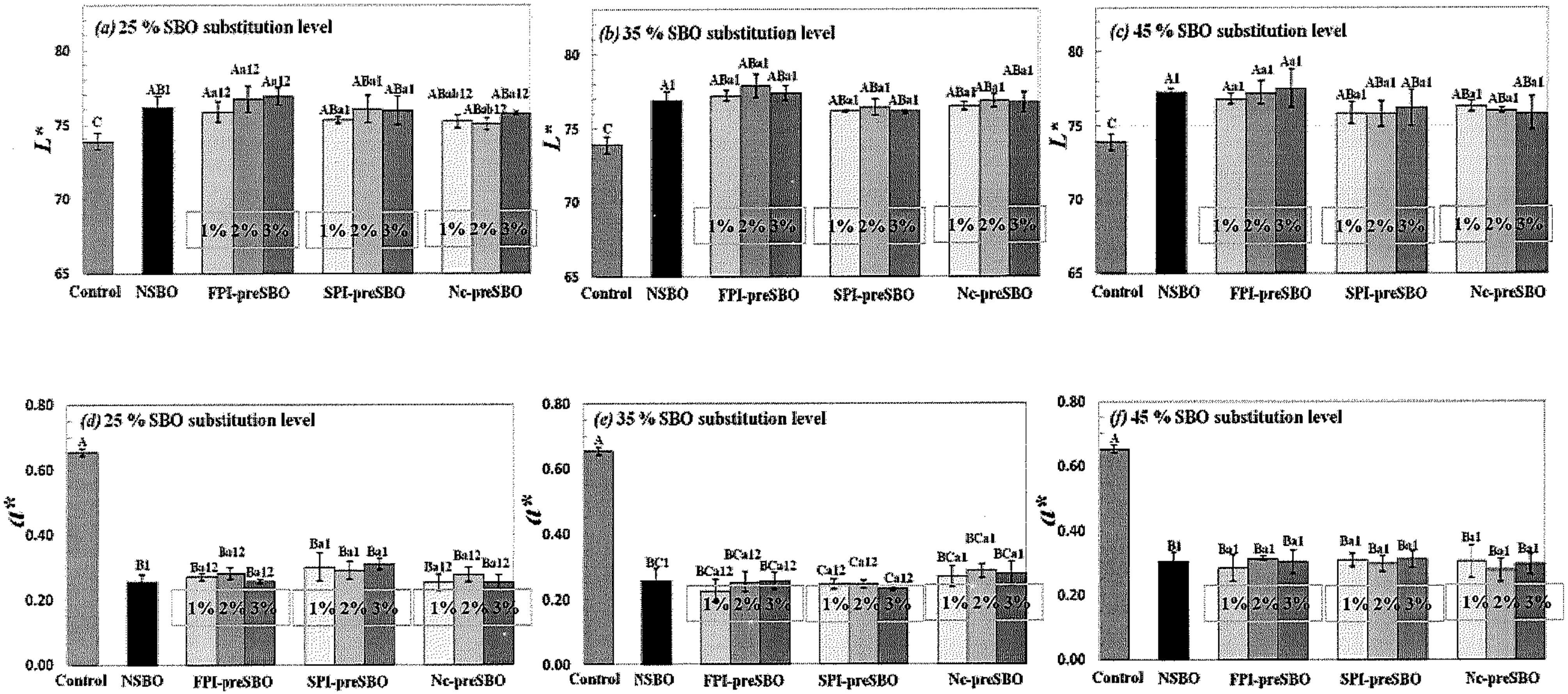
Fig. 6 Water holding capacity (WHC) of the sausages as affected by partial replacement of porcine fat with SBO at *(a)* 25 %, *(b)* 35 %, and *(c)* 45 % by weight of porcine fat. SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized pre-emulsified SBO, SPI stabilized pre-emulsified SBO, and Nc stabilized pre-emulsified SBO, which were referred to Control, NSBO, FPI-preSBO, SPI-preSBO, Nc-preSBO, respectively. FPI, SPI, and Nc were used at different levels–*i.e.*, 1 %, 2 %, and 3 %.

Means ± standard deviations (*n*=3) were shown.

In each subfigure, capital letters indicate significant difference between means at a same emulsifier concentration (p<0.05). Small letters indicate significant difference between means at a same SBO introducing form (p<0.05).

Different numeric letters indicate significant difference between means at a same emulsifier concentration and SBO introducing form (p<0.05).

Color parameters involving lightness (L^*), redness (a^*), and yellowness (b^*) of the sausages were examined and the result was shown in Fig. 7. Partial replacement of porcine fat with SBO affected to increase L^* and b^* of the sausages ($p<0.05$), whereas a^* was significantly reduced ($p<0.05$). The present result agreed with the previous study observing increased lightness and yellowness of the sausages when olive oil was employed to substitute porcine fat (Bloukas *et al.*, 1997). Vegetable oils tended to provide smaller droplet size compared to animal fat globules, and the smaller sized oil droplets with larger surface areas could better reflect light than did the animal fat globules, resulting in higher lightness of the products (Youssef and Barbut, 2011b). Lowered redness of the sausages formulated with SBO might be regarded due to a reduction in myoglobin present in meat source when vegetable oil was incorporated to the recipe. Markedly diminished redness was observed in meat emulsion when animal fat content was reduced (Youssef and Barbut, 2011b). By incorporation oil via pre-emulsication technique, reduction in redness of bologna was also observed (Bishop *et al.*, 1993). Increase in yellowness found for the sausages with SBO inclusion might be expected since a yellow color of the oil. This tendency was in accordance with development in yellowness of the frankfurters added with canola oil (Youssef and Barbut, 2011b). There was no noticeable difference on color appearance of the sausages incorporated with SBO in different forms ($p>0.05$). Emulsifier concentration also had no significant effect on color parameters of the sausages ($p>0.05$)


(a) 25 % SBO substitution level
(b) 35 % SBO substitution level
(c) 45 % SBO substitution level
(d) 25 % SBO substitution level
(e) 35 % SBO substitution level
(f) 45 % SBO substitution level
L*
a*
80
75
70
65
0.80
0.60
0.40
0.20
0.00
1% 2% 3%
Control
NSBO
FPI-preSBO
SPI-preSBO
Nc-preSBO
C
AB1
Aa12
ABa1
ABab12
ABa12
A1
A
B1
BC1
Ba12
Ba1
BCa12
Ca12
BCa1

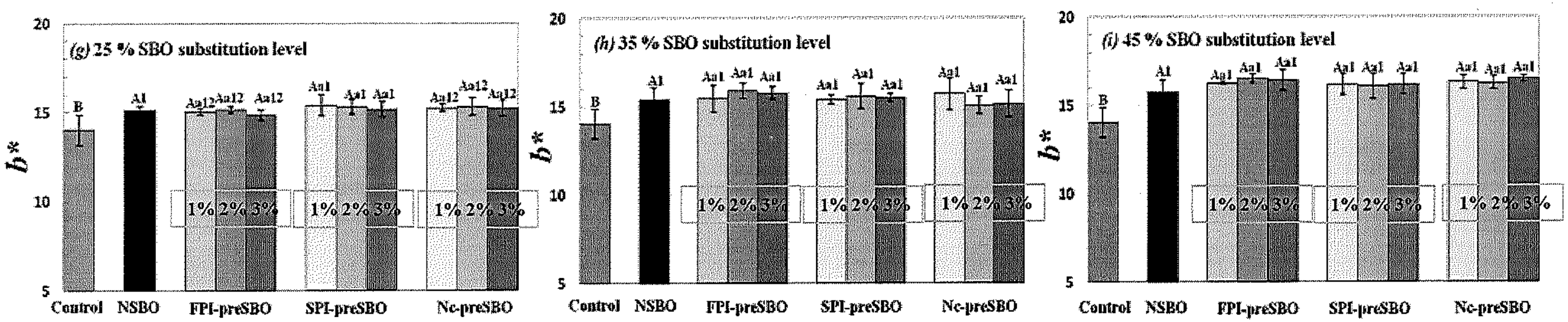


Fig. 7 Lightness (*L**), redness (*a**), and yellowness (*b**) of the sausages as affected by partial replacement of porcine fat with SBO at different levels–*i.e.*, 25 %, 35 %, and 45 %–by weight of porcine fat. SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized pre-emulsified SBO, SPI stabilized pre-emulsified SBO, and Nc stabilized pre-emulsified SBO, which were referred to Control, NSBO, FPI-preSBO, SPI-preSBO, Nc-preSBO, respectively. FPI, SPI, and Nc were used at different levels–*i.e.*, 1 %, 2 %, and 3 %.

Means ± standard deviations (*n*=3) were shown. *In each subfigure, capital letters indicate significant difference between means at a same emulsifier concentration (p<0.05). Small letters indicate significant difference between means at a same SBO introducing form (p<0.05). Different numeric letters indicate significant difference between means at a same emulsifier concentration and SBO introducing form (p<0.05).*

Next, texture attributes–*i.e.*, hardness (the maximum force during the first compression cycle), cohesiveness (the extension which the sample could be deformed before rupture), chewiness (the work required to chew a solid sample to a steady state of swallowing), springiness (the height that sample recovers during the time that elapses between the end of first compression and the begin of second compression), and gumminess (the force needed to disintegrated a semisolid sample to a steady state of swallowing)–were identified (Bourne 1978). The texture profiles of the sausage samples were illustrated in Table 7.

Partial replacement of porcine fat with SBO affected to texture profile of the sausages, depending on SBO added form and level. Higher hardness and gumminess were found for the NSBO, FPI-preSBO, and Nc-preSBO compared to Control and SPI-preSBO (p<0.05), suggesting to stronger gel networks of the former samples. Replacing beef fat with canola oils markedly increased hardness of the meat emulsion (Youssef and Barbut, 2011b). This behavior was postulated since smaller sized canola oil droplets compared to animal fat globules, so the canola oil droplets possessed larger surfaces areas to enhancing interactions between the protein residues, thereby reinforcing the protein gel network (Youssef and Barbut, 2010). In the present work, difference on springiness and cohesiveness of all samples was not noticeable (p>0.05). Emulsifier content also had no effect on texture profile of the samples, irrespectively of emulsifier type (p>0.05). Increase SBO substitution level affected to decrease protein gel strength, as suggested by lowered hardness, chewiness, and gumminess of the sausages formulated with SBO at 45 % (p<0.05).

