Characteristics and Functional Properties of Gelatin from Goat Skin as Affected by Extraction and Drying Conditions

Sulaiman Mad-Ali

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Technology

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ชื่อวิทยานิพนธ์	ผลของสภาวะการสกัดและการทำแห้งต่อคุณลักษณะและสมบัติเชิงหน้าที่ของเจลาตินจากหนังแพะ
ผู้เขียน	นายสุไลมาน หมัดอะหลี
สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีอาหาร
ปีการศึกษา	2558

บทคัดย่อ

หนังแพะสามารถใช้เป็นวัตถุดิบทางเลือกสำหรับการผลิตเจลาติน จากการศึกษาผลของสภาวะการสกัดและการทำแห้งต่อสมบัติของเจลาตินจากหนังแพะ โดยนำหนังแพะมาแช่ด้วยสารละลาย NaOH (เข้มข้น 0.50 และ 0.75 โมลาร์) ที่ระยะเวลาต่างๆ (1-4 วัน) ก่อนสกัดเจลาตินที่อุณหภูมิ 60 องศาเซลเซียส ระยะเวลา 5 ชั่วโมง พบว่าค่าความแข็งแรงของเจลเจลาตินที่ได้เพิ่มขึ้นเมื่อความเข้มข้นของสารละลาย NaOH และระยะเวลาที่ใช้เพิ่มขึ้น ($p < 0.05$) เจลเจลาตินมีค่า L^* ลดลงในขณะที่ค่า b^* เพิ่มขึ้นเมื่อระยะเวลาในการแช่เพิ่มขึ้น เจลาตินประกอบด้วยสายโซ่ชนิดแอลฟาเป็นองประกอบหลัก รองลงมาคือสายโซ่ชนิดบีตา การแช่หนังแพะด้วยสารละลาย NaOH ที่ระดับความเข้มข้น 0.75 โมลาร์ ระยะเวลา 2 วัน ให้ผลผลิตของเจลาตินสูงสุด (ร้อยละ 15.95, โดยน้ำหนักเปียก) รวมทั้งค่าความแข็งแรงเจลที่สูง (222.42 กรัม) ซึ่งสูงกว่าเจลจากเจลาตินจากวัว (199.15 กรัม) เพื่อเพิ่มผลผลิตของเจลาตินจึงได้ทดลองนำหนังแพะที่แช่ด่างแล้วไปผ่านการแช่ในสารละลาย Na_2SO_4 (เข้มข้น 0-1 โมลาร์) พบว่าผลผลิตของเจลาตินเพิ่มขึ้นเมื่อความเข้มข้นของ Na_2SO_4 เพิ่มขึ้น และจากการศึกษาผลของการฟอกจางสีหนังแพะด้วยสารละลาย H_2O_2 (เข้มข้น 0-2 โมลาร์) พบว่าหนังแพะที่ผ่านการแช่ด้วยสารละลาย Na_2SO_4 ก่อนการฟอกจางสีให้ผลผลิตเจลาตินและค่าความแข็งแรงของเจลสูงกว่าเจลาตินจากหนังแพะที่ไม่แช่ด้วย Na_2SO_4 และพบว่าเจลาตินที่ได้ประกอบด้วยสายโซ่ชนิดแอลฟาเป็นองค์ประกอบหลัก รองลงมาคือสายโซ่ชนิดบีตา นอกจากนี้หนังแพะที่ผ่านการแช่ด้วย Na_2SO_4 มีระดับการสลายตัวของเจลาตินที่เหนี่ยวนำด้วย H_2O_2 ในระดับที่ต่ำกว่าเมื่อเทียบกับหนังแพะที่ไม่ผ่านการแช่ด้วย Na_2SO_4 ค่า L^* ของเจลเจลาตินเพิ่มขึ้นเมื่อความเข้มข้นของ H_2O_2 เพิ่มขึ้น ($p < 0.05$) อันเป็นผลจากการฟอกจางสีของ H_2O_2 เมื่อศึกษาโครงสร้างทางจุลภาคของเจลเจลาตินจากหนังแพะที่ผ่านการฟอกจางสีด้วย H_2O_2 และแช่ด้วยสารละลาย Na_2SO_4 พบว่าเจลมีโครงสร้างทางจุลภาคแบบละเอียดและเป็นระเบียบ ดังนั้นสภาวะที่เหมาะสมในการสกัดเจลาตินจากหนังแพะคือการแช่หนังแพะในสารละลาย NaOH เข้มข้น 0.75 โมลาร์ ตามด้วยการใช้สารละลาย Na_2SO_4 เข้มข้น 0.75 โมลาร์ และฟอกจางสีด้วย H_2O_2 เข้มข้น 2

โมลาร์ เจลาตินที่ได้มีปริมาณกรดอิมิโนเท่ากับ 217 หน่วยต่อ 1,000 หน่วย และมีอุณหภูมิการเกิดเจลและอุณหภูมิการหลอมละลายเท่ากับ 22.49 และ 32.28 องศาเซลเซียส ตามลำดับ

เมื่อนำหนังแพะที่ผ่านการปฏิบัติเบื้องต้นด้วยสภาวะที่เหมาะสมมาสกัดเจลาตินที่อุณหภูมิต่างๆ (50-70 องศาเซลเซียส) เป็นระยะเวลาต่างกัน (2.5 และ 5 ชั่วโมง) พบว่า ผลผลิตของเจลาตินที่ได้อยู่ในช่วงร้อยละ 22.1 ถึง 23.1 (โดยน้ำหนักเปียก) เจลาตินประกอบด้วยสายโซ่ชนิดแอลฟาเป็นองค์ประกอบหลักและสายโซ่ชนิดบีตาเป็นลำดับรอง อย่างไรก็ตามเมื่อสกัดเจลาตินที่อุณหภูมิ 70 องศาเซลเซียส พบว่าไม่มีการเหลืออยู่ของสายโซ่ชนิดบีตา เจลาตินที่ได้จากการสกัดที่อุณหภูมิ 50 องศาเซลเซียส เป็นระยะเวลา 2.5 ชั่วโมง มีค่าความแข็งแรงเจลสูงสุด (267 กรัม) ($p < 0.05$) อุณหภูมิการเกิดเจลและอุณหภูมิการหลอมละลายของเจลาตินอยู่ในช่วง 21.18-25.17 และ 30.69-34.12 องศาเซลเซียส ตามลำดับ ค่าความแข็งแรงของเจล อุณหภูมิการเกิดเจลและอุณหภูมิการหลอมละลายของเจลาตินลดลงเมื่ออุณหภูมิและระยะเวลาในการสกัดเพิ่มขึ้น และพบว่าเจลาตินที่ได้จากการสกัดที่อุณหภูมิและระยะเวลาที่เพิ่มขึ้นมีโครงสร้างที่ประกอบด้วยเส้นสายที่หนาขึ้น รวมทั้งมีช่องว่างขนาดใหญ่ที่เพิ่มขึ้น เจลาตินที่ได้จากการสกัดที่อุณหภูมิ 50 องศาเซลเซียส เป็นระยะเวลา 2.5 ชั่วโมง มีปริมาณกรดอิมิโนเท่ากับ 225 หน่วยต่อ 1,000 หน่วย และมีค่าความแข็งแรงเจลสูงกว่าเจลาตินจากวัว

จากการศึกษาจำแนกคุณลักษณะของผงเจลาตินจากหนังแพะที่ผ่านการทำแห้งด้วยวิธีพ่นฝอยที่อุณหภูมิขาเข้าต่างๆ (160-200 องศาเซลเซียส) พบว่าขนาดอนุภาคของผงเจลาตินส่วนใหญ่อยู่ในช่วง 4.65-5.14 ไมโครเมตร อนุภาคมีรูปทรงโค้งเว้าและขนาดที่แตกต่างกันขึ้นกับอุณหภูมิขาเข้าที่ใช้ ผงเจลาตินที่ได้มีสีขาวครีมและมีสีเหลืองมากขึ้นเมื่ออุณหภูมิขาเข้าของการทำแห้งเพิ่มมากขึ้น ($p < 0.05$) เจลาตินมีสายโซ่ชนิดแอลฟาเป็นองค์ประกอบหลัก อย่างไรก็ตามเมื่ออุณหภูมิขาเข้าของการทำแห้งเท่ากับ 200 องศาเซลเซียส สายโซ่ชนิดแอลฟาในเจลาตินหายไปเกือบหมด นอกจากนี้ค่าความแข็งแรงเจลของเจลาตินลดลงเมื่ออุณหภูมิขาเข้าของการทำแห้งเจลาตินด้วยวิธีพ่นฝอยเพิ่มขึ้น ($p < 0.05$) เจลาตินที่ผ่านทำแห้งด้วยวิธีพ่นฝอยที่อุณหภูมิขาเข้า 160 องศาเซลเซียส มีค่าความแข็งแรงเจลสูงสุด (260 กรัม) และมีค่าเทียบเท่ากับค่าความแข็งแรงเจลของเจลาตินที่ผ่านการทำแห้งด้วยวิธีระเหิด (268 กรัม) ($p > 0.05$) นอกจากนี้ทั้งเจลาตินที่ผ่านการทำแห้งแบบพ่นฝอยที่อุณหภูมิขาเข้า 160 และ 180 องศาเซลเซียส มีค่าความแข็งแรงเจลสูงกว่าค่าความแข็งแรงเจลของเจลาตินทางการค้าจากวัว ($p < 0.05$) และเจลาตินที่ได้มีค่าการละลายสูงกว่าร้อยละ 90 ที่พีเอชช่วงกว้าง (1-10)

จากการศึกษาคุณลักษณะและสมบัติเชิงหน้าที่ของเจลาตินที่ผ่านการทำแห้งด้วยวิธีพ่นฝอย (SDGG) และเจลาตินที่ผ่านการทำแห้งด้วยวิธีระเหิด (FDGG) เปรียบเทียบกับเจลาตินทางการค้าจากวัว (BG) พบว่า SDGG มีค่าไฮโดรโฟบิซิตีบริเวณพื้นผิวสูงสุดเมื่อเทียบกับเจลาตินอื่น ($p < 0.05$) SDGG ให้เจลที่มีค่า a^* และ b^* สูงกว่าเล็กน้อยรวมทั้งมีค่าความขุ่นของสารละลายที่สูงกว่าเมื่อเทียบกับ FDGG ($p < 0.05$) สารละลายของ SDGG และ FDGG สามารถเกิดเจลได้ที่อุณหภูมิห้อง (25-28 องศาเซลเซียส) ภายในระยะเวลา 18.52-19.30 นาที เจลาตินทั้งสองมีอุณหภูมิการเกิดเจลและอุณหภูมิการหลอมละลายอยู่ในช่วง 25.14- 25.23 และ 34.09- 34.18 องศาเซลเซียส ตามลำดับ เจลของเจลาตินจาก SDGG และ FDGG มีโครงสร้างทางจุลภาคที่แน่นกว่าและมีช่องว่างขนาดเล็กกว่าเมื่อเทียบกับเจลของ BG สมบัติการเกิดฟองของเจลาตินทุกชนิดเพิ่มขึ้นเมื่อความเข้มข้นของเจลาตินเพิ่มขึ้น (ร้อยละ 1-3) SDGG มีค่าการเกิดฟองและค่าความคงตัวของฟองสูงกว่า FDGG อิมัลชันที่เติม SDGG มีขนาดอนุภาค (d_{32}, d_{43}) และค่า flocculation factor สูงกว่าอิมัลชันที่เติม FDGG อีกทั้งยังมีค่าความคงตัวของอิมัลชันในระดับที่ต่ำกว่าโดยบ่งชี้จากค่า coalescence index ที่สูงกว่าและประจุลบที่ต่ำกว่าภายหลังการเก็บรักษานาน 10 วัน ดังนั้นวิธีการทำแห้งมีผลบางส่วนต่อคุณลักษณะและสมบัติเชิงหน้าที่ของเจลาตินที่ได้ และเพื่อลดค่าใช้จ่ายในการดำเนินการ การทำแห้งด้วยวิธีพ่นฝอยโดยใช้อุณหภูมิขาเข้า 160 องศาเซลเซียสจึงเป็นวิธีแนะนำสำหรับการผลิตเจลาตินจากหนังแพะ

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ABSTRACT

Goat skin was used as an alternative raw material for gelatin extraction and the impacts of extraction and drying conditions on properties of gelatin were investigated. When the skins were pretreated with NaOH solutions (0.50 and 0.75 M) for various times (1-4 days) prior to extraction at 60 °C for 5 h, gel strength of gelatins increased as NaOH concentration and pretreatment time increased ($p < 0.05$). Pretreatment for a longer time yielded the gelatin gel with the decrease in L^*-value but increase in b^*-value. All gelatins contained α-chains as the predominant component, followed by β-chain. Pretreatment of goat skin using 0.75 M NaOH for 2 days rendered the highest yield (15.95%, (wet weight basis)) as well as high gel strength (222.42 g), which was higher than bovine gelatin (199.15 g). To improve the extraction yield, alkali-treated skins were subsequently subjected to treatment using Na_2SO_4 (0-1 M). The yield increased as concentration of Na_2SO_4 increased ($p < 0.05$). When skins with prior Na_2SO_4 treatment were bleached using H_2O_2 (0-2 M), the resulting gelatin showed the higher yield and gel strength than those without prior Na_2SO_4 treatment. All gelatins had α-chain as major components, followed by β-chain. The degradation induced by H_2O_2 was lower in gelatin with prior Na_2SO_4 treatment. L^*-values increased with increasing H_2O_2 concentrations ($p < 0.05$) due to the bleaching effect of H_2O_2. With Na_2SO_4 and H_2O_2 pretreatments, gelatin gel had finer and more ordered microstructure. The optimal pretreatment condition for gelatin extraction from goat skin included soaking the skin in 0.75 M NaOH, followed by treatment using 0.75 M Na_2SO_4 and subsequent bleaching with 2 M H_2O_2. Gelatin had the imino acid content of 217 residues/1000 residues with gelling and melting temperature of 22.49 and 32.28 °C, respectively.

When skin pretreated under the selected condition was extracted at different temperatures (50-70 °C) for various times (2.5 and 5 h), the yield of gelatin ranged from 22.1 to 23.1% (wet weight basis). All gelatins contained α-chains as the predominant components, followed by β-chain. However, no β-chain was retained when extracted at 70 °C. Gelatin extracted at 50 °C for 2.5 h exhibited the highest gel strength (267 g) ($p < 0.05$). Gelling and melting temperatures for goat skin gelatin were in the ranges of 21.18-25.17 and 30.69-34.12 °C, respectively. Gel strength, gelling and melting temperatures of gelatin gels generally decreased with increasing extraction temperatures and times. Gels of gelatin extracted at a higher temperature for longer time had larger strands and voids. Gelatin extracted at 50 °C for 2.5 h contained imino acids of 225 residues/1000 residues and showed higher gel strength than bovine gelatin.

Gelatin powder from goat skin prepared by spray drying at various inlet temperatures (160-200°C) was characterized. Predominant particle sizes were in the range of 4.65-5.14 μm. Gelatin powder was mostly concave in shape with varying sizes, depending on inlet temperatures used. All gelatin powders were creamy whitish. Powder generally became more yellowish as the inlet temperature of spray drying increased ($p < 0.05$). All gelatins contained α- chain as the dominant constituent. Nevertheless, α- chain of gelatin spray-dried at 200°C almost disappeared. Gel strength of gelatin decreased as the inlet temperature for spray drying increased ($p < 0.05$). Gelatin with inlet temperature of 160°C had the highest gel strength (260 g) ($p < 0.05$), which was comparable to the freeze-dried counterpart (268 g) ($p > 0.05$). Goat skin gelatin spray-dried with inlet temperatures of 160 or 180°C had higher gel strength than commercial bovine gelatin ($p < 0.05$). All gelatins had solubility greater than 90% in the wide pH ranges (1-10).

Characteristics and functional properties of spray-dried goat skin gelatin (SDGG) and freeze-dried counterpart (FDGG) were determined, in comparison with commercial bovine gelatin (BG). SDGG had the highest surface hydrophobicity, compared with others ($p < 0.05$). SDGG gel showed slightly higher

*a** and *b** values as well as the higher solution turbidity than those of FDGG ($p < 0.05$). Both SDGG and FDGG solutions could set at room temperature (25-28 °C) within 18.52-19.30 min. Both gelatins showed the gelling and melting temperatures of 25.14- 25.23 °C and 34.09- 34.18 °C, respectively. Gels from SDGG and FDGG had the denser structure with smaller voids than those from BG. Foaming properties of all gelatins increased with increasing concentrations (1-3%). SDGG had a higher foam expansion and stability than FDGG. Emulsion containing SDGG had the higher droplet size *(d_{32}, d_{43})* and flocculation factor than that containing FDGG ($p < 0.05$) and showed the lower stability of emulsion as indicated by the higher coalescence index with lower negative charge after 10 days of storage. Thus, drying methods affected characteristics and functional properties of obtained gelatin to some extent. To reduce the operating cost, spray drying with inlet temperature of 160 ° C was recommended to produce gelatin powder from goat skin.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Gelatin is protein obtained by thermal denaturation or partial hydrolysis of collagen. Gelatin is widely used in food, pharmaceutical, cosmetic and photographic applications because of its unique functional properties. Gelatin has been used in confections (mainly for providing chewiness, texture, and foam stabilization), low-fat spreads (to provide creaminess, fat reduction, and mouthfeel), dairy (to provide stabilization and texturization), baked goods (to provide emulsification, gelling, and stabilization), and meat products (to provide water-binding) (Schrieber and Gareis, 2007). In the pharmaceutical and medical fields, gelatin is used as a matrix for implants, in injectable drug delivery microspheres, and in intravenous infusions (Panduranga Rao, 1996; Pollack, 1990). Gelatin, being low in calories, is normally recommended for use in foodstuffs to enhance protein levels, and is especially useful in body-building foods. In addition, gelatin is also used to reduce carbohydrate levels in foods formulated for diabetic patients (Karim and Bhat, 2009). In general, commercial gelatins are produced from bovine and porcine skin (Hao *et al.*, 2009). The type and source of different materials yield gelatin with varying amino acid composition, thereby influencing the properties of resulting gelatins. Gelatin with high imino acid (proline and hydroxyproline) always relatively has high gel strength (Cho *et al.*, 2006), due to high hydrogen bond formation in gel network (Arnesen and Gildberg, 2002). Gelatin from mammalian skin often has higher gel strength than marine gelatin such as fish (Norland, 1987). Mammal gelatin typically has a bloom value with the range of 200-240, which is regarded as high bloom (Karim and Bhat, 2009)

In general, gelatin from mammalian skin can be extracted by pre-treatment using alkaline solution (lime) and the obtained gelatin is considered as “B type” (Hinterwaldner, 1977). Alkaline solution plays an important role in breaking of internal structure cross-linked by strong bond and contributes to more efficient

extraction (John and Courts, 1977). Although gelatin has a wide range of useful applications, pessimism and strong concerns still persist among consumers with regard to its usage (Asher, 1999). This is mainly due to religious sentiments. Both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products (Badii and Howell, 2003). Therefore, the extraction of gelatin from alternative source, especially by-products from slaughter house, e.g skin or bone, should be conducted.

Goats have been raised in Thailand for their meat and milk. The number of goats increased annually since their meat has become promising for consumption, especially for Muslims. When goats are slaughtered, by-product including skin accounts for 6.4-11.6% (weight/body weight) (FAO, 2015). The skin can be used as an alternative raw material for gelatin extraction. However, a little information regarding gelatin from goat skin has not been reported, especially in terms of processing parameters determining property of gelatin. Extraction under optimized condition is a means to bring about gelatin from goat skin with quality equivalent or superior to commercially available gelatin, in which goat quality gelatin can be produced and used as a replacer for available commercial gelatin.

1.2 Review of literature

1.2.1 Goats in Thailand

Goats are the economically important animals raised in Thailand for their meat and milk. The number of goats increases from 130,904 heads in 1998 to 491,779 heads in 2012 and is still gradually increasing **(**Department of Livestock Development, 2014). Goats are the animal domesticated to produce meat and milk for consumption and commonly raised in the province of southern border such as Songkhla, Pattani, Yala, Narathiwas, etc. Nowadays, those areas have goats raised up to 62.5 % of all goats in country (Department of Livestock Development, 2014). Goats in Thailand are predominantly used for meat (90%), while milk production is a smaller industry (10 %) (Pralomkarn and Boonsanit, 2011). Native goats are genetic resources that can be crossed with exotic breeds to produce rangeland goats for more

extensive production. Approximately 10 goat breeds exist in Thailand: 2 indigenous and 8 exotic (Table 1). There are 4 meat breeds and 4 dairy breeds. The 2 local goat breeds are the Northern Thai referred to as ‘Bangala’, which has a large but thin body, long pendulous ears (similar to the Anglo-Nubian) and a straight face profile, and the other is the Southern Thai referred to as ‘Katjang’, which is small in size with short upright ears (similar to the Katjang goat found throughout Southeast Asia).

Extensively raised and consumed meat goats are the crossbreeding of Anglo-Nubian with Boer (Figure 1) because they are easy to raise and have high growth and desired weight (Maming, 2010).

Figure 1 Two years Aglo-Nubian × Boer
Source : Cevenda and Solaiman (2010)

1.2.2 Goat skin

Goat carcass from slaughtering accounts for approximately of 41.2% for non-obese goat and 51.4% for obese goat. Based on percentage of alive weight, skin accounts for 6.4-11.6% (weight/body weight) (FAO, 2015).

Goat skins have a very tight fibre network, compared with those from sheep and cattle (Stosic, 1994). Glycoaminoglycan content and relaxation of shrinkage tension decrease rapidly up to the age of 10 months and thereafter more slowly. Collagen content increases more slowly up to age of 20 months and thereafter level off. Collagen content of goat skin increases from 59 % at age of 3 months up to

Table 1 Breeds of goats in Thailand

No.	Breed	Developed by/country of origin	Imported to Thailand	Description and Distribution in Thailand
	Local breeds			
1	Northern Thai Native goat (Bangala)	Northern region, Thailand	Descended from nomadic goats of India, Myanmar	Large size, using as a base resource, mainly found in the north and central regions.
2	Southern Thai Native goat (Katjang)	Southern region, Thailand	Descended from nomadic goats of Malaysia	Small size, large numbers have been graded up, mainly found in south region.
	Meat breeds			
3	Anglo-Nubian	Britain	1982 (UK) 1988, 1993, 1997 (USA) 2007 (Australia)	It is a useful dual purpose breed that has a good capacity to adapt to hot climate, found all the country

Table 1 Breeds of goats in Thailand (cont.)

No.	Breed	Developed by/country of origin	Imported to Thailand	Description and Distribution in Thailand
	Meat breeds			
4	Boer	South Africa	1996-1997 (South Africa) 2007 (Australia)	Small size, quality for leather Bred by Royal project.
5	Black Bengal)	India, Bangladesh	2005 (Bangladesh)	Small size, large numbers have been graded up, mainly found in south region.
6	Jamunapari	India	2007 (Malaysia)	Large size, tallest and longest ears. Bred by Royal project
	Dairy breeds			
7	Saanen	Saanen Valley, Switzerland	1984 (Australia) 1985 (Netherlands) 1997 (USA) 2007 (Australia)	Most popular dairy goat in Thailand. Heavy milk producers, mainly found in central and south regions

Table 1 Breeds of goats in Thailand (cont.)

No.	Breed	Developed by/country of origin	Imported to Thailand	Description and Distribution in Thailand
	Dairy breeds			
8	Alpine	Britain	1997 (USA)	Suited to cooler climates with low humidity, found in central and south regions
9	Toggenburg	Obertoggenburg, Switzerland	1997 (USA)	Suited to cooler climates with low humidity, found in central and south regions.
10	Laoshan	Shangdong, China	2001, 2006 (China)	Similar to Saanen, bred by Farm model, Royal project

Source: Anothaisinthawee *et al.* (2010).

70 % at age greater than 20 months (Wang and Attenburrow, 1994). Collagen fibre bundles become more compact with age. Stosic (1994) reported the dependence of the fat content of the goat skin (on a moisture free basis) on the age of animal. No significant differences were found between the sexes, where the fat contents are found approximately by 2.5 % and are less than other mammals. Collagen contents of goat skin ranges from 52.74 % to 68.88 % (Stosic, 1994).

1.2.2.1 Histological characteristics

The normal size of the skin varies from 23×12 inch and 33×18 inch. Skins of sizes below the former and above the latter are called “kids” and “heavies”, respectively. In many aspects, the structure of goat skins can be considered between a cow calf and a sheep skin. Composition and structure of goat skin are shown in Figure 2 (Stosic, 1994).