Table 7 Texture parameters of the sausages as affected by partial replacement of porcine fat with SBO in different forms at various substitution levels

sample	Emulsifier concentration (%)	SBO level (% by wt of pork fat)	Hardness (N/cm2)	Cohesiveness	Chewiness (Ns)	Gumminess (N/cm2)	Springiness (cm)
Control	-	-	2351.2±108.4 B	0.652±0.056 A	1062.4±12.3 B	1271.1±45.3 C	0.823±0.015 AB
NSBO	-	25	3039.4±121.0 A1	0.676±0.046 A1	1505.7±140.1 A1	1979.0±17.0 A1	0.852±0.013 A1
FPI-preSBO	1		3022.5±74.3 Aa1	0.680±0.006 Aa1	1497.8±79.7 Aa1	1872.6±84.4 ABa1	0.839±0.005 Aa1
	2		3015.9±151.2 Aa1	0.691±0.028 Aa1	1581.9±60.1 Aa1	1869.3±104.8 ABa1	0.847±0.010 Aa1
	3		2988.3±74.1 Aa1	0.688±0.001 Aa1	1455.0±139.8 Aa1	1828.2±109.6 ABa1	0.852±0.012 Aa1
SPI-preSBO	1		2518.2±198.8 Ba1	0.681±0.004 Aa1	1385.4±76.2 Aa1	1566.8±96.0 Ba1	0.844±0.018 Aa1
	2		2399.0±116.5 Ba1	0.685±0.003 Aa1	1375.1±125.2 Aa1	1539.6±63.5 Ba1	0.841±0.023 Aa1
	3		2302.5±168.9 Ba1	0.683±0.022 Aa1	1328.6±118.8 Aa1	1540.3±16.4 Ba1	0.835±0.019 Aa1
Nc-preSBO	1		2938.4±88.7 Aa1	0.685±0.001 Aa1	1485.4±65.9 Aa1	1921.6±83.5 Aa1	0.853±0.016 Aa1
	2		2950.7±143.6 Aa1	0.682±0.017 Aa1	1461.4±180.7 Aa1	1964.6±119.8 Aa1	0.861±0.023 Aa1
	3		2889.7±162.2 Aa1	0.684±0.029 Aa1	1441.2±160.8 Aa1	1996.1±50.4 Aa1	0.856±0.023 Aa1
NSBO	-	35	2840.4±218.8 B	0.702±0.014 A1	1520.2±112.4 A1	1939.5±42.1 A1	0.843±0.042 A1
FPI-preSBO	1		2885.3±90.3 ABa1	0.684±0.030 Aa1	1557.7±52.3 Aa1	1851.5±98.3 Aa1	0.862±0.021 Aa1
	2		2815.8±89.2 ABa1	0.689±0.039 Aa1	1455.6±134.5 Aa1	1871.7±88.0 Aa1	0.841±0.035 Aa1
	3		2798.5±141.9 ABa1	0.684±0.028 Aa1	1525.5±59.3 Aa1	1858.8±102.5 Aa1	0.864±0.009 Aa1
SPI-preSBO	1		2454.8±98.6 Ba1	0.680±0.020 Aa1	1420.0±76.7 Ba1	1436.0±93.4 Ba1	0.842±0.018 Aa1
	2		2193.5±153.6 Ba1	0.685±0.011 Aa1	1348.1±125.2 Ba1	1527.4±72.3 Ba1	0.845±0.023 Aa1
	3		2113.0±224.1 Ba1	0.686±0.042 Aa1	1278.6±118.8 Ba1	1403.2±61.6 Ba1	0.832±0.021 Aa1

Nc-preSBO	1		2833.6±49.4 ABa1	0.682±0.017 ABa1	1524.9±65.9 Aa1	1885.4±111.6 Aa1	0.842±0.044 Aab1
	2		2787.5±164.6 ABa1	0.696±0.009 ABa1	1503.2±180.7 Aa1	1918.4±119.0 Aa1	0.857±0.028 Aab1
	3		2842.5±99.7 ABa1	0.670±0.039 ABa1	1589.1±160.8 Aa1	1920.2±145.4 Aa1	0.858±0.031 Aab1
NSBO	-	45	2925.7±127.8 A1	0.675±0.016 A1	1429.6±221.5 A1	1508.9±47.4 A2	0.842±0.018 A1
FPI-preSBO	1		2748.4±54.5 ABa2	0.674±0.025 Aa1	1375.3±130.1 Aa2	1440.0±52.2 ABa2	0.848±0.005 Aa1
	2		2825.4±123.6 ABa2	0.683±0.027 Aa1	1350.4±134.5 Aa2	1409.8±77.0 ABa2	0.843±0.007 Aa1
	3		2677.8±42.0 ABa2	0.673±0.012 Aa1	1416.4±26.7 Aa2	1441.2±73.7 ABa2	0.853±0.003 Aa1
SPI-preSBO	1		2229.3±29.5 Ba2	0.674±0.012 Aa12	1198.8±28.7 Ba2	1333.3±25.3 Ba2	0.851±0.011 Aa1
	2		2168.6±217.7 Ba2	0.673±0.014 Aa12	1174.2±173.1 Ba2	1350.4±57.1 Ba2	0.841±0.010 Aa1
	3		2101.4±168.1 Ba2	0.655±0.036 Aa12	1150.9±140.7 Ba2	1292.6±34.7 Ba2	0.846±0.022 Aa1
Nc-PreSBO	1		2743.1±191.1 ABa2	0.686±0.013 Aa1	1223.5±122.1 ABa2	1431.7±111.9 ABa2	0.851±0.007 Aa1
	2		2635.6±142.6 ABa2	0.679±0.008 Aa1	1264.1±158.1 ABa2	1476.3±62.3 ABa2	0.849±0.009 Aa1
	3		2570.8±195.0 ABa2	0.689±0.015 Aa1	1316.3±136.9 ABa2	1395.0±23.1 ABa2	0.834±0.009 Aa1

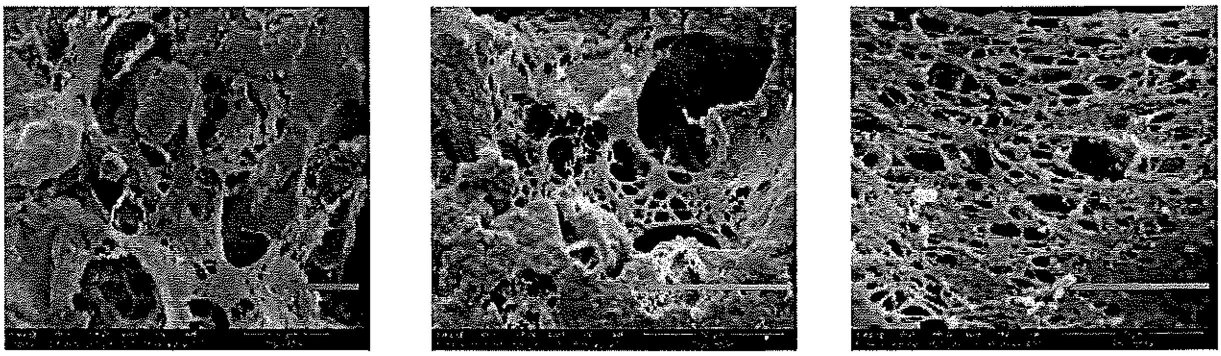
SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized pre-emulsified SBO, SPI stabilized pre-emulsified SBO, and Nc stabilized pre-emulsified SBO, which were referred to Control, NSBO, FPI-preSBO, SPI-preSBO, Nc-preSBO, respectively. FPI, SPI, and Nc were used at different levels–*i.e.*, 1 %, 2 %, and 3 %.

In each column, different capital letters indicate significant difference between means (n=3) at a same SBO level (p<0.05). Different small letters indicate significant difference between means (n=3) at a same SBO level and emulsifier type (p<0.05). Different numeric letters indicate significant difference between means (n=3) at a same oil introduction method and emulsifier type (p<0.05).

From the texture profile analysis, stronger protein matrix generally observed for the NSBO, FPI-preSBO, and Nc-preBSO. From the previous result, better emulsifying ability of Nc and SPI compared to FPI was observed as indicated by the smaller dispersed droplet size. Nonetheless, better ability of FPI based emulsion to strengthen the sausage matrix was suggested. Gelling ability and water retention capacity of the added non-meat proteins played a greater role rather than emulsion formation in determining the stability of emulsified meat products (Su *et al.*, 2000). By employing different emulsifiers, texture profile of the comminuted meat products incorporated with pre-emulsified vegetable oils could be affected. In the present work, the higher cooking loss and less retained texture attribute were evident for the SPI-preSBO, which might be supposed since a restrict network formation of SPI in the meat matrix. Processed meats are usually heated to the final temperature of 65 to 73 °C, which might not sufficient to denature the main components–*i.e.*, 7S and 11S–of SPI (Feng and Xiong, 2002), especially when salt (Nagano *et al.,* 1996; Feng and Xiong, 2002) and/or sugar (Kulmyrzaev *et al.,* 2000) was present. Restricted partial denaturation of SPI affected to hinder its interactions with myofibrillar proteins (Feng and Xiong, 2002), thereby limiting its gelling property in emulsified meat matrix (McCord *et al.,* 1998). Enhancement of gelling properties of SPI could be achieved by increasing surface hydrophobicity via a pre-heating process (Feng and Xiong, 2002).

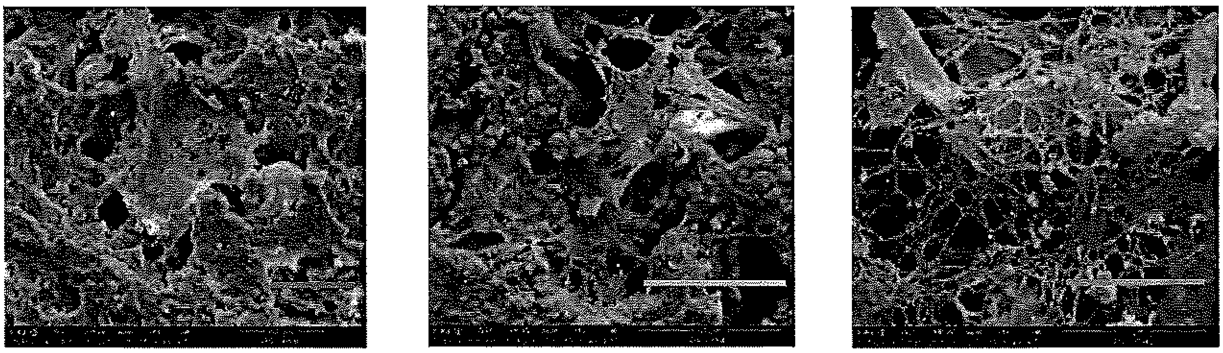
From the present results, higher matrix strength of the sausages could be found for the FPI-preSBO as suggested by lowered cooking loss and restored texture attributes. Non-meat protein employed as emulsifier for preSBO preparation played a crucial role on stability and characteristics of comminuted meat products. Partial replacement of beef fat with pre-emulsified canola oil using whey protein isolate as emulsifier could lower cooking loss of the sausages (Youssef and Barbut, 2011a). Nonetheless, replacing porcine fat with pre-emulsified olive oil stabilized by SPI led to higher weight loss of the sausages (Bloukas *et al.* 1997). The effective concentrations of the employed emulsifiers to provide sausages with the lowest cooking loss and restored textural attributes were 1 %, 2 %, and 1 % for FPI, SPI, and Nc, respectively. The most suitable SBO substitution level was 25 % as implied by the highest stability of the sausages. Therefore, the microstructure of the sausages added with SBO in different forms was determined at 25 % back-fat substitution to elucidate the effect of SBO inclusion on microstructure of the protein gel, and the result was illustrated in Fig. 8.