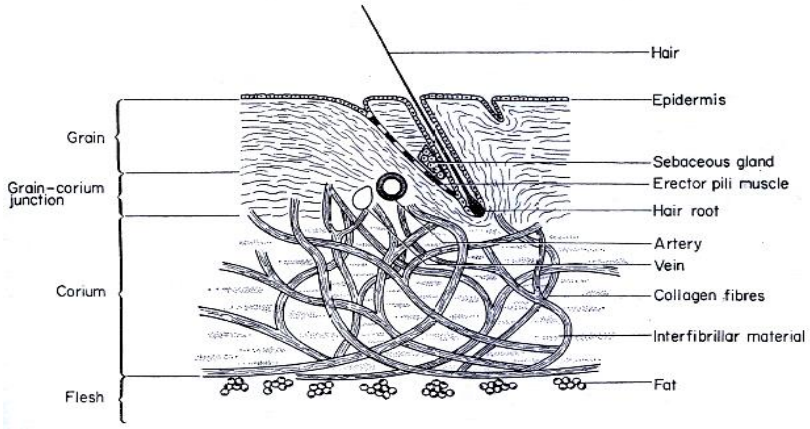

Figure 2 Schematic cross-section of goat skin

Source: Stosic (1994)

Epidermis: The epidermis of a goat skin covers approximately 1 to 2.6 % of the total thickness of the skin. The average number of hairs varies from 8000 to 18,000 hairs per square inch. Two types of hair are present (coarse and fine hairs). Like the calf, the goat has straight hair follicles and hence straight hairs. The hair follicles are quite deeply rooted. The glands and fat cells are very much less in

number in goat skins. The erectorpili muscle is well developed and long in goat skin (Mtenga, 1979).

Grain layer: The grain layer of the goat skin usually occupies approximately 24 to 54 % of the total thickness of the skin. In the grain layer, the collagen fibre bundles are compactly woven. Because of lesser number of cellular components and fat glands, the goat skin is comparatively compact than sheep skin (Mtenga, 1979). In the goat skins, there is more elastin and its covers approximately is 2/3 rd of the entire layer of the grain. The presence of larger amount of elastin tissue in perhaps one of the reasons is why greater efforts are needed to open up the grain structure during pre-tanning process. In good quality goat skins, corium and grain layer merges uniformly (Mtenga, 1979).

Corium layer: The corium of the goat skin occupies approximately 45 to 75 % of the total thickness of the skin. The collagen fibres present in this layer are firmer and fuller than the corresponding ones in the sheep skins. It is nearly equal to that of calf skin (Mtenga, 1979). For goat skins, a very low angle of weave is present. Fat cells and fat droplets are rarely found in the corium proper of goat skin. A considerable amount of reticular tissue is present in goat skin.

Flesh layer: It roughly covers 1 to 2 % of the total thickness of the skin. A considerable amount of elastin tissue is present in the layer. Natural fats and fat cells are present in this layer.

1.2.3 Collagen

Collagen is an important constituent of the supporting structures of both vertebrates and invertebrates. Collagen is the main protein of connective tissue in animals, making up about 25-35 % of the whole-body protein content (Berillis *et al.*, 2011; Di Lullo *et al.*, 2002). It is naturally found exclusively in metazoa, including sponges (Muller, 2003). Collagen is abundant in tendons, skin, bone, the vascular system of animals and the connective tissue sheaths surrounding muscle. In muscle tissue, it serves as a major component of endomysium. Collagen comprises one-third

or more of the total protein of mammals. About 10 % of mammalian muscle protein is collagen (Foegeding *et al.*, 1996).

Collagen has unusually high content of glycine and imino acids (proline and hydroxyproline), with very small amounts of aromatic and sulphur containing amino acids. Collagen is only protein reported to contain more than 0.1 % hydroxylysine (Ballian and Bowes, 1977).

1.2.3.1 Types of collagen

Various distinct types of collagen exist, each with its own genes, which express their characteristic polypeptide chains. The various types of tropocollagen molecules include type I, II, III, IV and VI (Table 2). The best known types, which are important in gelatin manufacturing are types I and III (Asghar and Henrickson, 1982)**.**

Table 2 Different types of collagen

Type	**Description**
Type I	This type occurs widely, primarily in connective tissue such as skin, bone and tendons.
Type II	This type of collagen occurs practically exclusively in cartilage tissue.
Type III	This type is strongly dependent on age: very young skin can contain up to 50%, but in the course of time this is reduced to 5-10%
Other types	The other types of collagen are present in very low amounts only and mostly organ-specific.

Source: Karim and Bhat (2009)

1.2.3.2 Structure of collagen

All collagen molecules are built up from three polypeptide (α) chains, each with a left-handed helical conformation, that are coiled around each other to form a characteristic right-handed collagen triple helix (Burghagen, 1999). In addition, they all contain noncollagenous sequences at their termini, and some

collagens also have these sequences as interruptions separating adjacent triple-helical regions to make the molecules more flexible. All collagen α-chains have repeating -Gly-X-Y- sequences. The occurrence of glycine in every third position is an absolute requirement as glycine is the only residue with a side-chain small enough to fit the restricted space in the center of the triple helix. Proline is frequently in the X-position and Hyp in the Y-position (Kielty and Shuttleworth, 1993). These residues are required for the correct conformation of the helix because they limit rotation of the polypeptide chains, and their hydrophobic and charged side-chains are located on the surface of the molecule making collagens polymerize into precisely ordered structures. Hyp is also essential for the thermal stability of the helix (Prockop and Kivirikko, 1995; Rehn and Pihlajaniemi, 1995).

1.2.3.3 Collagen cross-linking

Collagen molecules are arranged into groups of four or five to create fibrils. These fibrils are further joined with other fibrils to form groups with a diameter of 100 to 2000 Å depending on the type of collagen and tissue sources (Voet and Voet, 1995). The collagen fibrils are stabilized by the formation of intermolecular cross-links between each other. This produces bonds between the different amino acids, mainly the lysine and/or hydroxylysine residues bond with adjacent lysine/hydroxylysine and histidine residues in similar regions (Figure 3). These bonds produce stable covalent cross-links which ensure that collagen is insoluble in water and cannot be attacked by most enzymes (Johnston-Banks, 1990). There are various different bond structures which have been proposed for the cross-link bonds between collagen fibrils. These include a head to tail, which is an overlapping of the α-chains, as well as side to side bonding structures (Figure 4). The arrangement of bonds between fibrils varies with different tissue sources. As the animal ages, the degree of cross-linking in the tissue increases and the proportion of procollagen, a soluble predecessor in collagen development, reduces significantly as the collagen matures (Johnston-Banks, 1990). The covalent cross-links involved in the β and γ component of collagen and the intermolecular cross-links between collagen molecules are formed spontaneously by the condensation of aldehyde groups. This may involve an aldol condensation-type reaction, or formation of a Schiff base when the aldehyde react

with an amino group. When hydroxylysine reacts with hydroxylysine aldehyde, the reaction product undergoes an Amadori-type rearrengment to form a “keto” structure, hydroxylysino-5-keto-norleucine (Foegeding *et al.*, 1996).

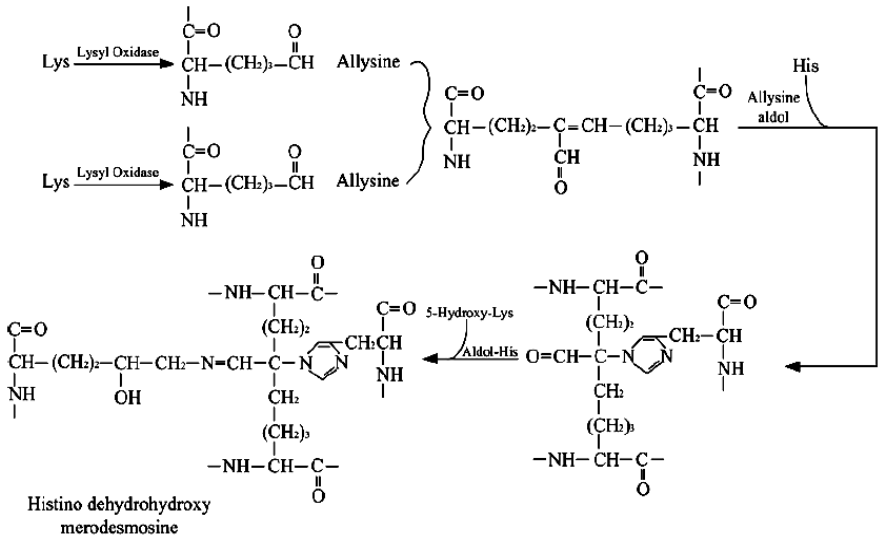

Figure 3 The biosynthetic pathway for cross-linking of lysine with histidine side chains in collagen.

Source: Voet and Voet (1995)

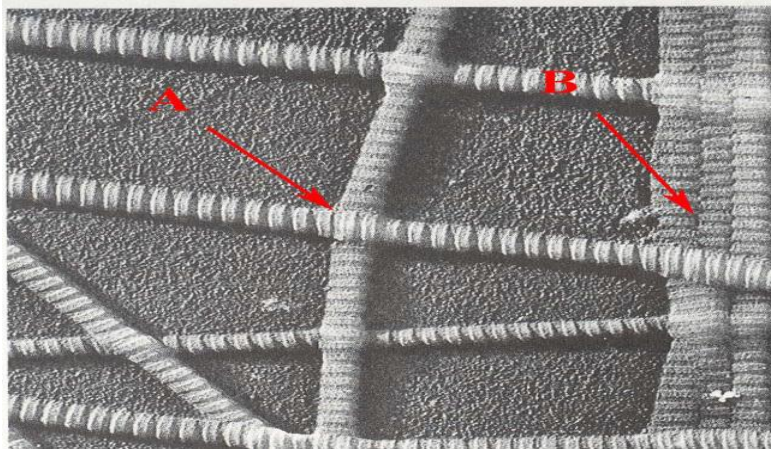

Figure 4 Collagen skin fibrils showing overlapping (A) and side to side (B) cross-linking

Source: Voet and Voet (1995)

1.2.3.4 Collagen in mammals

Collagen and gelatin consists of 18 different amino acids (Table 3). Although some differences in amino acid composition are apparent across collagens derived from different sources, there are certain features that are common and unique for all collagens. It is the only mammalian protein, which contains the large amounts of hydroxyproline and hydroxylysine, and the total imino acid (proline and hydroxyproline) content is high (Ballian and Bowes, 1977). The amino acid composition of gelatin is very close to that of its parent collagen.

Table 3 Amino acid composition of collagen and gelatin from mammal

Amino acids	No. residues/1000 residues			
	Bovine collagen	Pig skin gelatin	Calf skin gelatin	Bone gelatin
Alanine	107	86-107	93-110	101-142
Arginine	48	83-91	86-88	50-90
Aspartic acid	47	62-67	66-69	46-67
Glutamic acid	72	113-117	111-114	85-116
Glycine	337	264-305	269-275	245-288
Histidine	5	8-10	7-8	4-7
Hydroxylysine	5	10	9-12	7-9
Hydroxyproline	94	135	140-145	119-134
Isoleucine	11	14	17-18	13-15
Leucine	24	31-33	31-34	28-35
Lysine	25	41-52	45-46	21-44
Methoionine	4	8-9	8-9	0-6
Phenylalanine	13	21-26	22-25	13-25
Proline	129	162-180	148-164	135-155
Serine	39	29-41	32-42	34-38
Threonine	17	22	22	20-24
Tyrosine	5	4-9	2-10	0-2
Valine	20	25-28	26-34	24-30

Source: Adapted from Arvanitoyannis (2002)

1.2.4 Gelatin

Gelatin is polypeptide produced by the thermal denaturation or partial hydrolysis of collagen derived from animal skin, connective tissue and bone (Johnston-Banks, 1990). The process involves the disruption of noncovalent bonds and it is partially reversible when gelation takes place (Bigi *et al.*, 1998).

The simplest way to transform collagen to gelatin is to denature soluble collagen. It involves hydrolysis catalyzed by enzymes, acid or alkaline. Thermal denaturation takes place by heating the collagen in neutral or slightly acidic conditions to about 40°C (Giménez *et al.*, 2005). The transition is sharp and complete within a few minutes over a small temperature interval. The activation energy for denaturation is approximately 81 Kcal (Jussila, 2004). Only the hydrogen bonds and hydrophobic bonds that help to stabilize the collagen helix are broken causing the fibers and fibrils of collagen to dissociate into tropocollagen units. The next step, in the hydrolysis of collagen, consists of breaking the intramolecular bonds between three chains of the helix (Nishimoto *et al.*, 2005).

1.2.4.1 Structure of gelatin

The primary structure and composition of gelatin resembles the parent collagen. This similarity has been substantiated for several tissues and species (Fernández-Díaz *et al.*, 2001). Gelatin has a width of ~14 Å and length of 3,000 Å, which is nearly identical to that of collagen. The molecular weight of gelatin varies from 20,000 to 250,000 (Ballian and Bowes, 1977). Slight differences are due to the source of raw material in combination with the pretreatment and extraction procedures used (Mariod and Adam, 2013). Gelatin is a mixture of different polypeptide chains including α-chains, β (dimers of α-chain) and γ (trimers of α-chain) components (Rbii *et al.*, 2011). The molecular weight of α form varies from 80,000 to 125,000 and for the β form 160,000 to 250,000 Da. The molecular weight variation for the γ form is from 240,000 to 375,000 (Nishimoto *et al.*, 2005) (Figure 5).