(a) Control



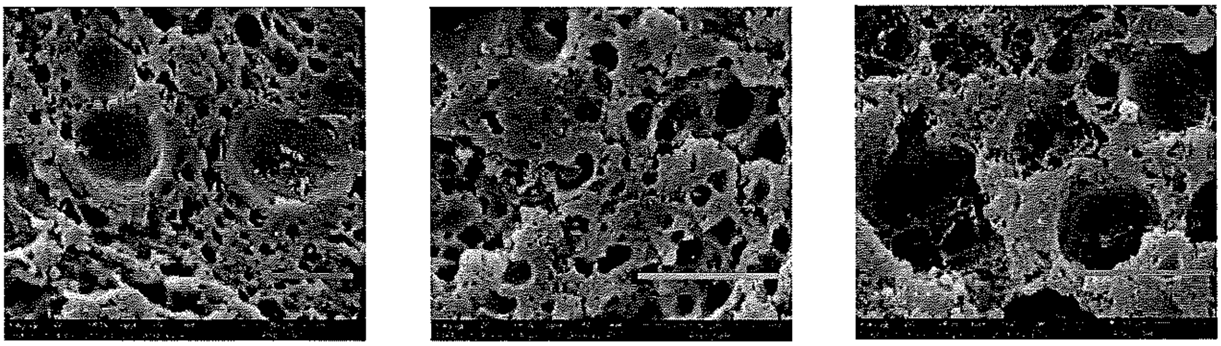
600x *1000x* *5000x*

(b) NSBO at 25% SBO substitution level



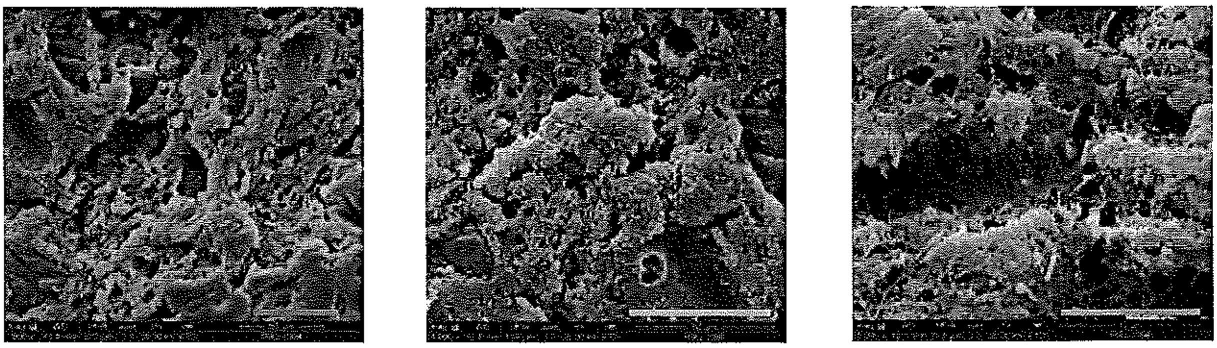
600x *1000x* *5000x*

(c) Nc-preSBO at 25% SBO substitution level (the concentration of Nc used to prepare preSBO was 1%)



600x *1000x* *5000x*

(d) SPI-preSBO at 25% SBO substitution level (the concentration of SPI used to prepare preSBO was 1%)



600x *1000x* *5000x*

(e) FPI-preSBO at 25% SBO substitution level (the concentration of FPI used to prepare preSBO was 2%)

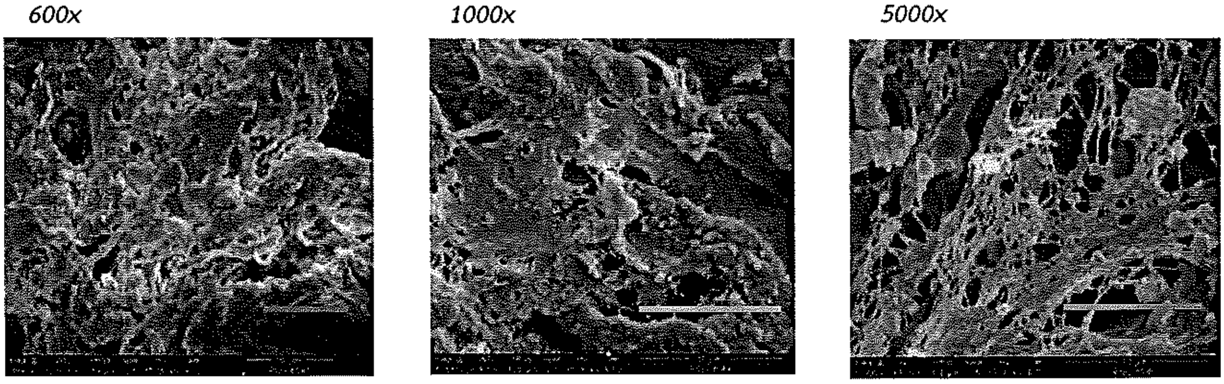
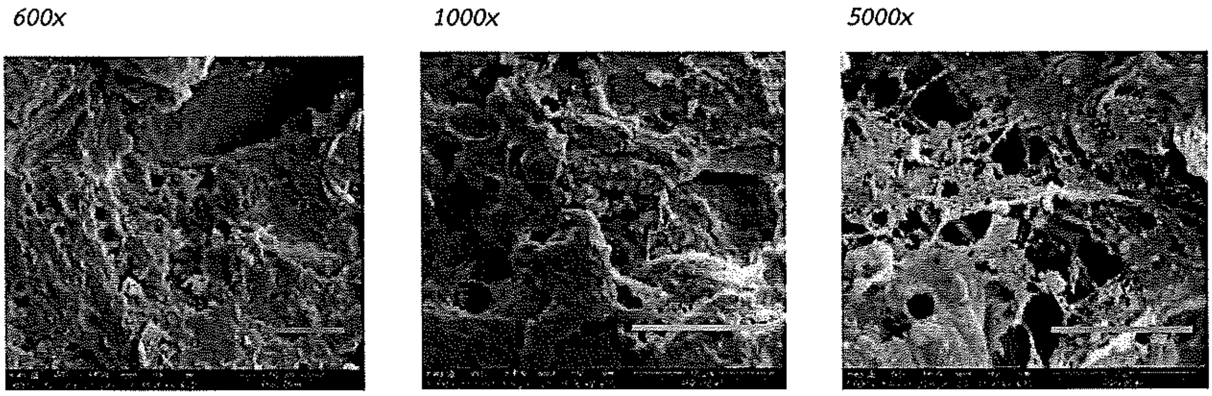


Fig.8 Microstructures of the sausages added with SBO at 25 % by wt of porcine fat in alternative forms (red and green bars = 50 μm; blue bars = 10 μm).

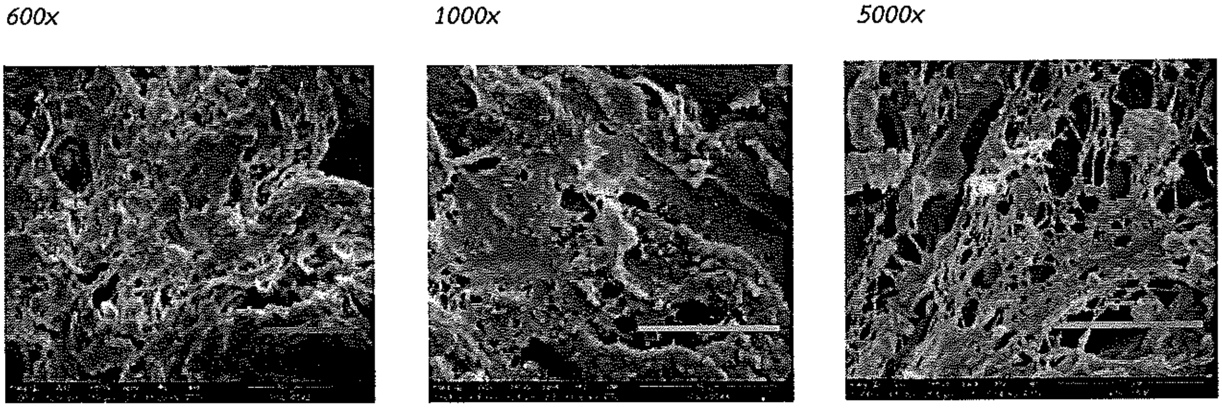
Considering on the NSBO and FPI-preSBO, microstructure with smoother and more homogeneity compared to the Control could be observed. This might explain better meat matrix stability of the former samples. A spongy-like structure with a presence of several large voids was found for the Nc-preSBO. This might affect to a higher cooking loss of the samples. For the SPI-preSBO, denser but less homogeneous structure than did the control was found.

From the results, one can see that the sausages with the highest protein matrix strength could be observed for the FPI-preSBO. The concentration of FPI, however, also played important role on stability of the products. To more elucidate this behavior, microstructures of the FPI-preSBO were observed at different FPI concentrations, and the result was depicted in Fig. 9.

(a) FPI-preSBO at 25% SBO substitution level (the concentration of FPI used to prepare preSBO was 2%)



(b) FPI-preSBO at 25% SBO substitution level (the concentration of FPI used to prepare preSBO was 2%)



(c) FPI-preSBO at 25% SBO substitution level (the concentration of FPI used to prepare preSBO was 3%)

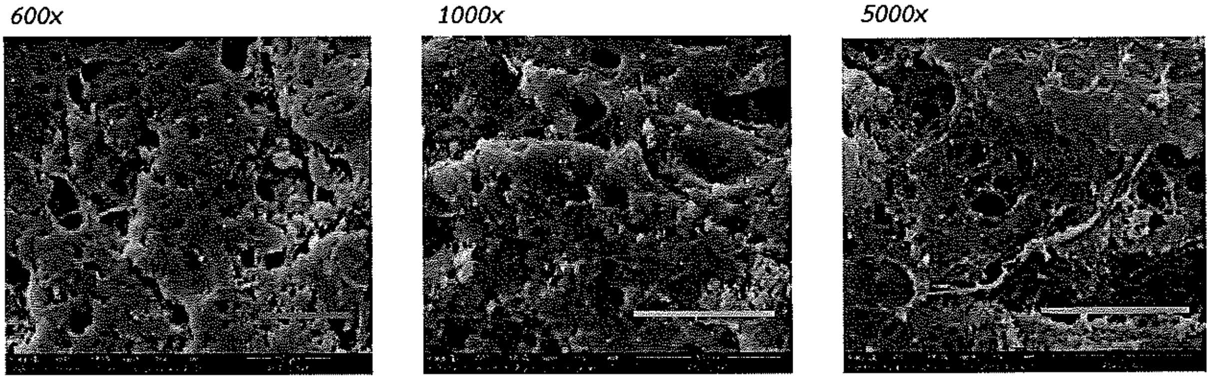
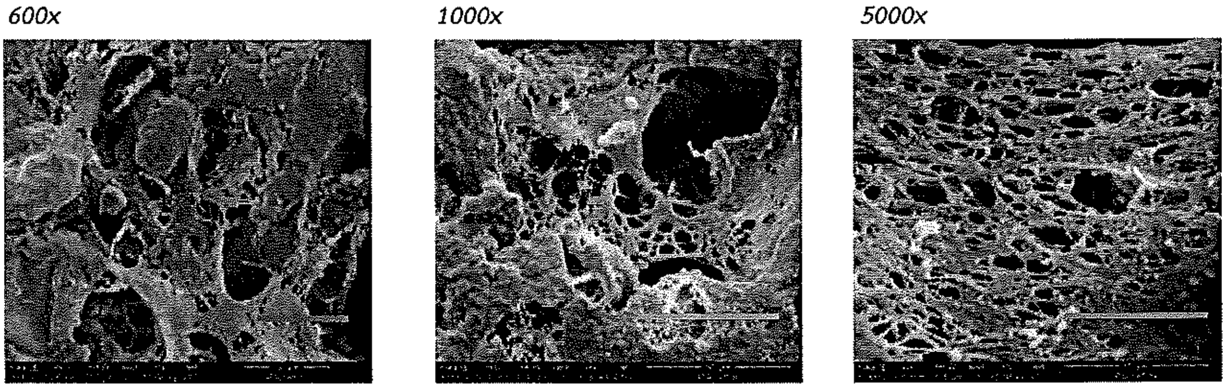


Fig. 9 Microstructure of the FPI-preSBO at different FPI concentrations (1, 2, and 3%), and the SBO substitution level was 25 % (red and green bars = 50 µm; blue bars = 10 µm).

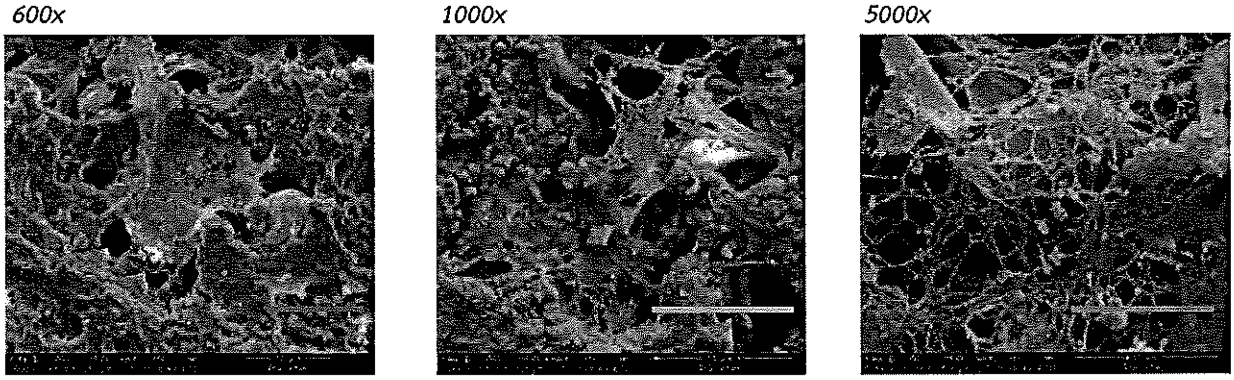
By increasing FPI concentration from 1 % to 2 %, the microstructure became smoother. When the FPI concentration increased to 3 %, however, less homogeneity structure was observed. This behavior was coincidental with the higher cooking loss of the 3 % FPI-preSBO compared to the 2% FPI-preSBO. By adding pea protein isolate at too high concentration, decreasing in a strength of muscle protein gel was reported, expected due to self association between muscle proteins and the isolated proteins, thereby reducing a stabilizing effect of pea protein isolate (Sun *et al.*, 2012).

Next, the effect of SBO substitution level on the microstructure of the sausages was observed by selecting the FPI-preSBO as a model, regarded due to their appreciable stability. In order for comparison, the microstructures of the Control and NSBO were also shown (see Fig. 10).

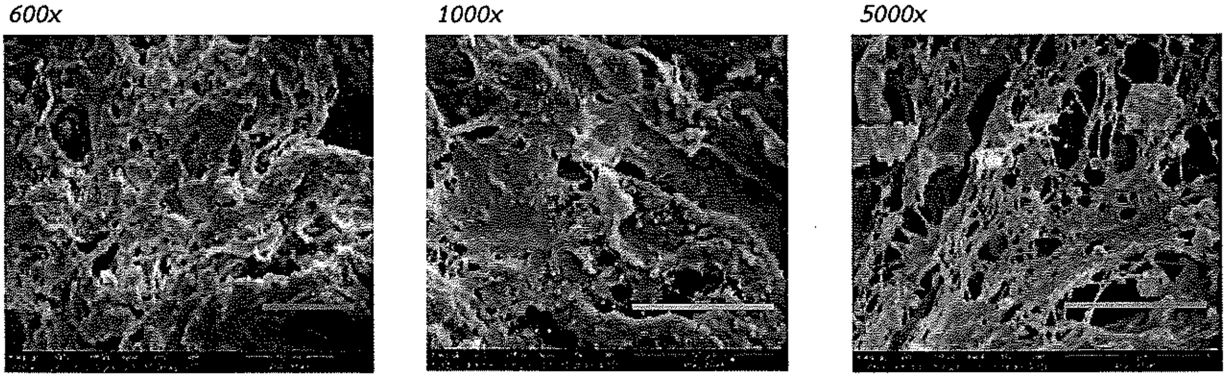
(a) Control



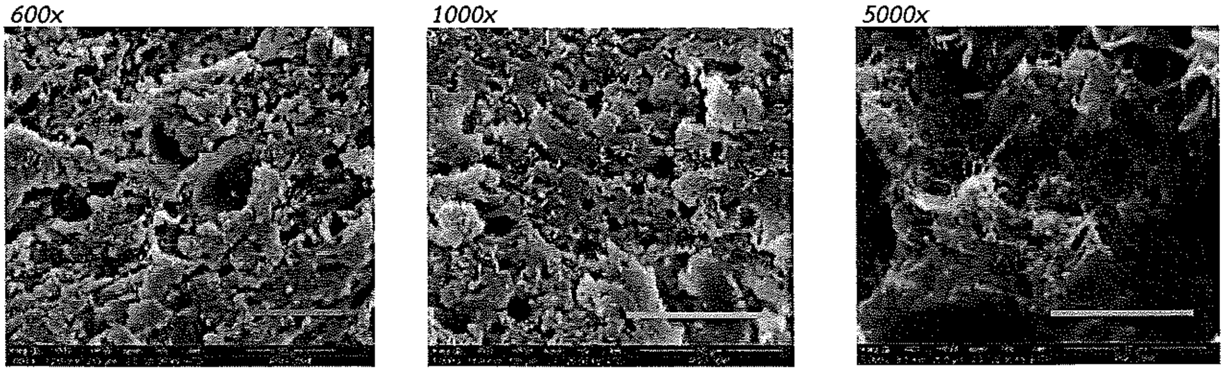
(b) NSBO at 25% SBO substitution level



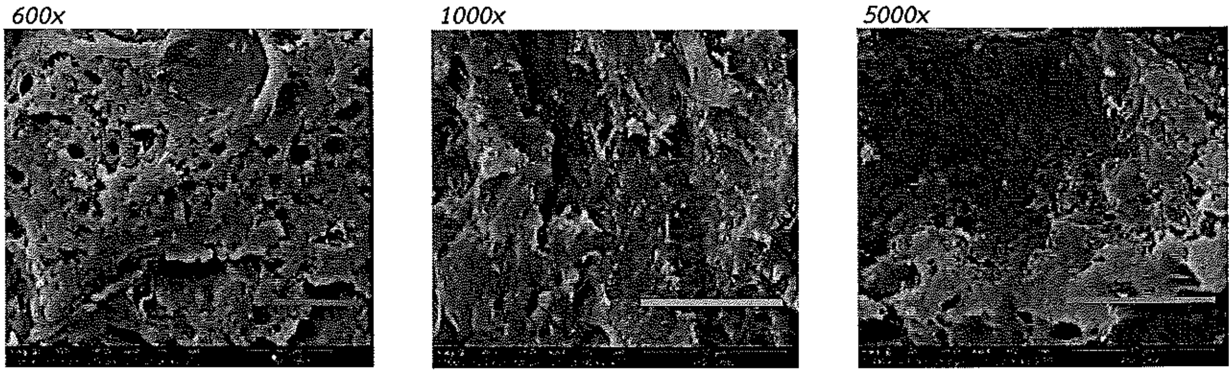
(c) FPI-preSBO at 25% SBO substitution level (the concentration of FPI used to prepare preSBO was 2%)



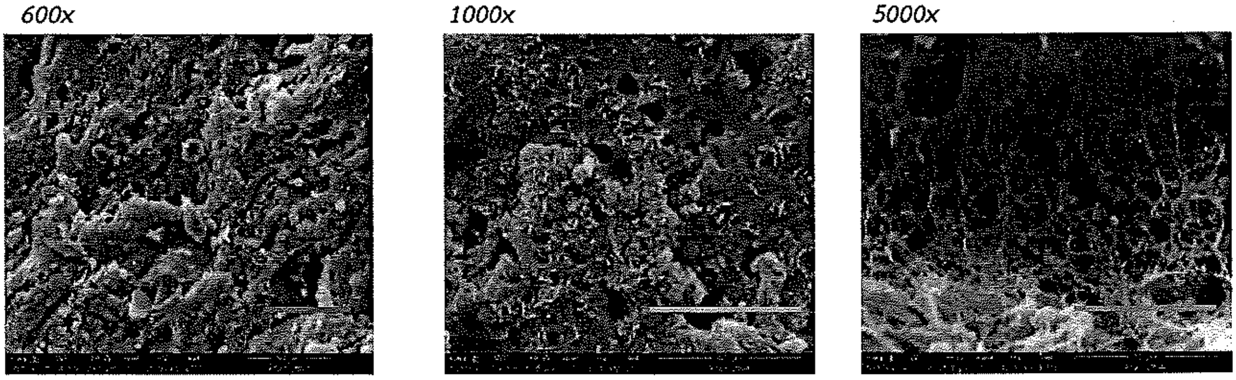
(d) NSBO with 35% SBO substitution level



(e) FPI-preSBO at 35% SBO substitution (the concentration of FPI used to prepare preSBO was 2%)



(f) NSBO with 45% SBO substitution level



(g) FPI-preSBO at 45% SBO substitution level (the concentration of FPI used to prepare preSBO was 2%)

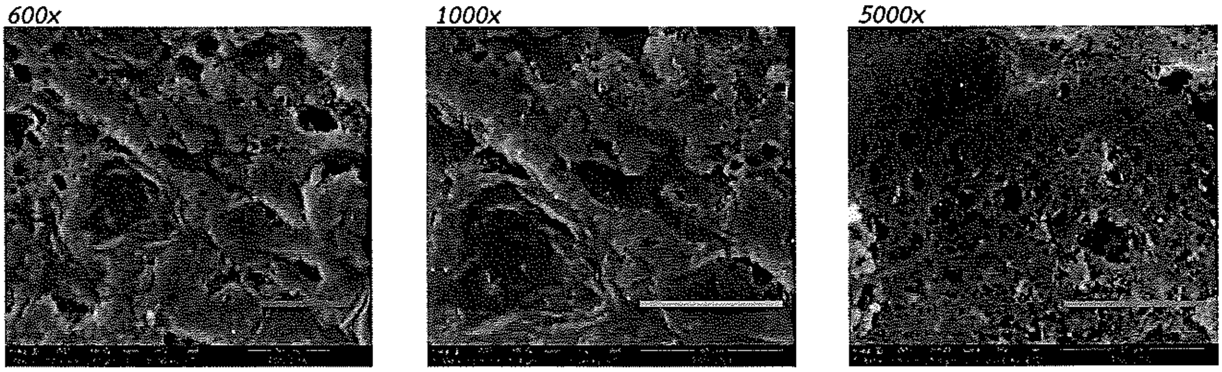


Fig. 10 Microstructure of the Control, NSBO, and FPI-preSBO at various SBO substitution levels (red and green bars = 50 μm; blue bars = 10 μm).

In case of the NSBO, increase SBO substitution level resulted in a larger amount of voids in the protein gel matrix. Nonetheless, increasing SBO amount seemed to have less effect on the morphology of the FPI-preSBO. Interestingly, the microstructures with more homogeneity, smoother, and less amount of voids could be obviously seen for the FPI-preSBO compared to the NSBO at the corresponding SBO substitution level.

Overview from the present results, SBO inclusion at the level of 25 % (by wt of porcine fat) via pre-emulsification technique using FPI at 2 % as emulsifier could successfully improved strength of the muscle protein gel, thereby providing the sausages with lowered cooking loss and most retained textural attributes.

5.2.4 Study on properties of pork sausages as affected by reducing fat content

Next, effect of fat reducing on characteristics of the sausages was studied. The control sausages contained 30 % of fat, and fat contents were reduced to 20 and 10 % for the studied samples. SBO was employed to partially replace animal fat at the level of 25 % by weight of porcine fat in alternative forms–*i.e.*, native and preSBO. To prepare preSBO, FPI at the concentration of 2 % was employed as emulsifier, by comparing SPI (1 %) and Nc (1 %). The concentrations of the used emulsifiers were selected according to their ability to provide the highest stabilizing effect as suggested by the previous results. Protein content of all sausages was fixed at 15 %.