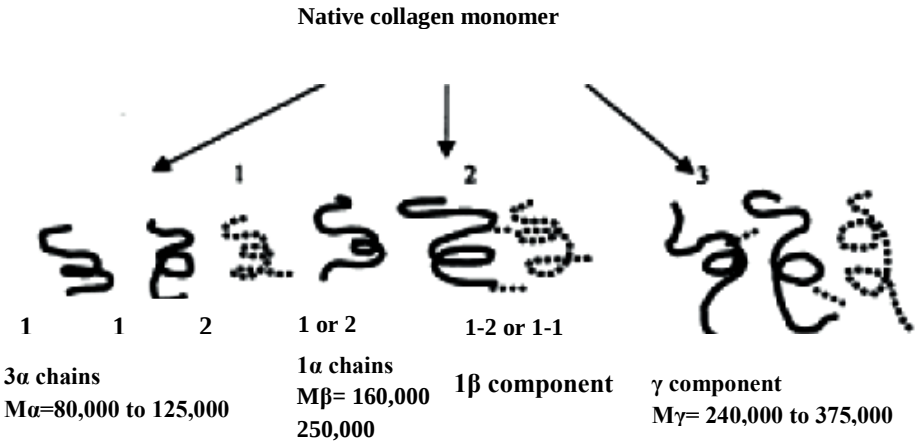

Figure 5 Possible paths of collagen conversion to gelatin

Source : Ofori (1999)

1.2.5 Production of gelatin

The ultimate aim in gelatin production is to convert the different insoluble collagenous raw material into a maximum soluble and highly purified gelatin with good physiochemical properties including high gel strength, high clarity and viscosity. Figure 6 shows a flow diagram of gelatin production process.

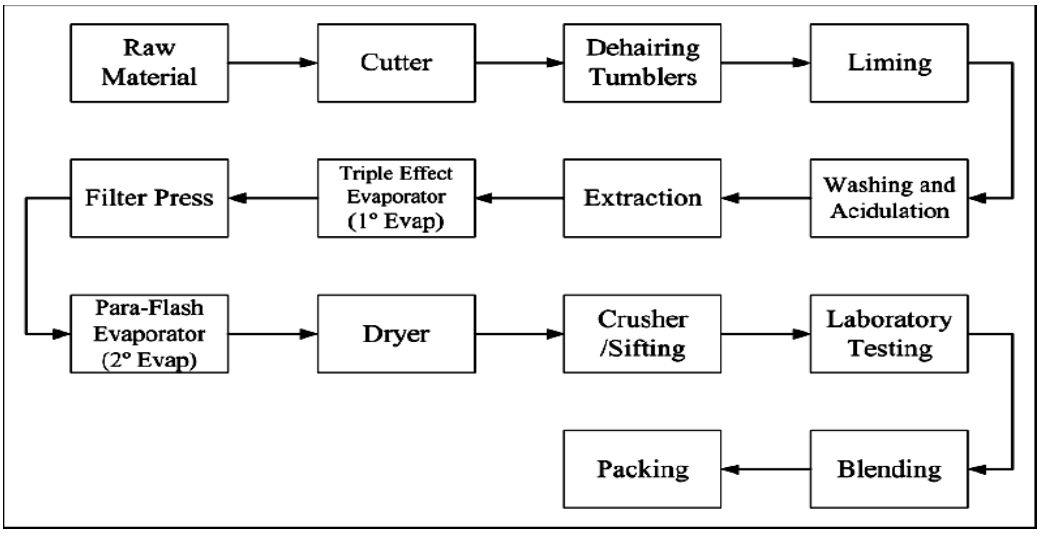

Figure 6 Summary of gelatin production process

Source: Wittich (2005)

There are three basic stages involved in the manufacturing of gelatin (Ofori, 1999), including preparation of the raw material, conversion of the purified soluble collagen into gelatin and refinement and recovery of the gelatin in dried form.

1.2.5.1 Raw material

The raw material availability both in quantity and at a reasonable cost is very critical to the manufacturer. Commercially, the only sources of practical importance are skin, bones and pig skins. Qualities of the gelatins produced and to some extent the process that can be used depend on the species, breed, age and the storage conditions to which the raw materials have been exposed (Leach and Eastoe, 1977).

1.2.5.2 Pretreatment of raw materials

1.2.5.2.1 Type of pretreatment

To convert insoluble collagen, two pretreatments are in current use, alkaline pretreatment and acid pretreatment (Johnston-Banks, 1990):

A) Acid process for type A gelatin

Acid hydrolysis is a milder treatment that will effectively solubilize collagens of animals slaughtered at a young age, such as pigs (Foegeding *et al.*, 1996). The pretreatment process is designed to convert the collagen into a form suitable for extraction. A sufficient number of the covalent cross-links in the collagen must be broken in order to enable the release of free α-chains (Kittiphattanabawon *et al.*, 2010). The process is also designed to remove other organic substances, such as proteoglycan, blood, mucin, sugar, etc., that also occur naturally in the raw material. It is optimized by each manufacturer to give the required and chemical properties to the galatins that are produced (Johnston-Banks, 1990). Normally, 18-19 h soaking in dilute acid is sufficient to bring about the conversion. Sulphuric and hydrochloric acids are used, often with the addition of phosphoric acid to retard color devepopment (Johnston-Banks, 1990). The resulting gelatin has an isoionic point of 7 - 9 based on

the severity and duration of the acid processing of the collagen which causes limited hydrolysis of the asparagine and glutamine side chains (Cole, 2000).

B) Alkaline process for type B gelatin

Type B gelatin are produced by alkaline hydrolysis of beef material, which results in deamidation and generation of a greater range of molecular weight species (Foegeding *et al.,* 1996). Alkaline pretreatment is normally applied to bovine skin and ossein. Lime - limimg in gelatin manufacture refers to a much longer period of treatment (John and Courts, 1977). Unfortunately, 8 weeks or more are required for complete treatment. Concentrations of up to 3% lime are used in conjunction with small amounts of calcium chloride or caustic soda. Frequent renewal of solution is practiced in order to remove extracted impurities and to maintain the degree of alkalinity present. If caustic soda is used, a 10-14 day pretreatment is possible (Johnston-Banks, 1990). The propose of liming process is to destroy certain chemical cross-linkages present in the collagen, as well as to remove unwanted material, other proteins, carbohydrates, etc. Fats are converted into insoluble lime soaps.

In addition, the time of alkaline treatment was easily reduced to five days and high yields of high grade gelatin obtained when sodium sulfate was added or sodium thiosulfate was incorporated (Ward, 1953). Pospísilová (1966) treated rat tail tendon fibres with hydrogen peroxide at concentrations range of 10^{-3}- 1.0 M. Breakage of internal bonding led to elongation of the fibre. Hydrogen peroxide at high concentration was used (up to 30% w/w) by Deasy (1965) for extraction of mature steer skin collagen. The collagen was completely solubilized after 5 days of treatment. John and Courts (1977) stated that Smejkal and Blajez treated calf skin with hydrogen peroxide in the presence of copper ions. The oxidative deamination occurred when copper ions bind to collagen and the complex reacts with atomic oxygen from hydrogen peroxide. This lead to the cleavage of inter- and intramolecular crosslinks and peptide bond breakage.

One of the most noticeable changes in the conversion of collagen to gelatin is the hydrolysis of amide groups of asparagine and glutamine. This

conversion leads to an increase of aspartic and glutamic acid residues in gelatin. On average, there is an approximately a 1.5% increase of aspartic acid and a 2-3% increase in glutamic acid (Wittich, 2005). Pigskin is pretreated in a mild acid and is a relatively short process as the collagen from pigskins and fish skins have no high degree of cross-linking. There are fewer amide groups removed. Type B gelatin from bovine skin requires a much more aggressive alkaline pretreatment and pretreatment takes a considerably longer time (Johnston-Banks, 1990). This is because the raw material comes primarily from mature cattle whose tissue has developed a higher degree of cross-linking. Throughout the course of this process, there will be almost a complete hydrolysis of the amide groups of asparagine and glutamine which releases ammonia. The pretreatment in an alkaline solution also causes the alkaline cleavage of the complex carbohydrates (glycosaminoglycans or GAGs) associated with the collagen extra-cellular matrix. The removal of GAGs from the skin results in the loosening of the collagen fibrils (Wittich, 2005).

The alkaline pretreatment also converts arginine to ornithine, which occurs when urea groups are removed from arginine (Wittich, 2005). This is a slow reaction; gelatin produced from a pretreatment period of 8-10 weeks has only a 3% conversion of arginine. Where gelatin is produced from a longer pretreatment period, 4-8 months, it has a higher conversion of about 34%. There are other subtle changes in amino acid composition in gelatin. Cysteine, tyrosine, isoleucine, and serine residues are usually lower than those found in collagen. This is due to the removal of some telopeptides during the cleavage of collagen cross-links, and is lost into the pretreatment solution (Johnston-Banks, 1990). Gelatin type-A or acid-processed gelatin generally has isoelectric points varying from 6.5 to 9.0 (Foegeding *et al.*, 1996), while type-B or alkaline processed gelatin e.g. bovine bone gelatin had the lowest solubility at pH 5 (Ahmad and Benjakul, 2011). Bovine gelatin with pI of approximately 4.88 was also reported (Zhang *et al.*, 2006).

1.2.5.2.2 Bleaching during pretreatment

Bleaching is another important process required for color improvement of gelatin, particularly when raw material contains the pigments or coloring

compounds. Mostly, oxidizing agents have been widely used to oxidize the pigments, yielding the fader color or white appearance. Hydrogen peroxide is a potent oxidant that is widely used as bleaching agent. Kołodziejska *et al.* (1999) reported that soaking squid skin in 1% H_2O_2 in 0.01 M NaOH for 48 h could improve the color of the resulting collagen. Gelatin from splendid squid skin bleached with H_2O_2 at a concentration of 2% also had the improved color (Nagarajan *et al.*, 2012). Aewsiri *et al.* (2009) found that bleaching of cuttlefish skin with 5% H_2O_2 not only improved the color of resulting gelatin but also enhanced its bloom strength effectively. H_2O_2 can be decomposed in aqueous solution via dissociation and hemolytic cleavage of O–H or O–O bonds, with the formation of highly reactive products: hydroperoxyl anion (HOO^-), and hydroperoxyl (HOO•) and hydroxyl (OH•) radicals, which can react with many substances, including chromatophores (Aewsiri *et al.*, 2009).

1.2.5.3 Extraction of gelatin

The conversion of pretreated raw material into gelatin takes place in five basic stage: (I) washing, (II) extraction, (III) purification, (IV) concentration and (V) drying (Johnston-Banks, 1990). The extraction process is designed to maximize the yields of gelatin. This is achieved by optimizing the balance between pH, temperature and time of extraction (Saunders and Ward, 1955). The extraction pH and level of conditioning influence the extraction rate, which results in different bloom strength and viscosity (Wittich, 2005). It can be selected either for the maximum extraction rate (low pH) or for the maximum physical properties (neutral pH) (Kittiphattanabawon, 2004). To extract collagens with high cross-linking at neutral pH, a substantial proportion of the cross-links need to be cleaved, necessitating a longer liming pretreatment. If shorter liming is used, a lower extraction pH is necessary in order to achieve acceptable conversion rates (Johnston-Banks, 1990). However, owing to the acidity present, the resultant gelatins will have lower viscosities (lower molecular weight) than those extracted at neutral pH. More efficient pretreatment conditions also allow the manufacturer to use lower extraction temperatures, resulting in gelatins of greater strength (bloom). Shorter treatments generally require higher extraction temperatures if neutral pH levels are chosen, resulting in gelatins of lower gel strength (Johnston-Banks, 1990). Nasrallah *et al.*

(1993) reported that the mild agitation can aid removal of the gelatin from the skin surface and allow faster and greater extraction of solids as shown by 8% absolute increase in gelatin recovered during extraction compared with no agitation.

Traditional extraction methods require the use of successive warm water extractions at strictly controlled temperature, varying from 55 ˚C to 90 ˚C to digest the pretreated collagenous raw material. There is a progressive decrease in gel strength for each successive extraction. On the contrary, the viscosity begins to fall each successive extraction (Hinterwaldner, 1977). Gelatin is extracted over a 16-20 hour period starting at 40 ˚C and increasing to 80 ˚C over this time. Kaewruang *et al.* (2013) achieved gelatin from the skin of unicorn leatherjacket extracted at different temperatures (65 and 75 ˚C) for various times (9, 12 and 15 h) and found that yield and recovery increased with increasing temperatures and times. Kittiphattanabawon *et al.* (2010) also reported that higher yields were obtained for gelatin extracted from shark skin when temperature increased from 45 to 75 °C.