To evaluate meat matrix stability, cooking loss and WHC of the sausages were measured, and the result was shown in Fig. 11. Lowering fat content resulted in higher cooking loss of the samples, which might be postulated due to higher amount of water added to replace some contents of fat for the fat reduced formulations. In a presence of higher water amount, meat proteins might not insufficient to hold whole water, resulting in increased cooking loss (Youssef and Barbut, 2011b, c). Nonetheless, significant lowered cooking loss could be found for the NSBO and FPI-preSBO compared to the Control (p<0.05), suggesting to the improved meat matrix stability of the former sausages. Lowered cooking loss of beef sausages could be observed when canola oil was introduced to partially replace beef fat (Youssef and Barbut, 2011b). This phenomenon was expected due to higher unsaturation degree of composited fatty acids of canola oil than beef fat, which could exhibit water holding capacity (St. John *et al.,* 1986). Moreover, the smaller sized of fat globules in canola oil treatments might create larger surface areas to be covered by proteins, leading to lower cooking loss (Youssef and Barbut, 2009). Incorporation of vegetable oil in pre-emulsified form affected to stability of comminuted meat products, depending on the type of non-meat proteins employed as emulsifier. By incorporating the pre-emulsified canola oil stabilized by whey protein isolate and SPI, lowered cooking loss of beef sausages was observed, whereas the higher cooking loss was found for the recipe added with Nc stabilized pre-emulsified canola oil (Youssef and Barbut, 2011b). This behavior might be expected because Nc was not crucial in term of fat holding capacity and Nc could not able to form heat induced gel, so it could not facilitate fluid retaining in the matrix of comminuted meat products (Youssef and Barbut, 2011b).

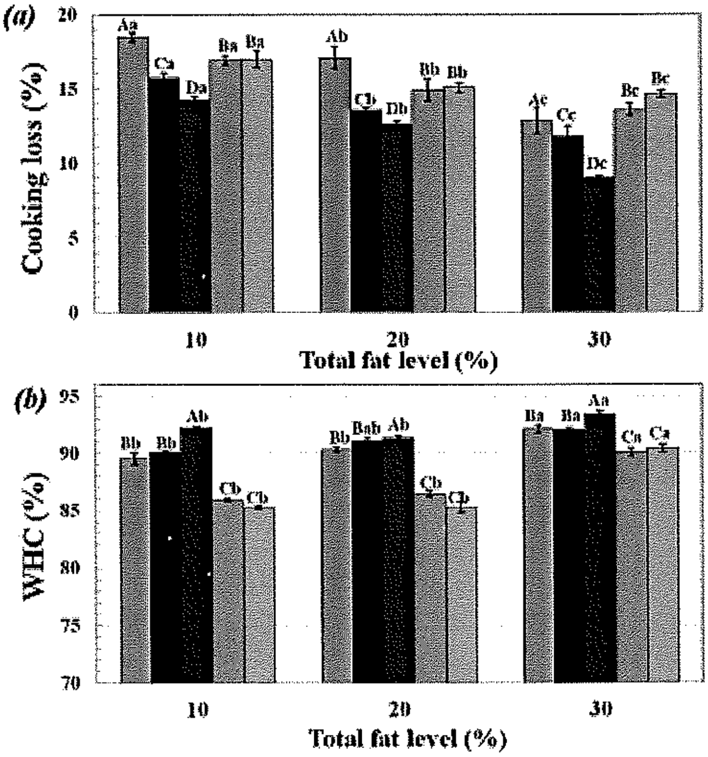


Fig. 11 Cooking loss *(a)* and WHC *(b)* of the sausages containing different total fat contents. SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized preSBO, SPI stabilized preSBO, and Nc stabilized preSBO, which were referred to Control (▨), NSBO (■), FPI-preSBO (■), SPI-preSBO (▨), and Nc-preSBO (▨), respectively.

Means ± standard deviations (n=3) were shown.

In each subfigure, capital letters indicate significant difference between means at a same fat level (p<0.05). Small letters indicate significant difference between means at a same SBO introducing form (p<0.05).

Considering on WHC, lowering fat content slightly affected to decrease WHC of the samples. Comparable WHC of the Control and NSBO was observed ($p>0.05$). By introducing SBO via pre-emulsification means, FPI-preSBO possessed higher WHC ($p<0.05$), whereas Nc-preSBO and SPI-preSBO showed a significantly lowered WHC compared to the Control ($p<0.05$). The present result suggested to capability of FPI to reinforce meat matrix stability, which was in agreement with the result of cooking loss. By incorporating pre-emulsified peanut oil, WHC of the cooked myofibrillar protein gels could also be improved (Wu *et al.*, 2009).

Fig. 12 illustrates color parameter of the sausages. Total fat level had no effect on lightness and yellowness of the sausage samples ($p>0.05$), which was in agreement with the result of previous studies (Ahmed *et al.*, 1990; Bishop *et al.*, 1993; Youssef and Barbut, 2011b). Partial substitution of porcine fat with SBO affected to increase L^* and b^* of the sausages ($p<0.05$). Higher lightness of the sausages could be found when beef fat was partially replaced with canola oil, supposed due to a smaller sized of canola oil globules with larger surface areas that could reflect more light that the larger beef fat globules (Youssef and Barbut, 2011b). Higher b^* of the sausages incorporated with SBO compared to the Control could be postulated due to a yellow color of the vegetable oil. Increasing in lightness and yellowness of the sausages was also reported when beef fat was substituted with olive oil in both of native and pre-emulsified (using SPI as emulsifier) forms (Bloukas *et al.*, 1997). For the sausages with SBO inclusion, reduction in redness of the sausages was observed, especially when the level of total fat was increased. Lowered redness of the sausages with SBO inclusion than did the Control might be postulated since a replacing part of meat proteins which is an origin of myoglobin with non-meat proteins in the SBO incorporated treatments. However, difference in all color parameters of the sausages added with SBO in dissimilar forms was not noticeable in the present work ($p>0.05$). It has been suggested that beef frankfurter color was affected by the fat content, and not by the oil treatments (Marquez *et al.*, 1989).

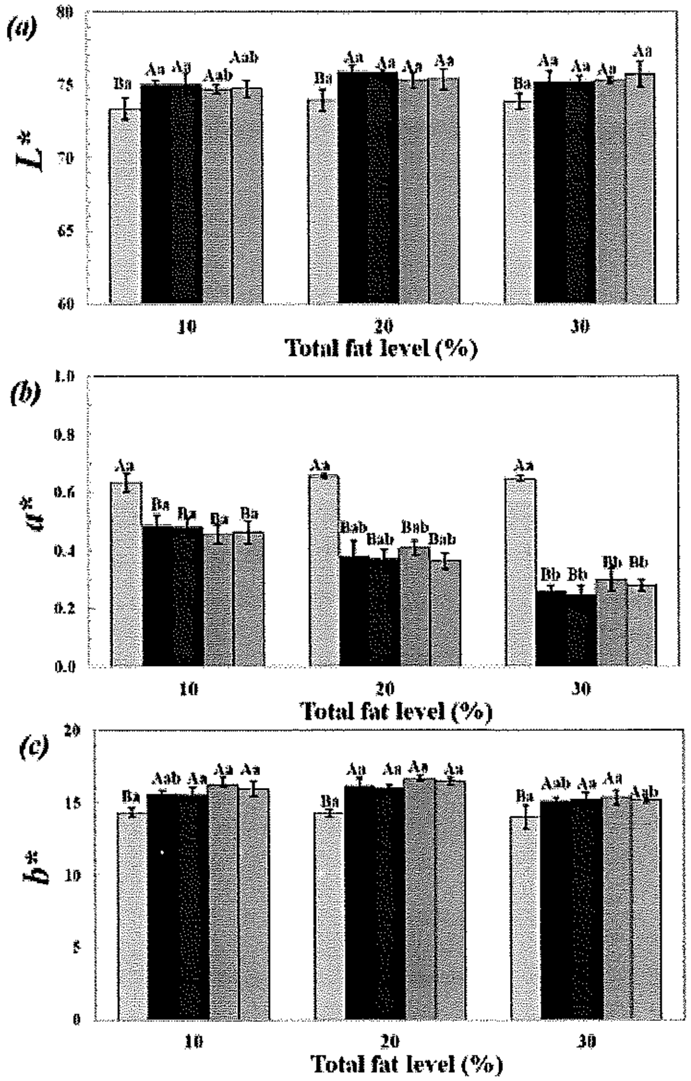


Fig. 12 Color parameters including *L* (a)*, *a* (b)*, and *b* (c)* of the sausages containing different total fat contents. SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized preSBO, SPI stabilized preSBO, and Nc stabilized preSBO, which were referred to Control (▨), NSBO (■), FPI-preSBO (■), SPI-preSBO (▨), and Nc-preSBO (▨), respectively.

Means ± standard deviations (*n*=3) were shown.

In each subfigure, capital letters indicate significant difference between means at a same fat level (p<0.05). Small letters indicate significant difference between means at a same SBO introducing form (p<0.05).

Next, texture attributes of the sausages were observed and the results were shown in Table 8. Partial substitution of porcine fat with SBO led to higher hardness compared to the Control recipe ($p<0.05$), which was in consistent with the previous results (Youssef and

Barbut, 2011b). It has been suggested that vegetable oils could provide smaller sized oil droplets compared to animal fat globules (Youssef and Barbut, 2010). Fat globules with smaller size had larger surface areas to be covered by proteins, thereby allowing more interaction of proteins residues in the matrix. This led to enhance a resistance to compression of meat products (Youssef and Barbut, 2011b). Irrespectively of non-meat protein types, incorporate of preSBO affected to increase hardness of the samples, especially at the increased fat content. Higher hardness was also observed for the fermented sausages, when SPI stabilized pre-emulsified olive oil was added (Bloukas *et al.*, 1997). Considering on the effect of Nc based pre-emulsified oils, however, a contradiction behavior was reported; Incorporation of Nc based pre-emulsified olive oil led to higher hardness of the sausages (Youssef and Barbut, 2011b), whereas Nc based pre-emulsified corn oil adding affected to decrease hardness of the reduced fat bologna (Bishop *et al.*, 1993).

Increasing of springiness, gumminess, and chewiness was observed when the total fat level was increased up to 30 % ($p<0.05$). This might be expected since ability of fat to provide elasticity for the formulation with higher fat content (Youssef and Barbut, 2011b, c). SBO inclusion led to higher springiness, gumminess, and chewiness of the sausages than did the Control ($p<0.05$), irrespectively of oil introducing forms. This tendency was correlated with the study of Park *et al.* (1989) reporting higher gumminess and chewiness of the frankfurters when high-oleic sunflower oil was introduced to partially replace regular beef fat.