After the extraction, there are left-over materials consisting of skin, fats and other unextracted collagen fibers, which are referred to as “scutch” and is left in the extractors. The gelatin solution is then filtered to remove small fibers and insoluble particles. The gelatin solution is then further concentrated by a secondary evaporation stage. A para-flash evaporator unit is used for the secondary evaporation stage. This gives a 30-38% concentrated gelatin solution and is treated to give a pH of 5.0 in the final product (Wittich, 2005).

Cole and McGill (1988) carried out gelatin extraction from bovine skin using 0.57 M NaOH / 0.7 M Na_2SO_4 for 4 days at 21 to 22 ˚C. The pretreated skin was then converted to gelatin by sequential extraction for 5 h in water (45, 50 and 55 ˚C) and finally by boiling (93 ˚C) for 7 h from bovine skin with varying ages (6, 18 and 60 months). At extraction temperature of 45 ˚C, the yield was 42.5, 26.8 and 10.3, respectively, and gel strength was 300, 275 and 238 g, respectively. It was regarded as the high bloom level (Sarbon *et al.*, 2013). Cole and Roberts (1997) soaked bovine skin, which is the ages of 18 months in 0.54 M $CaOH_2$/0.026 M Na_2S for 1-6 weeks at a controlled temperature of 22 °C. Thereafter, the skin was washed

well and acidulated in 0.1 M H_2SO_3 (sulphurous acid). The gelatin was then extracted by warming the skin in water, in a water-bath at controlled temperatures starting at 45 °C for the first extraction. It was found that the extractable gelatin continually increased by 3.3-39.5% yield. Yield and bloom in various conditions of extraction from bovine skin as shown in Table 4. Barbooti *et al.* (2008) carried out gelatin extraction using bovine skin soaked in 10 % $Ca(OH)_2$ solution for 5 weeks. The extraction was conducted in four stages using hot water. Furthermore, bovine split wastes soaked with 3-4% lime and 0.5% NaOH for 2 weeks were used for gelatin extraction. Gelatin was extracted about five times from 70 to 90 °C at a 5°C interval. Resulting gelatin had molecular weight distribution of gelatin less than 300 kDa with wide distribution and the pI of gelatin was 4.88 (Zhang *et al.*, 2006). Duerr and Earle (1974) comparatively extracted gelatin from bovine bone using 0.1 M KCl, 0.1 M NaOH and tap water. It was found that the highest yield (0.38% on wet weight basis) was observed when 0.1 M NaOH was used, followed by those from 0.1 M KCl and tap water (0.09%, wet weight basis).

Alkaline pretreatment has been also implemented for several fish skin, prior to gelatin extraction. Cho *et al.* (2006) studied the effects of $Ca(OH)_2$ on properties of gelatin from skate (*Raja Kenojei)*. Skin was soaked in $Ca(OH)_2$ at different levels (0.5, 1, 1.5 and 2 %) for 2 days at 4 °C and gelatin was extracted at 50 °C for 3 h. It was found that 1.5% $Ca(OH)_2$ pretreatment rendered gelatin with the highest yield and gel strength. Jamilah *et al.* (2011) reported that yield of gelatin (wet weight basis) from skins of red tilapia, walking catfish and striped catfish were 12.92, 13.06 and 11.17, respectively, when the skins were pretreated with $Ca(OH)_2$ at the concentration of 27 g/L, at 20 °C for 14 days before extraction. For gelatin from catfish skin, it was extracted from skin soaked with 10 volumes (v/w) of 1 g/L $Ca(OH)_2$ solution for 68 h. The extraction temperature was 43.2 °C and the extraction time was 5.73 h. The resulting gelatin showed a higher gel strength (276 g), compared with porcine skin gelatin (Liu *et al.*, 2008).

Table 4 Yield and bloom in various conditions of extraction from bovine skin with varying ages

Animal age (months)	Conditions	Extraction yield (%)			Bloom (g)			References
		1st	2nd	3th	1st	2nd	3th	
6	0.575 M NaOH/ 0.7 M Na_2SO_4 for 4 days, sequentially extracted (45, 50 ˚C) for 5 h	45.2	31.4	-	300	311	-	Cole and McGill (1988)
10	0.540 M $Ca(OH)_2$/0.026 M Na_2S for 2 weeks, sequentially extracted (45, 50, 55 ˚C) for 5 h	35.3	26.6	20.9	326	313	286	Cole and Roberts (1996)
18	0.575 M NaOH/ 0.7 M Na_2SO_4 for 4 days, sequentially extracted (45, 50, 55 ˚C) for 5 h	35.3	26.6	20.9	310	324	299	Cole and McGill (1988)
18	0.540 M $Ca(OH)_2$ /0.026 M Na_2S for 4 weeks, sequentially extracted (45, 50, 55 ˚C) for 5 h	22.5	28.4	27.7	310	324	299	Cole and McGill (1988)
60	0.575 M NaOH for 4 days, sequentially extracted (45, 50, 55 ˚C) for 5 h	10.3	17.3	21.4	283	279	259	Cole and Roberts (1997)
-	15% $Ca(OH)_2$ for 4 weeks	-	-	-	-	-	-	Shin and Song (1999)
-	1.5 g/l of Esperase protease, pH 9 for 17 h and extracted at 70 ˚C for 3 h	46.6	-	-	-	-	-	Petersen and Yates (1977)

- : Data not available; extraction temperatures of 45, 50 and 55 ˚C for first, second and third extraction, respectively.

1.2.5.4 Clarification of gelatin

The turbidity and dark color of gelatin is commonly caused by inorganic, protein and mucosubstance contaminants, introduced or not removed during its extraction (Zarai *et al.*, 2012). Gelatin manufacture generally has a good process to clarify the impurities from the gelatin solution, such as chemical clarification and filtration processes. Both color and clarity of gelatin gel are important aesthetic properties, depending on the application for which the gelatin is intended. Diatomaceous earth, perlite, activated carbon and cellulose filter were commonly used for clarification of gelatin (Schrieber and Gareis, 2007). For gelatin production associated with low molecular weight, microfiltration technique was used instead of filter or centrifugation (Schrieber and Gareis, 2007).

A) Removal of particles and dyes

Diatomaceous earth is a promising source for contaminant filtration in several industries. It was used in drinking water industry as filter, separating cysts, algae and asbestos contaminated with starting water (Bhardwaj and Mirliss, 2004). For sugarcane production, diatomaceous earth could help cane syrup clearer when it was crystallized, which met consumer demand (Gil and Wright, 1994). Al-Ghouti *et al.* (2003) used diatomite for the removal of the problematic reactive dyes as well as basic dyes from textile wastewater. The surface area of diatomite was found to be 27.80 m^2/g and the pH_{ZPC} occurred around pH of 5.4. The surface charge of diatomite decreased as the pH of the solution increased. The maximum methylene blue removal from aqueous solution was achieved at basic pH of around (10–11). Diatomaceous earth with the range of 0.5-5% and activated carbon ranging from 0.1 to 5% have been employed for clarification of gelatin extracted from bovine skin or bone (Schrieber and Gareis, 2007). Cho *et al.* (2006) treated gelatin with 3% activated carbon (250-350 mesh), followed by filtering through Whatman No.1 filter paper. Gelatin solution had turbidity of 6.98 ppm. Ockerman and Hansen (1999) used 5% activated carbon as bleaching agent for gelatin solution at 55-60 °C for 4-6 h. Gelatin solution obtained was clearer.

B) Removal of ions

Inorganic substances can be formed in gelatin and varied with the type of raw material and the processes used. Pork skin gelatins contain small amounts of chlorides or sulfates. Ossein or skin gelatins contain primarily calcium salts of those acids which are used in neutralization after liming (GMIA, 2012). For the regulation, specified ash content in gelatin does not to exceed 2 %. For pharmaceutical industry, ash content was not more than 1 %. Free salt gelatin was required for photographic industry (Schrieber and Gareis, 2007). Available salt might affect physical properties of gelatin, which might contribute to its sensory properties. Gelatin contained high amount of salt has poor quality. Gelatin with high sulfate content becomes turbid when dissolved in water containing calcium due to the formation of calcium sulfate residues. To tackle this problem, ion exchange treatment may be used for demineralizing or deashing of gelatins (Schrieber and Gareis, 2007).

1.2.5.5 Drying

After clarification, gelatin is generally subjected to drying. Drying should be carried out as quickly as possible to minimize the loss of properties (Johnston-Banks, 1990). Spray-drying is a unit operation by which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. The gas generally used is air or more rarely an inert gas as nitrogen. The initial liquid feeding to the sprayer can be a solution, an emulsion or a suspension. Spray-drying produces, depending on the starting feed material and operating conditions, a very fine powder (10–50 μm) or large size particles (2–3 mm) (Gharsallaoui *et al.*, 2007). Compared to freeze-drying method, spray-drying method is costly 30–50 times cheaper (Desobry *et al.*, 1997). Spray-drying has been considered as a solution for conventional drying problems because the process has usually been proved not only efficient but also economic. However, spray-drying is considered as an energy wasting operation because it is impossible to utilize all the heat going through the drying chamber (Gharsallaoui *et al.*, 2007). Spray-drying is also largely used for encapsulation of various food ingredients, often called microencapsulation (Shahidi and Han, 1993). This technique transforms liquid feed into dry powder in a one step, continuous

particle processing operation and can be applied to a wide variety of materials (Broadhead *et al.*, 1992) as shown in Table 5. Spray-drying technique has inconveniences related to processing variables that must be well controlled to avoid difficulties such as low yields, sticking, or high moisture content. These are often encountered with laboratory scale spray-dryers (Billon *et al.*, 2000). The optimization of spray-drying process involves the evaluation of parameters concerning both spray-dryer and feed formulation (Bhandari *et al.*, 2008). Gelatin extracted from shark cartilage was dried using three different drying methods, including freeze drying, hot-air drying and spray drying (Kwak *et al.*, 2009). Freeze-dried gelatin had the highest gel strength and foam forming ability, but the foam stability was lowest. Nonetheless, spray-dried gelatin showed the highest emulsion ability. Gudmundsson and Hafsteinsson (1997) evaporated gelatin solution from cod skin using vacuum at 43-45 °C until 85-90% of water was removed. The solution of concentrated gelatin was then air dried at 45 °C to eliminate the remaining water. Another portion of gelatin solution was freeze-dried. Freeze-dried gelatin had notably higher gel strength value than air-dried counterpart. Air drying was prone to protein degradation than freeze-drying, thereby lowering gel forming properties (Gudmundsson and Hafsteinsson, 1997).

1.2.6 Functional and physical properties of gelatin

The important properties of gelatin are gel strength, viscosity, gelling and melting points. These properties are affected by many factors, such as molecular weight distribution, concentration of the gelatin solution, gel maturation time, gel maturation temperature, pH and salt content (Karim and Bhat, 2009). The most remarkable characteristics of gelatin are its solubility in water and ability to form thermally reversible gels (Simon *et al.*, 2002).

The physiochemical and functional properties of fish gelatin have been studied extensively (Ahmad and Benjakul, 2011; Gómez-Estaca *et al.*, 2009; Karim and Bhat, 2009). The source and type of collagen influence the properties of the resulting gelatins. Generally, fish gelatins have the lower concentration of imino acids (proline and hydroxyproline), compared to mammalian gelatins. Warm water fish gelatins such as bigeye-tuna and tilapia contain a higher imino acid content than

Table 5 Experimental conditions optimized for different foods by spray-drying

Samples	Inlet temperature (°C)	Out temperature (°C)	Hot air flow rate	Feed flow	References
Chicken meat protein hydrolysate	120-200	88.8-128.6	0.6 m^3/h	0.10–0.38 kg/h	Kurozawa *et al.* (2009)
Microencapsulated eggs powder with gelatin, lactose, pullulan, and their mixtures	171.8	72.5	1.54 m^3/min	NR	Koç *et al.* (2011)
Gelatin microparticles contaminating propolis extractive solution	140-160	NR	NR	4-6 %	Bruschi *et al.* (2003)
Casein hydrolysate encapsulated with soy bean protein isolate	140	110	NR	3.6 L/h	Ortiz *et al.* (2009)
Emulsion made of whey protein, lactose and soybean oil	180	78-90	0.8 m^3/min	15 mL/min	Fäldt and Bergenståhl (1996)
Whey protein hydrolysate encapsulated with moltdextrin or maltodextrin/β-cyclodextrin mixture	200	90 ± 5	0.7 m^3/h	1000 ml/ h	Yang *et al.* (2012)

NR: not report

gelatin from cold-water fish such as cod, whiting and halibut. The imino acid contents are approximately 22-25% in warm water fish gelatin and 17% in cold water fish gelatin (Núñez-Flores *et al.,* 2013). Apart from amino acid composition, the properties of gelatin are also influenced by the distribution of the molecular weights, structure and compositions of its subunits. Generally, gel strength and gel melting point are the major physical properties of gelatin gels. The lower content of imino acid in fish gelatin gives the low gel strength, low gelling and melting temperature (Haug *et al.,* 2004)**.**

1.2.6.1 Solubility

Solubility is a prerequisite for other functional properties of proteins or peptides. Soluble proteins provide a homogeneous dispersibility of the molecules in colloidal systems and enhance the interfacial properties (Thiansilakul *et al.,* 2007; Zayas, 1997). Gelatin with high solubility, especially in the wide pH range (1-10) can be used widely and effectively. Gelatin swells upon contact with cold water forming large visible swollen particles. When heated above the melting point, the hydrated gelatin will rupture and go into solution, and form a gel upon cooling. Gelatin is practically insoluble in alcohol and non-polar solvents such as sorbitol, mannitol and glycerine (Jamilah and Harvinder, 2002). Kittiphattanabawon *et al.* (2012) reported that the solubility of gelatin from shark skin extracted at higher extraction temperatures was generally higher than that of gelatin extracted at lower temperatures, in which the lowest solubility of the resulting gelatins was observed at pH about 8–9 Benjakul *et al.* (2009) reported that solubility of gelatin extracted from bigeye snapper skin with two species, including *Priacanthus tayenus* and *Priacanthus macracanthus* was greater than 90% at all pHs tested (1-10) and gelatin from both species showed the lowest solubility at pH 8.0.