Table 8 Texture parameters of the sausages as affected by partial replacement of porcine fat with SBO in different forms at various total fat levels

Total fat level (%)	sample	Hardness (N/cm^2)	Cohesiveness	Chewiness (Ns)	Gumminess (N/cm^2)	Springiness (cm)
30	Control	2282.5±163.4 Da	0.652±0.056 Bb	1018.6±88.1 Ba	1271.1±45.3 Ba	0.823±0.015 Ba
	NSBO	2839.4±121.0 ABa	0.676±0.015 Ab	1505.7±140.1 Aa	1979.0±17.0 Aa	0.852±0.013 Aa
	FPI-preSBO	3022.5±74.3 Aa	0.691±0.018 Ab	1581.9±60.1 Aa	1869.3±104.8 Aa	0.847±0.005 Aa
	SPI-preSBO	2518.2±198.8 Ca	0.681±0.004 Ab	1385.4±76.7 Aa	1781.8±127.7 Aa	0.844±0.018 Aa
	Nc-preSBO	2738.4±88.7 Ca	0.685±0.001 Ab	1485.4±65.9 Aa	1921.6±83.5 Aa	0.853±0.016 Aa
20	Control	2175.4±144.3 Db	0.709±0.003 Bab	908.6±79.5 Bab	1320.9±82.4 Bab	0.745±0.008 Bab
	NSBO	2643.1±125.4 ABb	0.744±0.005 Aab	1287.0±47.2 Aab	1490.5±121.5 Aab	0.795±0.003 Aab
	FPI-preSBO	2940.9±53.6 Ab	0.749±0.004 Aab	1278.2±8.3 Aab	1561.4±84.1 Aab	0.796±0.015 Aab
	SPI-preSBO	2577.3±73.9 Cb	0.751±0.007 Aab	1247.6±87.7 Aab	1514.8±34.4 Aab	0.787±0.007 Aab
	Nc-preSBO	2562.8±43.5 Cb	0.748±0.008 Aab	1258.2±62.0 Aab	1921.6±59.0 Aab	0.792±0.003 Aab
10	Control	1862.2±84.6 Dc	0.730±0.010 Ba	865.5±36.5 Bb	995.1±158.9 Bb	0.723±0.002 Bb
	NSBO	1982.0±106.7 ABc	0.767±0.001 Aa	1038.0±13.2 Ab	1129.7±86.8 Ab	0.743±0.011 Ab
	FPI-preSBO	2092.6±159.5 Ac	0.775±0.016 Aa	1139.1±37.5 Ab	1102.8±76.0 Ab	0.740±0.003 Ab
	SPI-preSBO	1895.9±90.4 Cc	0.766±0.011 Aa	1127.9±61.0 Ab	1115.3±34.4 Ab	0.747±0.003 Ab
	Nc-preSBO	1928.1±102.9 Cc	0.764±0.008 Aa	1141.0±36.2 Ab	1098.9±30.9 Ab	0.741±0.003 Ab

SBO was added in different forms–*i.e.*, Control (no SBO inclusion), native SBO, FPI stabilized preSBO, SPI stabilized preSBO, and Nc stabilized pre SBO, which were referred to Control, NSBO, FPI-preSBO, SPI-preSBO, Nc-preSBO, respectively.

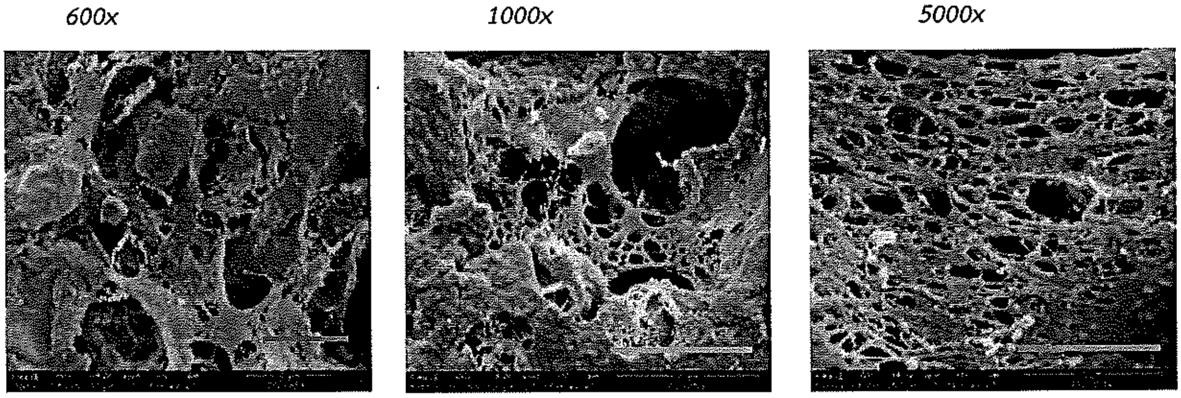
In each column, different capital letters indicate significant difference between means (*n*=3) at a same total fat level ($p<0.05$). Different small letters indicate significant difference between means (*n*=3) at a same SBO inclusion form ($p<0.05$).

Decreasing of cohesiveness was observed when total fat content of the sausages was increased to 30 % ($p<0.05$), which was in correspond with results of other studies (Barbut and Mittal, 1992; Pietrasik, 1999; Youssef and Barbut, 2011b). By using different fat sources, textural attributes of meat products was affected, depending on saturation degree of composited fatty acids of the fats/oils and/or pre-emulsifying the oil with non-meat proteins (Youssef and Barbut, 2011c). SBO inclusion led to higher cohesiveness compared to the control ($p<0.05$), and different cohesiveness between the sausages added with SBO in dissimilar forms was not noticeable in the present work ($p>0.05$).

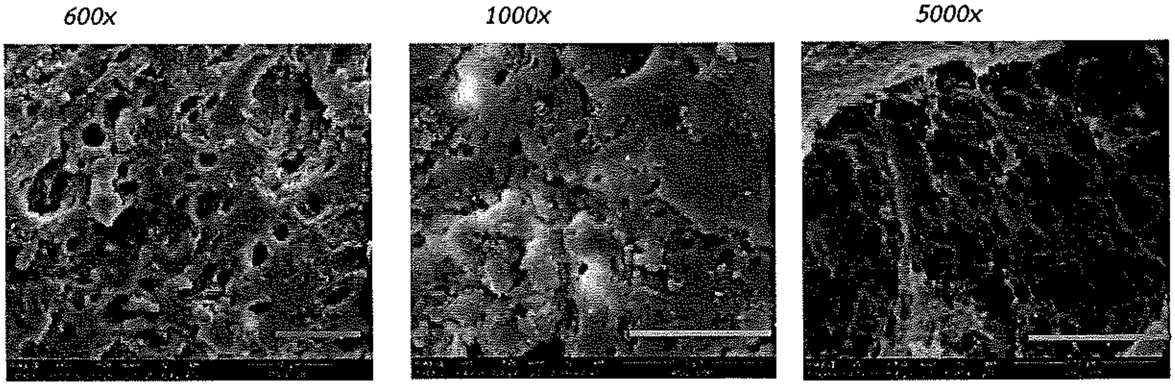
Overview from the present results, one can see that reduction of fat level markedly affected to characteristics of the sausages. Higher cooking loss and less restored textural attributes tended to be observed in the sausages with lower fat contents. Incorporation of SBO led to improved product stability, especially in a form of FPI stabilized preSBO as implied by lowered cooking loss and most restored texture attributes than those others. To more elucidate the characteristics of the sausages as affected by fat reduction, microstructures of the sausages incorporated with SBO in dissimilar forms at various total fat levels were observed as shown in Fig. 13.

Generally, the microstructure with less protein network formation was observed with decreased total fat level, especially for the sausages formulated with preSBO stabilized by Nc. Considering on the sausages incorporated with preSBO, the sausages formulated with Nc stabilized preSBO obviously possessed protein matrix with higher heterogeneity and presence of several large voids compared to the others. This might be used to explain less stability of this sausage formula as indicated by higher cooking loss and less retained textural attributes. For the samples added with preSBO stabilized by FPI, the protein network with more compact and higher homogeneity was observed, which was correspond to their higher stability compared to the others as previously observed by lowered cooking loss and better retained textural attributes. This phenomenon might be supposed since more pronounced interactions between FPI and muscle proteins compared to the other employed non-meat proteins (Sun *et al.*, 2012). By isolating in alkaline condition via the pH shift method, partial denaturation of the FPI might be supposed due to a hydrolysis reaction accelerated by alkaline environment (Rawdkuen *et al.*, 2009; Yongsawatdigul and Park 2004). Unfolding of FPI might affect to enhance hydrophobic interaction between protein residues, thereby strengthen the protein gel network (Kim *et al.*, 2005; Knudsen *et al.*, 2008). Better gelling ability of the protein isolated from short-bodied mackerel via the pH-shift method was previously reported as indicated by higher gel strength of the gel compared to the gel of protein recovered by conventional treatment (Chaijan *et al.*, 2010).

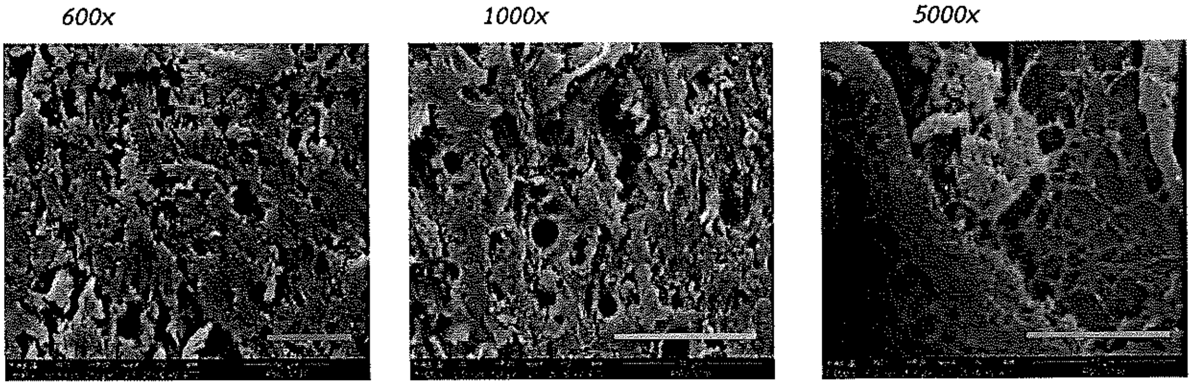
(a) Control (the sausages without SBO inclusion and total fat level of 30 %)



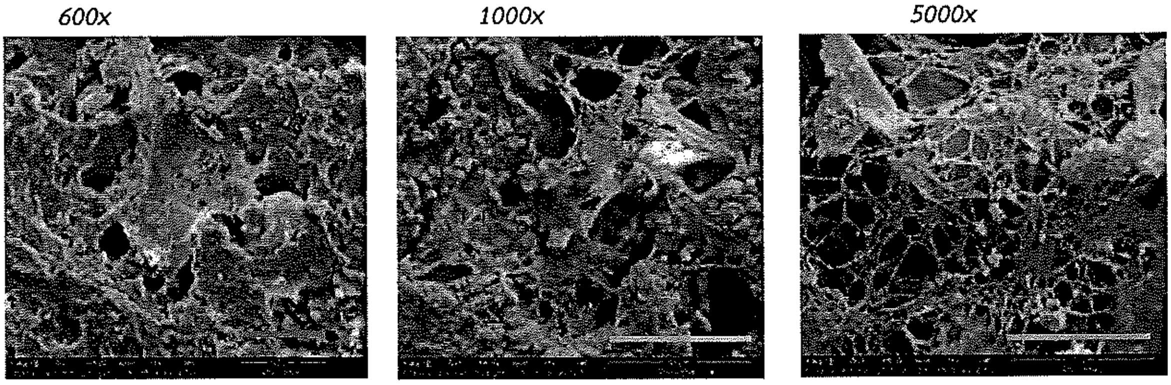
(b) Control (the sausages without SBO inclusion and total fat level of 20 %)



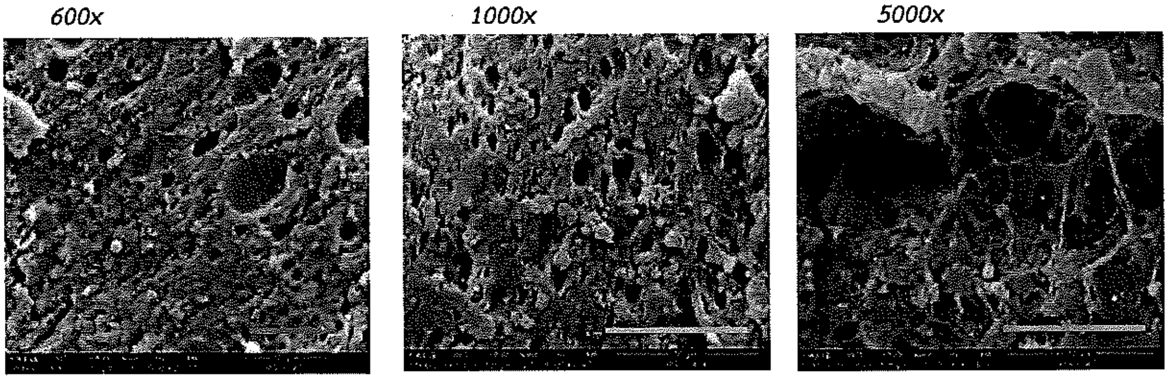
(c) Control (the sausages without SBO inclusion and total fat level of 10 %)



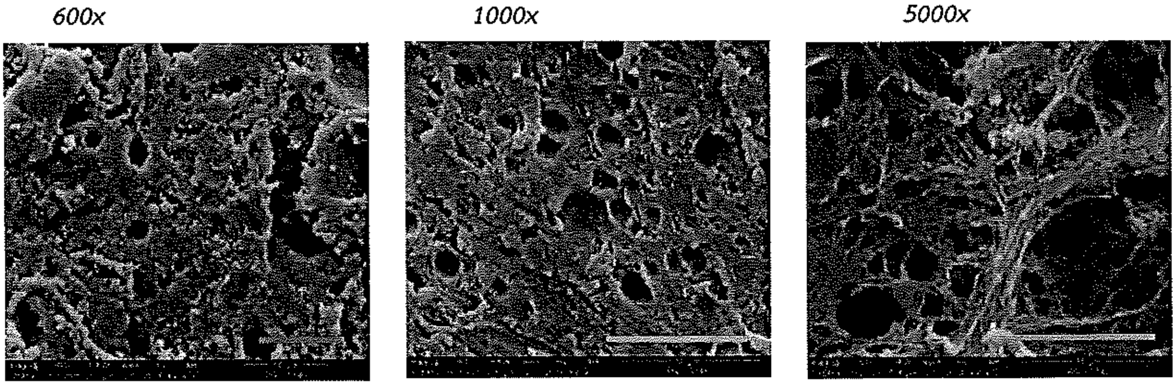
(d) NSBO at 25% SBO substitution level and total fat level of 30 %



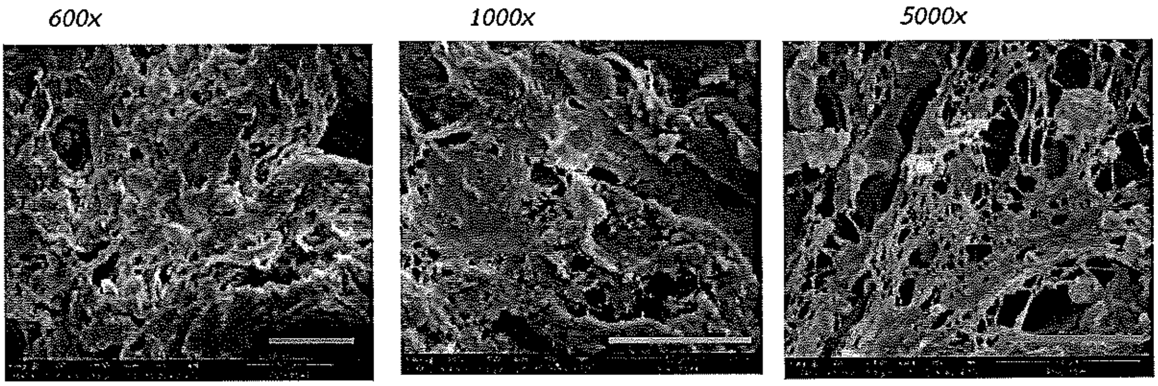
(e) NSBO at 25% SBO substitution level and total fat level of 20 %



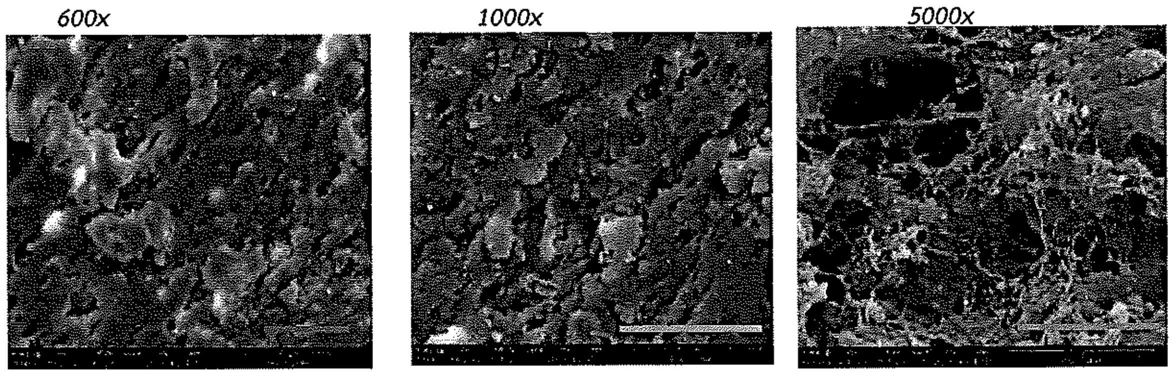
(f) NSBO at 25% SBO substitution level and total fat level of 10 %



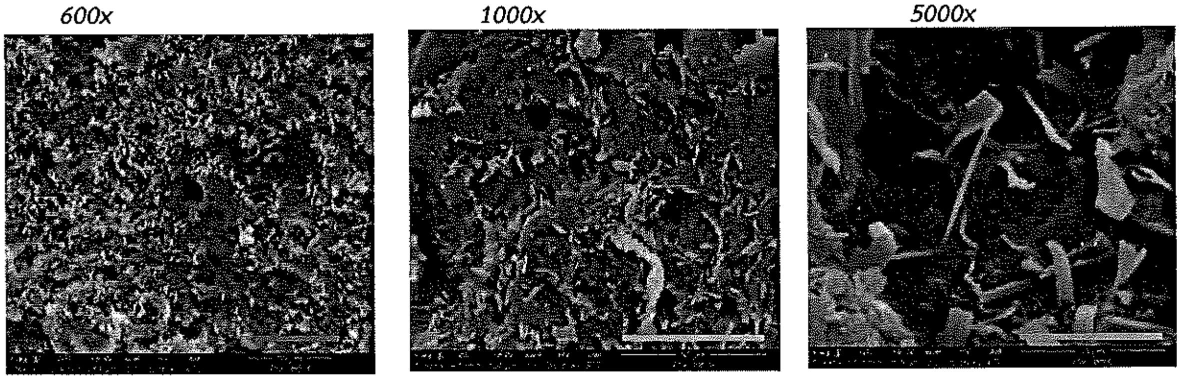
(g) FPI-preSBO at 25% SBO substitution level and total fat level of 30 %


600x *1000x* *5000x*

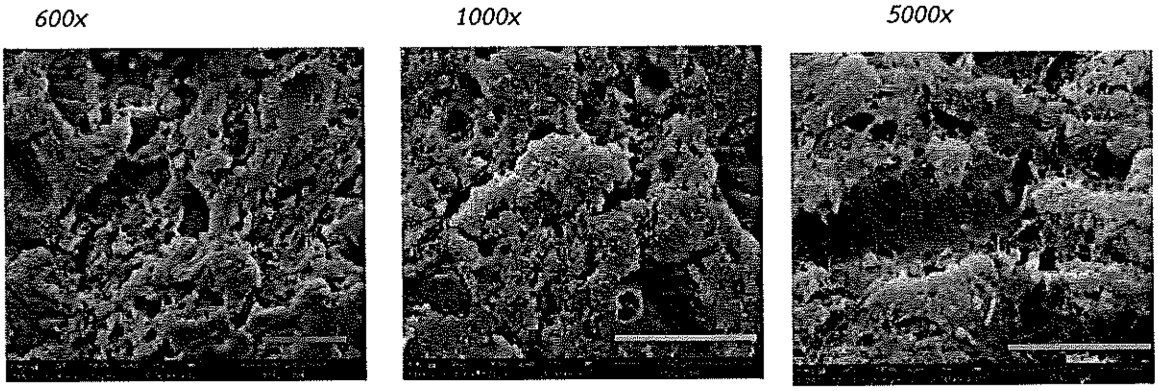
(h) FPI-preSBO at 25% SBO substitution level and total fat level of 20 %