1.2.6.2 Gelation

The rheological properties of thermo reversible gelatin gels are primarily a function of temperature (below the melting point of the gel) and the concentration of gelatin for a given gelatin type (Zhou *et al.,* 2006). The gel converts

to a solution as the temperature rises to 30°C to 40°C, thus gelatin gels tend to melt in the mouth (Morimura *et al.*, 2002). This is the desirable properties in ready to eat food such as clear dessert jellies and marshmallows. The well accepted mechanism of gelatin gelation is the random coiled helix reversion. The amino acid, which is rich regions of the different polypeptide chains serve as potential junction zones. Upon cooling, they take up a helical conformation resulting in the three-dimensional gel (Nishimoto *et al.*, 2005). Upon controlled cooling below the melting temperature, T_m, the reformation of the helical form occurs (Wong, 1989). The energy barrier for folding is ~ 4 kJ/mol. The initial refolding is rapid and involves the Gly-I-I- regions of the polypeptide chain, forming a single turn of a left-handed helix. This nucleation along the polypeptide chain is structurally stabilized by a certain type of water bridging. The nucleated polypeptide then (1) folds back into loops, with the nucleated regions aligned to form triple strands, or (2) has its nucleated region aligned with that of the other nucleated polypeptide chain. At high enough concentrations, interchain alignment becomes possible and association of polypeptide chains to form triple-helical collagen molecules can occur (Wong, 1989).

Gel strength properties are related to the α- and β-chain components in the gelatin. Cho *et al.* (2006) reported that gel strength is governed by molecular weight, amino acid composition and the ratio of α/β-chain presents. The gel strength, which is also related to viscosity, is an important property in the food industry as it is a good guide to the behavior of the gel. Liu *et al.* (2008) reported that gelatin with more α chain content and having molecular weight of approximately 100,000 g/ mol showed the higher gel strength. On the other hand, a high ratio of peptides with molecular weights lower than the α-chains decreased the gel strength.

1.2.6.3 Gelling and melting temperature

Gelatin gels will start melting when the temperature increases above a certain point, which is called the gel melting point, and is usually lower than human body temperature (Karim and Bhat, 2009; Kim and Mendis, 2006). This melt-in-the-mouth property has become one of the most important characteristics of gelatin gels, and is widely exploited in the food and pharmaceutical industries. The melting point

is the temperature at which a gelatin gel softens sufficiently and allowing carbon tetrachloride drops to sink through it. Factors such as the maturing temperature and the concentration of the gelatin gel tend to affect its melting point (Gómez-Guillén *et al.*, 2002). The main differences in the properties of mammalian and fish gelatins are that fish gelatins have lower gelling and melting temperatures, but relatively higher viscosities (Leuenberger, 1991). Typical gelling and melting points for porcine and bovine gelatins range from 20 to 25 ˚C and 28 to 31 ˚C, respectively while typical gelling and melting points for fish gelatins were in the range from 8 to 25 ˚C and 11 to 28 ˚C, respectively (Karim and Bhat, 2009). The wide range of gelling temperatures is greatly influenced by the origin of the raw material used in the process. According to Gilsenan and Ross-Murphy (2000), the rheological properties and melting points of mammalian gelatin with gelatin fish from different types were compared, in which gelatins from cold-water fish with a much higher critical concentration and lower melting point than mammalian samples were observed, due to the lower imino acid contents.

1.2.4.4 Emulsifying and foaming properties

Gelatin is used as a foaming, emulsifying, and wetting agent in food, pharmaceutical, medical, and technical applications due to its surface-active properties. Gelatin with surface-active is capable of acting as an emulsifier in oil-in-water emulsions (Lobo, 2002). The hydrophobic areas on the peptide chain are responsible for giving gelatin its emulsifying and foaming properties (Cole, 2000; Galazka *et al.*, 1999). However, gelatin is generally a weaker emulsifier than other surface-active substances such as globular proteins and gum arabic. When used on its own, gelatin often produces relatively large droplet sizes during homogenization (Chesworth *et al.*, 1985; Dickinson and Lopez, 2001). Gelatin was either hydrophobically modified by the attachment of nonpolar side-groups (Toledano and Magdassi, 1998), or used in conjunction with anionic surfactants to improve its effectiveness as an emulsifier (Müller and Hermel, 1994; Olijve *et al.*, 2001; Surh *et al.*, 2005). In some products, its surface active and film-forming characteristics can be successfully exploited during the emulsification process (Kläui *et al.*, 1970). For marshmallows, the gel-forming properties of gelatins are used to stabilize the foam

upon cooling. In most applications, gelatin is chosen not only for its surface-active properties, but rather because of its unique combination of surface active, chemical, rheological, and gelling properties.

Several factors have been known to govern interfacial properties. Gelatin concentration and the sources of raw material determine the property of gelatin. Aewsiri *et al.* (2008) stated that higher emulsifying and foaming properties of gelatin from precooked tuna fin increased with increasing gelatin concentration. On the contrary, the emulsifying capacity of gelatin from bigeye snapper and unicorn leatherjacket decreased when the gelatin concentration increased (Binsi *et al.*, 2009; Kaewruang *et al.*, 2013). In addition, Aewsiri *et al.* (2009) studied the emulsifying properties, emulsion activity index (EAI) and emulsion stability index (ESI), foaming properties, foam expansion (FE) and foam stability (FS) of gelatin from cuttlefish skin with and without bleaching using H_2O_2. Emulsion with gelatin from bleached dorsal and ventral skin was more stable, compared with those of gelatin without bleaching. With the extended bleaching time and higher H_2O_2 concentration, the lower ESI of gelatin all gelatin samples was obtained, except for gelatin from dorsal skin, in which the highest ESI was noted when the skin was bleached with 5% H_2O_2 for 48 h ($p < 0.05$). Compared with low molecular weight fish gelatin (~ 50 kDa), emulsion containing high-molecular weight counterpart (~ 120 kDa) was more stable (Surh *et al.*, 2006). For foam- forming ability, unbleached skin gelatin, both from dorsal and ventral skin, showed a slightly lower FE than that from bleached skin, while bleaching had no impact on the FS of gelatin from ventral skin, but the highest FS was found in gelatin from dorsal skin bleached with 5% H_2O_2 for 48 h (Aewsiri *et al.*, 2009). Jongjareonrak *et al.* (2010) found that foam capacity and foam stability of gelatin from farmed giant catfish were higher, compared with those of gelatin from calf skin. Shark cartilage and precooked tuna fin gelatins had the lower foam capacity and foam stability than gelain from porcine skin.

1.3 Objectives

1.3.1 To study the effect of alkaline concentration and curing time during pretreatment of goat skin on characteristics and properties of resulting gelatin

1.3.2 To study the impact of sodium sulfate and hydrogen peroxide used during pretreatment on yield and properties of resulting gelatin

1.3.2 To investigate the impact of extraction condition on yield and properties of gelatin from goat skin

1.3.3 To elucidate the effect of spray-drying condition on characteristics and properties of gelatin from goat skin

1.3.4 To study characteristics and functional properties of gelatin from goat skin obtained from different drying methods

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CHAPTER 2

CHARACTERISTICS AND GEL PROPERTIES OF GELATIN FROM GOAT SKIN AS INFLUENCED BY ALKALINE-PRETREATMENT CONDITIONS

2.1 Abstract

Characteristics and properties of gelatin from goat skin pretreated with NaOH solutions (0.50 and 0.75 M) for various times (1-4 days) were investigated. All gelatins contained α–chains as the predominant component, followed by β-chain. Gelling and melting temperatures of those gelatins were 23.02-24.16 and 33.07-34.51 °C, respectively. Gel strength of gelatins increased as NaOH concentration and pretreatment time increased ($p < 0.05$). Pretreatment for a longer time yielded the gelatin with the decrease in L^*-value but increase in b^*-value. Pretreatment of goat skin using 0.75 M NaOH for 2 days rendered the highest yield (15.95%, (wet weight basis)) as well as high gel strength (222.42 g), which was higher than bovine gelatin (199.15 g). Gelatin obtained had the imino acid content of 226 residues/1000 residues and the gelatin gel had the fine and ordered structure. Therefore, goat skin gelatin could be used as a potential replacer of commercial gelatin.

2.2 Introduction

Gelatin is the protein obtained by thermal denaturation or partial hydrolysis of collagenous materials (Mohtar *et al.*, 2010). It has many applications in food and non-food industries. In food industry, gelatin is one of the water soluble polymers that can be used to improve stability and consistency of food. For medical and pharmaceutical industries, it can be used to produce soft and hard capsules, wound dressing and adsorbent pads (Widyasari and Rawdkuen, 2014). Gelatin is mainly produced from bovine and porcine skins and demineralized bones (Mohtar *et al.*, 2010). The global demand of gelatin for food and non-food applications was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018 (Sheela, 2014). Different types and sources of materials may yield gelatin with varying properties associated with different amino acid compositions. Gelatin with high imino acid (proline and hydroxyproline) has relatively high gel strength (Cho *et al.*, 2006),

due to high hydrogen bonding in gel network (Arnesen and Gildberg, 2007). Gelatin from mammalian skin generally has higher gel strength than fish gelatin (Benjakul *et al.*, 2012). Mammalian gelatin typically has a Bloom value with the range of 200-240 g, which is regarded as high Bloom (Karim and Bhat, 2009).

Alkaline pretreatment (liming process) is particularly established for gelatin extraction from mammalian skins and bones, which normally takes a few days to four months, depending on the type and concentration of lime used (Schrieber and Gareis, 2007). The obtained gelatin is considered as "type B" (Hinterwaldner, 1977). Alkaline solution plays an important role in breaking cross-links in the skin matrix stabilized by strong bonds, thereby increasing extraction efficiency (John and Courts, 1977). NaOH is normally preferred due to its ability to regulate the desired alkalinity and the pretreatment is complete in a very short time (John and Courts, 1977). Nevertheless, the yield and gel strength of gelatin are governed by liming conditions. Gel strength and viscosity of gelatin are governed by alkaline type and concentration as well as curing time (Jamilah *et al.*, 2011; Stainsby, 1977). Stronger liming condition normally renders the gelatin with higher viscosity, but generally leads to the lower yield (Schrieber and Gareis, 2007).

Although gelatin has a wide range of applications, the pessimism and strong concerns still persist among consumers, mainly due to religious sentiments (Sinthusamran *et al.*, 2014). Both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products (Badii and Howell, 2003). Poultry-related product has been also concerned in terms of avian influenza. Therefore, the extraction of gelatin from alternative mammals, especially by-products from goat slaughtering, e.g. skin or bone, should be taken into consideration.

Goat is one of economically important animals raised in Thailand for their meat and milk. The number of goats increased from 130,904 heads in 1998 to 491,779 heads in 2012 and is still gradually increasing (Department of Livestock Development, 2014). The number of goats increased annually since their meat has become promising for consumption, especially for Muslims. When goats are

slaughtered, by-product including skin accounts for 6.4-11.6% (based on the body weight) (Warmington and Kirton, 1990). The skin can be used as an alternative raw material for gelatin extraction. However, a little information regarding gelatin from goat skin has been reported. Extraction under the optimized condition is a means to bring about gelatin from goat skin with high yield and quality equivalent or superior to commercially available gelatin. The present study aimed to investigate the impact of alkaline pretreatment conditions on characteristics and gel properties of gelatin from goat skin obtained from the slaughter house in the southern part of Thailand.