600x *1000x* *5000x*

(i) FPI-preSBO at 25% SBO substitution level and total fat level of 10 %

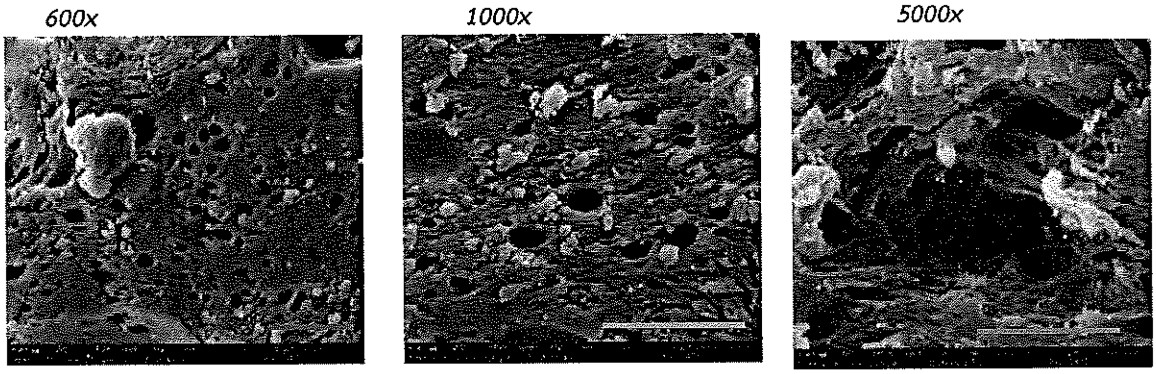

600x *1000x* *5000x*

(j) SPI-preSBO at 25% SBO substitution level and total fat level of 30 %



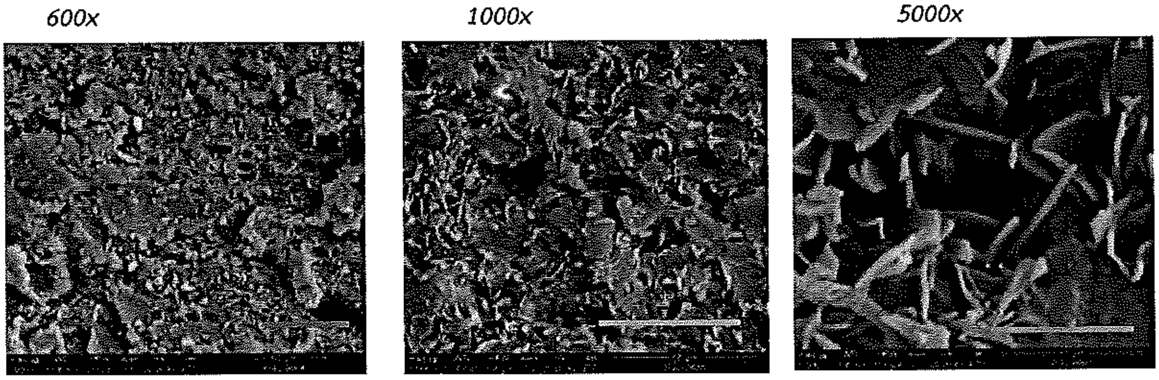
600x *1000x* *5000x*

(k) SPI-preSBO at 25% SBO substitution level and total fat level of 20 %



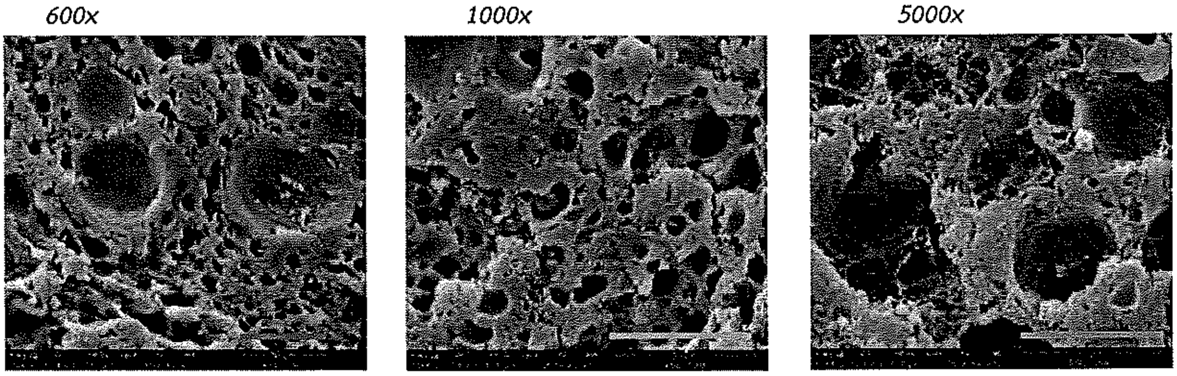
600x *1000x* *5000x*

(l) SPI-preSBO at 25% SBO substitution level and total fat level of 10 %

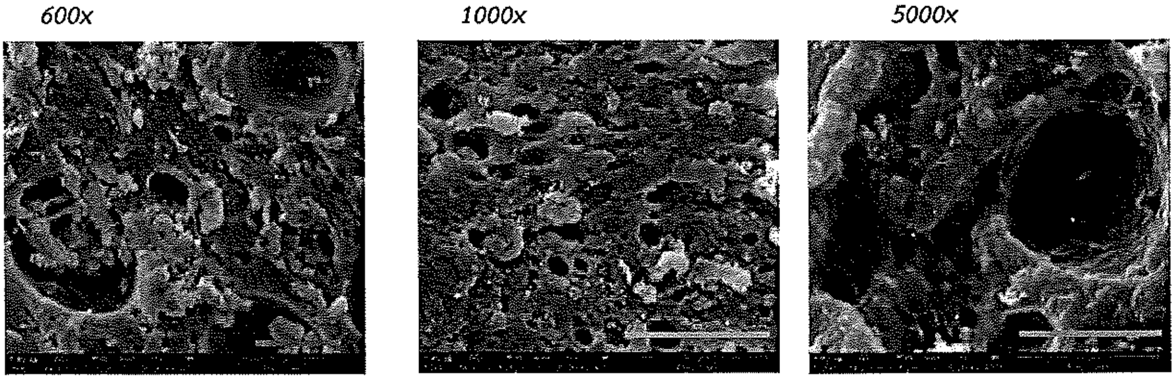


600x *1000x* *5000x*

(m) Nc-preSBO at 25% SBO substitution level and total fat level of 30 %



(n) Nc-preSBO at 25% SBO substitution level and total fat level of 20 %



(o) Nc-preSBO at 25% SBO substitution level and total fat level of 10 %

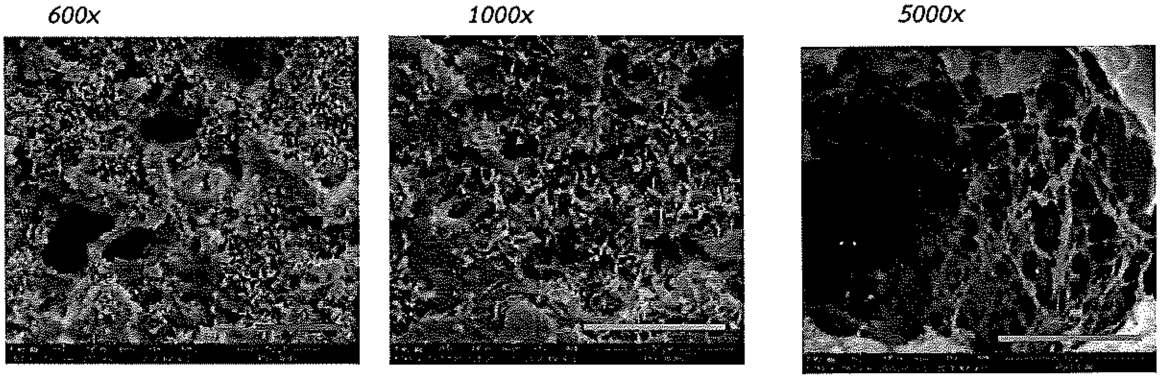


Fig. 13 Microstructures of the sausages incorporated with SBO in dissimilar forms at various total fat levels (red and green bars = 50 μm; blue bars = 10 μm).

6. Conclusion

By using the pH shift method, protein isolated from a yellow stripe trevally (*Selaroides leptolepis*) could be prepared with a recovery yield of *ca.* 64 %. The fish protein isolate (FPI) possessed the major protein bands with a molecular weight of *ca.* 35–50 kDa supposed to be actin myofibrillar protein. The FPI showed a great solubility in alkaline condition with the lowest solubility at pH 5.5. Considering on functional properties of the FPI, it was found that FPI possessed better emulsifying ability than soy protein isolate (SPI) and Na-caseinate (Nc), when the emulsions were exposed to heat treatment.

Further, characteristics of the pork sausages as affected by partial replacement of porcine fat with SBO were observed. SBO in alternative forms–*i.e.,* native and pre-emulsified form–was added to substitute porcine fat at different levels (25, 35, and 45 % by wt of porcine fat). The pre-emulsified SBO (preSBO) was prepared using different emulsifiers–*i.e.,* FPI, SPI and Nc–at varying concentrations (1–3 %). The sausages without SBO, with SBO adding in native, FPI stabilized preSBO, SPI stabilized preSBO, and Nc stabilized preSBO were referred as the Control, NSBO, FPI-preSBO, SPI-preSBO, and Nc-preSBO, respectively. Properties of the sausages involving cooking loss, water holding capacity (WHC), color, texture parameters, and microstructure were observed. There was no significant difference on cooking loss between the Control and NSBO, irrespectively of SBO inclusion levels ($p>0.05$). Introducing SBO in pre-emulsified form influenced cooking loss of the sausages in different manners depending on the employed emulsifier type and SBO substitution levels. As compared to the Control, higher cooking loss was observed for SPI-preSBO and Nc-preSBO ($p<0.05$), but FPI-preSBO possessed lowered cooking loss ($p<0.05$), suggesting to an important role of non-meat proteins on stability of the emulsified meat matrix. Higher hardness and gumminess were found for the NSBO, FPI-preSBO, and Nc-preSBO compared to Control and SPI-preSBO ($p<0.05$). Higher cooking loss and less retained texture attributes observed for the SPI-preSBO might be supposed due to a restrict network formation of SPI in the meat matrix at the present heating condition. Considering on the microstructure of the sausages, the NSBO and FPI-preSBO showed a smoother and more homogeneity protein network compared to the Control. This agreed with better protein matrix stability of the FPI-preSBO as suggested by lowered cooking loss than did the Control. A spongy-like structure with a presence of several large voids was found for the Nc-preSBO, which might be related with their higher cooking loss. For the SPI-preSBO, denser but less homogeneous structure than did the Control was found. Partial replacement of porcine fat with SBO affected to increase

L^* and b^* of the sausages ($p<0.05$), whereas a^* was significantly reduced ($p<0.05$). There was no noticeable difference on color appearance of the sausages incorporated with SBO in different forms ($p>0.05$).

Increase SBO substitution level affected to decrease WHC of the sausages. Moreover, lowered hardness, chewiness, and gumminess were observed when the sausages were formulated with SBO at 45 % ($p<0.05$). However, the FPI-preSBO could maintain a comparable WHC with the Control through 35 % SBO substitution level ($p>0.05$), suggesting to better capacity of the FPI to retain matrix strength compared to Nc and SPI.

Considering on effect of non-meat protein concentration, it was found that the effective concentrations of FPI, SPI, and NC to retain protein matrix strength were 2, 1, and 1 %, respectively. Excessive non-meat protein amount might lead to self association between the emulsifier and meat proteins, thereby restricting stabilizing effect of the non-meat protein emulsifier.

Next, the effect of fat reduction of characteristics of the sausages was studied. The total fat amount of the sausage samples was varied–*i.e.*, 30, 20, and 10 %. SBO in different forms was incorporated to partially replace porcine fat at 25 % by wt of porcine fat. Reduction of fat level markedly affected to stability of the sausages as evident by higher cooking loss and less restored textural attributes of the formulations with lower fat contents. Incorporation of SBO led to improved product stability, especially in a form of FPI stabilized preSBO as implied by lowered cooking loss and most restored texture attributes than those others. Generally, the microstructure with less protein network formation was observed with decreased total fat level, especially for the sausages formulated with preSBO stabilized by Nc. The Nc-preSBO possessed protein matrix with higher heterogeneity and presence of several large voids, which might be used to explain less stability of this sausage formulation as indicated by higher cooking loss and less retained textural attributes. For the FPI-preSBO, the protein network with more compact and higher homogeneity was observed, which was correspond to their higher stability compared to the others as previously observed by lowered cooking loss and better retained textural attributes. Effective ability of FPI to stabilize meat matrix was postulated due to more pronounced interaction between the FPI and meat protein residues. By isolating via the pH shift method, partial denaturation of the FPI could be expected due to a hydrolysis reaction accelerated by alkaline environment.

Overview from the present results, a yellow stripe trevally–a low price fish species abundantly present in the Southern part of Thailand–could be used to prepare FPI with effective functional property to be used in meat products. As employed as emulsifier to stabilize preSBO, the FPI possessed a good ability to retain sausage stability against fat reduction effect compared to the other commercially non-meat proteins-*i.e.,* SPI and Nc. In this work, partial substitution of porcine fat by preSBO could be accomplished by using the FPI at 2 % as emulsifier, by providing the sausages with lowered cooking loss, retained texture attributes, and microstructure with smoothness and high homogeneity.

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8. ข้อคิดเห็นและข้อเสนอแนะสำหรับการวิจัยต่อไป

- To more elucidate effect of the FPI on improvement of sausage stability, other properties of the FPI, such as hydrophobicity and thermal properties should be investigated.

- To more utilization of the FPI, observation on effect of the FPI to be used as emulsifier in other kinds of meat products, such as fermented sausages should be studied.

9. ภาคผนวก

9.1 ผลงานตีพิมพ์

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