2.3 Materials and Methods

2.3.1 Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulfate (SDS), Coomassie blue R-250 and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were obtained from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the bloom strength of 150-250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

2.3.2 Collection and preparation of goat skin

Skins from Anglo-Nubian goats with the age of approximately 2 years were collected from a local slaughter house in Chana district, Songkhla province, Thailand. Seven kilograms of goat skins were randomly taken from three goats, pooled and used as the composite sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice (a skin/ice ratio of 1:2, w/w) and transported to the Department of Food Technology, Prince of Songkla University, within 2 h. Upon arrival, the skins were cleaned and washed with running water (26-28 ˚C). Prepared skins were then cut into small pieces (2.5×2.5 cm^2) using knives, placed in polyethylene bags and stored at -20 ˚C until use. The storage time was not longer than 2 months.

2.3.3 Alkaline-pretreatment of goat skin

Prepared skins were soaked in NaOH solution (0.50 M and 0.75 M) with a skin/solution of 1:10 (w/v) at 25 ˚C. During pretreatment, the mixtures were stirred manually twice a day. Pretreatment was performed for 1, 2, 3 and 4 days. At every sampling time, the solutions were removed and replaced by the same volume of fresh solution. The treated skins were washed with running water until the pH of wash water became neutral or slightly alkaline. During pretreatment and washing, hair and other non-collagen materials released from the skin were removed. All pretreated skins were subjected to gelatin extraction.

2.3.4 Extraction of gelatin

To extract gelatin, the pretreated skins were placed in distilled water at 60 ˚C with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 5 h with a continuous stirring at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The mixture was then filtered using two layers of cheesecloth. The filtrate was further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea). The resultant filtrate was freeze-dried using a ScanvacModel Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). All gelatins were subjected to analyses.

2.3.5 Analyses

2.3.5.1 Yield and recovery

The yield of gelatin was calculated by the following equations:

$$\text{Yield (\%)} = \frac{\text{Weight of dried gelatin (g)} \times 100}{\text{Weight of initial skin (g)}}$$

Recovery (%) = [hydroxyproline content of filtrate (g/ml) × volume of filtrate (ml)]/[hydroxyproline content of initial skin (g/g) × weight of initial skin (g)]×100

Hydroxyproline content was determined according to the method of Bergman and Loxley (1963).

2. 3.5.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed following the method of Laemmli (1970). Gelatin samples were dissolved in 5% SDS and the mixtures were incubated at 85 ˚C for 1 h. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris HCl, pH 6.8. containing 4% SDS and 20% glycerol). The mixtures were boiled in boiling water for 2 min. Samples (15 µg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis. Gels were stained with 0.05% (w/v) Coomassie blue R-250 in 15 % (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% acetic acid. High MW markers were used for estimation of MW.

2.3.5.3 Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated l-alanine tri-glycine sulfate (DLATGS) detector. The horizontal attenuated total reflectance accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–650 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were rationed against a background spectrum recorded from the clean empty cell at 25 °C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

2.3.5.4 Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of Boran *et al.* (2010) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). Gelatin solution (6.67%, w/v) was prepared in the same manner as described previously. The solution was preheated at 35 °C for 30 min. The measuring geometry used was 3.5 cm parallel plate and the gap was set at 1.0 mm. The measurement was performed at a scan rate of 0.5 °C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 50 to 5 °C and heating from 5 to 50 °C. The gelling and melting temperatures were designated, where tan δ became 1 or δ was 45°.

2.3.5.5 Determination of gel strength

Gelatin gel was prepared as per the method of Kittiphattanabawon *et al.* (2010). Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis.

Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.3.5.6 Microstructure analysis of gelatin gel

Microstructure of gelatin gel (6.67%, w/v) was visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100 % (v/v). The samples were subjected to

critical point drying. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 15 kV.

2.3.5.7 Determination of color of gelatin gel

The color of gelatin gels (6.67% w/v) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated according to the following equation (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard (L^* = 93.63, a^* = -0.94 and b^* = 0.40).

2.3.5.8 Amino acid analysis

Amino acid composition of the selected gelatin sample was analyzed according to the method of Nagarajan *et al.* (2012) with a slight modification. The sample was hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115 °C for 24 h. The hydrolysate was neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

2.3.6 Statistical analysis

All experiments were run in triplicate using three different lots of skins. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the Duncan's multiple range test (Steel *et al.*, 1980). For pair

comparison, T-test was used. Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

2.4 Results and Discussion

2.4.1 Yield of extraction

The yields and recovery of gelatin extracted from goat skin pretreated using various NaOH concentrations are shown in Table 6. Gelatin from skin pretreated with NaOH solution at both concentrations (0.5 M or 0.75 M) had the increasing yield as the pretreatment times increased ($p < 0.05$). Nevertheless, gelatin from skin pretreated with 0.5 M NaOH for 4 days had the lower yield, compared with those pretreated for 2 and 3 days ($p < 0.05$). On the other hand, skins were not swollen when it was pretreated with 0.5 M NaOH for only 1 day (data not shown). For gelatin extracted from the skins pretreated using 0.75 M NaOH, the lower yield was obtained when pretreatment time was longer than 2 days (data not shown). Under the harsh alkaline condition, the repulsion between protein chains in skin matrix became more pronounced. As a result, solubilization was augmented and the loss of gelatin was obtained. The use of weak alkaline condition for a short time was not sufficient to destroy the cross-links in the skin matrix. In general, the higher yield was obtained when the skins were pretreated with 0.75 M NaOH, in comparison with that of 0.5 M NaOH. Yields of 11.38 - 13.23% and 11.60 - 15.95% (on wet weight basis) were found for gelatin extracted from skin pretreated with NaOH at concentrations of 0.5 M and 0.75 M, respectively. The recovery of gelatin from skin pretreated with NaOH at 0.5 M and 0.75 M were 22.50 - 30.26 % and 27.40 – 34.16 %, respectively. The highest yield from goat skin (15.95%) was obtained when the pretreatment of skin was carried out using 0.75 M NaOH for 2 days ($p < 0.05$). Jamilah *et al.* (2011) reported that yield of gelatin (wet weight basis) from skins of red tilapia, walking catfish and striped catfish were 12.92, 13.06 and 11.17, respectively, when alkaline pretreatment was used before extraction. Cho *et al.* (2006) reported that the highest extraction yield (16.80%) of gelatin from skate skin was found when the skin was pretreated with 1.5% calcium hydroxide ($Ca(OH)_2$) for 2 days. The different yields might be due to different skin matrixs between mammals and fish as well as gelatin

extraction process including pretreatment (Kittiphattanabawon *et al.*, 2010; Nagarajan *et al.*, 2012). Goat skin had the complex structures with strong fibrils, compared with those from fish skin. As a result, the harsher pretreatment conditions were required. The result suggested that higher alkaline concentration used for pretreatment more likely destroyed the covalent bond stabilizing the cross-links in the skin matrix. As a consequence, intermolecular cross-links between collagen fibrils were destabilized and could be extracted into the medium with ease, leading to the higher yield (Hinterwaldner, 1977). With longer pretreatment time, those bondings were more disrupted, in which free α-chains or β-chain was more released from skin complex during extraction.

2.4.2 Protein patterns

Protein patterns of gelatin from goat skin pretreated with NaOH at different concentrations for various times are illustrated in Figure 7. All gelatin samples contained α–chains as the major component. β- chain was also found in all samples. MWs of α_1- and α_2-chains were estimated to be 131 and 124 kDa, respectively. β- chain, a dimer, had MW of 216 kDa. For all gelatin samples, no marked differences in protein patterns were noticeable. The result indicated that α-chains and β-chain were not degraded by pretreatment conditions. However, proteins with MW greater than β-chain were slightly higher in band intensity as the gelatin was extracted from goat skin pretreated with NaOH for a longer time, particularly with 0.75 M NaOH. For alkaline pretreatment of chanel catfish skin using 1 g/ L ($Ca(OH)_2$) for 76 h, α–chains were more degraded (Liu *et al.*, 2008). Generally, gelatins with higher content of α-chains showed the better functional properties including gelling, emulsifying and foaming properties (Gómez-Guillén *et al.*, 2002). Thus, the components including α- chains and β-chains of gelatin from goat skin were quite tolerant to alkaline pretreatment, compared with other species, particularly fish.

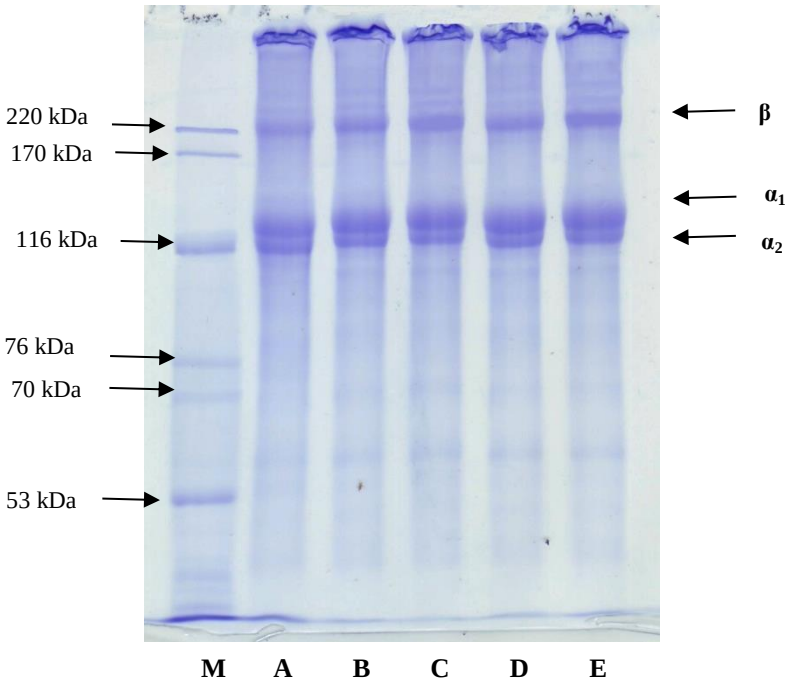

Figure 7 SDS-PAGE patterns of gelatin from goat skin pretreated with NaOH at different concentrations for various times. M denotes high molecular weight markers. A, B, C: gelatin from goat skin pretreated with 0.5 M NaOH for 2, 3 and 4 days, respectively and D, E: gelatin from goat skin pretreated with 0.75 M NaOH for 1 and 2 days, respectively**.**

2.4.3 Fourier transform infrared (FTIR) spectra

FTIR spectra of gelatin from the skins of goat pretreated with NaOH under different conditions are shown in Figure 8. In general, all gelatins showed similar spectra. Amide I band of gelatin pretreated with NaOH at concentrations of 0.5 and 0.75 M for various times appeared at 1630.42-1634.21 cm^{-1} and 1631.50-1638.30 cm^{-1}, respectively. The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the CN stretch, CCN deformation and in-plane NH bending modes (Bandekar, 1992). The absorption in the amide I region is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins (Nagarajan *et al.*, 2012). The absorption peak at amide I was characteristic for the coil structure of gelatin (Nagarajan *et al.*, 2012). When the wavenumber shifts to the lower frequencies, those functional groups might undergo interaction with some reactive groups. For amide II, it results from an out-of-phase combination of C-N

Table 6 Extraction yield, recovery, gel strength and gel color of gelatin from goat skin pretreated with NaOH at different concentrations for various times

NaOH concentration (M)	Time (days)	Yield (% wet weight basis)	Recovery (%)	Gel strength (g)	Color value			
					L^*	a^*	b^*	ΔE^*
0.5	2	13.23 ± 0.17^{Ab}	30.26 ± 0.50^{Ac}	211.71 ± 3.79^{Aab}	18.17 ± 0.50^{Ab}	2.89 ± 0.65^{Aa}	7.30 ± 0.19^{Aa}	75.85 ± 0.53^{Aa}
	3	14.79 ± 0.25^{Bc}	31.94 ± 0.55^{Bd}	216.46 ± 1.83^{Ab}	17.64 ± 0.48^{ABb}	2.80 ± 0.72^{Aa}	8.04 ± 0.38^{ABc}	76.43 ± 0.45^{Bb}
	4	11.38 ± 0.52^{Ca}	22.50 ± 0.37^{Ca}	229.51 ± 2.68^{Bd}	16.88 ± 0.24^{Ba}	2.30 ± 0.19^{Aa}	8.56 ± 0.54^{Bc}	77.22 ± 0.30^{Bb}
0.75	1	11.60 ± 0.11^{Aa}	27.40 ± 0.24^{Ab}	209.18 ± 3.05^{Aa}	17.83 ± 0.20^{Ab}	2.88 ± 0.12^{Aa}	7.16 ± 0.06^{Aa}	76.16 ± 0.20^{Aa}
	2	15.95 ± 0.08^{Bd}	34.16 ± 0.28^{Be}	222.42 ± 2.87^{Bc}	16.958 ± 0.30^{Ba}	2.81 ± 0.25^{Aa}	7.44 ± 0.08^{Bb}	77.05 ± 0.29^{Bb}

Values are presented as mean ± SD (n=3).
Different uppercase letters within the same column under the same NaOH concentration indicate significant differences ($p < 0.05$).
Different lowercase letters within the same column indicate significant differences ($p < 0.05$).

stretch and in-plane NH deformation modes of the peptide group (Bandekar, 1992). Amide II bands of all gelatin samples were noticeable at the wavenumbers of 1535.04-1541.03 cm^{-1} and 1527.25-1543.27 cm^{-1} for gelatin from skin pretreated with 0.5 M and 0.75 M NaOH, respectively. It was observed that they shifted to lower wavenumber in gelatin from skin pretreated for a longer time.

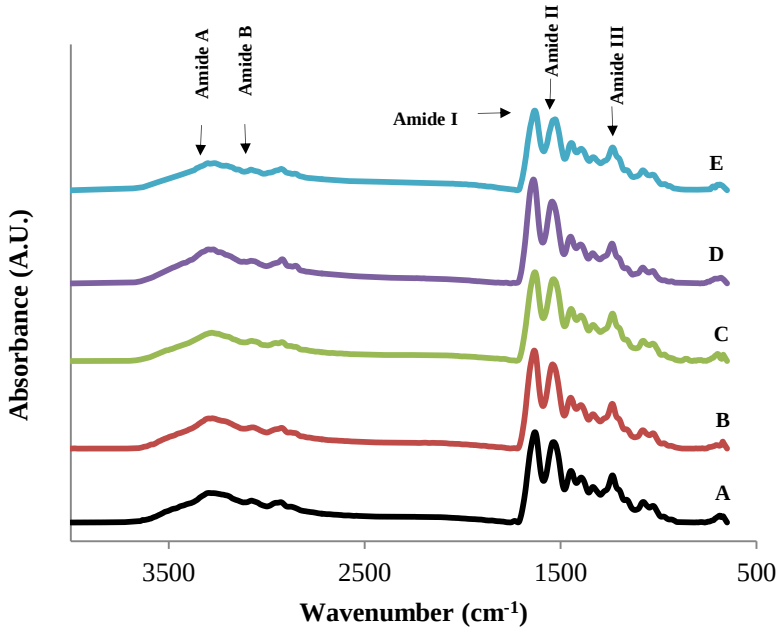

Figure 8 FTIR spectra of gelatin from goat skin pretreated with NaOH at different concentrations for various times. A, B, C: gelatin from goat skin pretreated with 0.5 M NaOH for 2, 3 and 4 days, respectively and D, E: gelatin from goat skin pretreated with 0.75 M NaOH for 1 and 2 days, respectively.

The amide III bands of all gelatin samples were observed at wavenumbers of 1234.30-1237.11 cm^{-1}, which indicated the disorder in gelatin molecules and were more likely associated with loss of triple helix state (Friess and Lee, 1996). The amide III band represents the combination peaks between CN stretching vibrations and NH deformation from amide linkages as well as absorptions taking place from wagging vibrations from CH_2 groups of glycine backbone and proline side-chains (Nagarajan *et al.*, 2012). However, no remarkable differences were observed in amide III among samples. During extraction at high temperature,

hydrogen bonds stabilizing triple helix were destroyed, leading to amorphous structure.

The amide A band of gelatin from skin pretreated with NaOH at concentrations of 0.5 and 0.75 M for various times was found at 3280.70-3295.72 cm^{-1} and 3268.89-3272.38 cm^{-1}, respectively, associated with stretching vibrations of NH group coupled with hydrogen bonding. Basically, a free NH stretching vibration is found in the range of 3400-3440 cm^{-1}. The position of this band shifts to lower frequencies because the NH group of a peptide is involved in a hydrogen bond (Nagarajan *et al.*, 2012). Gelatin from skin pretreated with NaOH at the same concentration for longer time showed the lower amplitude than other gelatin samples. Coincidentally, the shift to lower wavenumber was found in gelatin sample extracted from skin pretreated for a longer time. This might be due to free amino group released could undergo Maillard reaction to a higher extent. This could enhance the yellow color in the sample. The amide B band of gelatin from skin pretreated with 0.5 and 0.75 M NaOH for various times was found at 2922.60-2928.28 cm^{-1} and 2920.31-2925.38 cm^{-1}, respectively, corresponding to the asymmetric stretching vibration of =C-H as well as NH_3^+ (Friess and Lee, 1996). Among all samples, gelatin pretreated for longer time had the lowest wavenumber of amide-B peak. This suggested the interaction of free amino groups with other, especially, carbonyl compounds via glycation. Thus, the secondary structure and functional group of gelatins derived from skin of goat were affected by alkaline pretreatment.

2.4.4 Gelling and melting temperatures

The changes in the phase angle (δ) of gelatin solutions during cooling (50 to 5 °C) and subsequent heating (5 to 50 °C) are illustrated in Figure 9A and 9B, respectively. All gelatin samples with different pretreatment conditions formed a gel in the range of 23.02-24.16 °C. This was regarded as the sharp decrease and rapid transition in phase angle during cooling, due to the increase in amount of energy that is elastically stored in storage modulus (*G'*) (Kasankala *et al.*, 2007). It was found that gelling temperature of gelatins from skin pretreated with the same NaOH concentration increased non-significantly with increasing pretreatment time ($p >$

0.05). However, gelling temperature was not much affected by pretreatment conditions used in the present study. The gelling temperature of gelatin from goat skin was higher than those of gelatins from skin of bigeye snapper (10.0 °C) (Binsi *et al.*, 2009) and silver carp (18.7 °C) (Boran *et al.*, 2010). Moreover, gelling temperature was also higher than that of other mammal gelatin such as bovine (21.7 °C) (Kasankala *et al.*, 2007). Karim and Bhat (2009) reported that melting point and gelling point are governed mainly by the amino acid composition (pro + hyp content), molecular weight distribution and also the ratio of α/β chains contained in the gelatin. Melting points of gelatins from goat skin with various pretreatments were found in the range of 33.07-34.51 °C, which were higher than those reported for gelatin from bigeye snapper skin (16.8 °C) (Binsi *et al.*, 2009), silver skin (27.1 °C), porcine (31.5 °C) and bovine (30 °C) (Kasankala *et al.*, 2007). The high melting point in this study might be associated with the high content of imino acids (Pro and Hyp) (Kasankala *et al.*, 2007). The proline plays a crucial role in boosting the formation of polyproline II helix (Ross-Murphy, 1992). Thermal stability of gelatin gel is directly related with pro-rich regions in gelatin molecules (Gómez-Guillén *et al.*, 2002). Apart from imino content, the melting point of gelatin also increases with increasing MW (Jamilah and Harvinder, 2002). With higher melting temperature, gel could be retained for a longer time, thereby rendering the better mouth feel when consumed. The gelling and melting temperatures of gelatin depend on species used as raw material, which may have different living environments and habitat temperatures (Gómez-Guillén *et al.*, 2002). Age of animal in conjunction with cross-link formation is another important factor affecting the gelling and melting behavior of gelatin (Hinterwaldner, 1977).

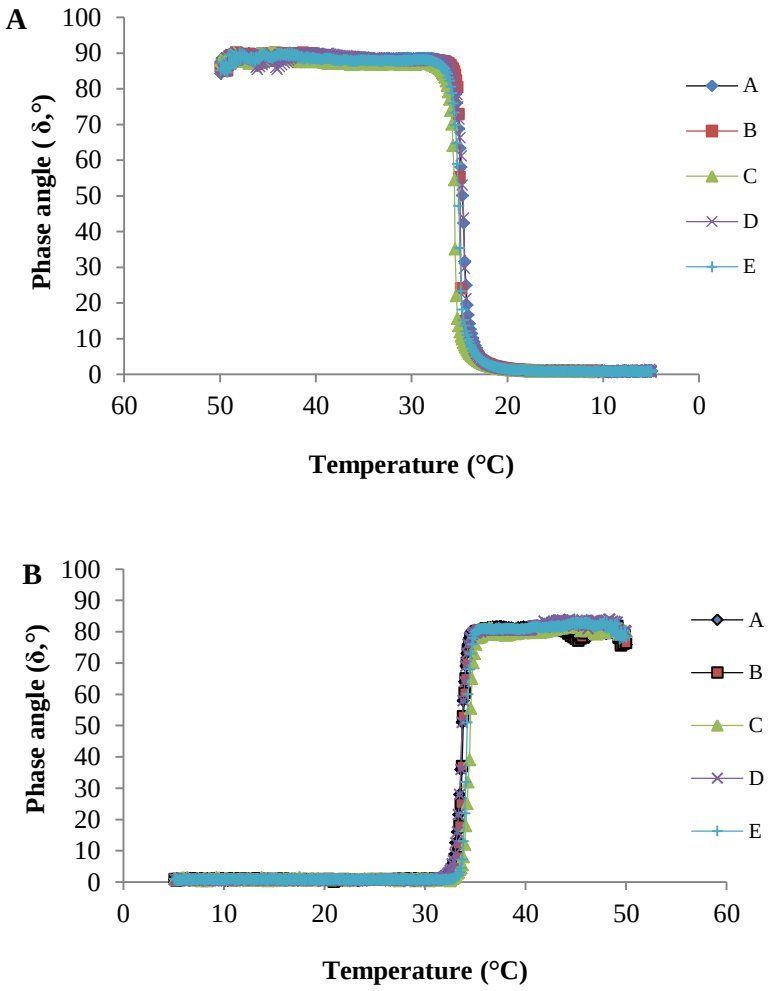

Figure 9 Changes in phase angle (δ, °) of gelatin solution (6.67%, w/v) from goat skin pretreated with NaOH at different concentrations for various times during cooling (A) and subsequent heating (B). M denotes high molecular weight markers. A, B, C: gelatin from goat skin pretreated with 0.5 M NaOH for 2, 3 and 4 days, respectively and D, E: gelatin from goat skin pretreated with 0.75 M NaOH for 1 and 2 days, respectively**.**

2.4.5 Gel strength of gelatin gel

Gel strength of gelatin gels from goat skin pretreated with NaOH solution under varying conditions is shown in Table 6. Gel strength is one of the most important functional properties of gelatins. The highest gel strength (229.52 g) was

found in gelatin from skin pretreated with 0.5 M NaOH for 4 days ($p < 0.05$), followed by that extracted from skin pretreated with 0.75 M NaOH for 2 days. Gel strength of gelatin gels more likely increased as the pretreatment times increased ($p < 0.05$). The amount of β- and γ-components of gelatin was one of the factors governing gelation of gelatin (Taheri *et al.*, 2009). However, no marked differences in α - and β - components of all gelatins were observed (Figure 7). It was observed that protein bands with MW higher than β- chain were slightly higher in intensity, when gelatin was extracted from skin pretreated using NaOH for a longer time. Those components might favor the network formation and strengthen the 3-dimentional network of gel. Goat skin gelatin had the higher gel strength (209.18-229.51 g) than bovine gelatin (199.15 g) (data not shown) ($p < 0.05$). Gelatin with different gel strength from various sources including brownbanded bamboo shark and blacktip shark (206-214 g) (Kittiphattanabawon *et al.*, 2010), seabass (282-369 g) (Sinthusamran *et al.*, 2014) and chicken feet (79-185 g) (Widyasari and Rawdkuen, 2014) were reported. Pretreatment condition and type of raw material have the influence on chemical compositions of gelatin, which directly affect the functional properties, especially gelation (Benjakul *et al.*, 2012). Molecular weight distribution was considered as a factor determining the gelling property of gelatin (Gómez-Guillén *et al.*, 2002). Thus, pretreatment process associated with both NaOH concentration and pretreatment time affected gel strength of gelatin.

2.4.6 Microstructures of gelatin gels

The microstructures of gelatin gels from goat skin with different pretreatment conditions are illustrated in Figure 10. Generally, the conformation and chain length of proteins in gel matrix directly determine the gel strength of gelatin (Benjakul *et al.*, 2009). With the same NaOH concentration, gelatin gel showed the finer network with very small voids as the pretreatment time increased. Among all samples, gelatin from skin pretreated with 0.5 M NaOH for 4 days and that extracted from skin pretreated using 0.75 M NaOH for 2 days had the fine structure. Nevertheless, the former showed the larger strands, which could be more resistant to force applied. This was evidenced by the higher gel strength (Table 6). Conversely, the larger voids were found in the gel of gelatin from skin pretreated for the shorter

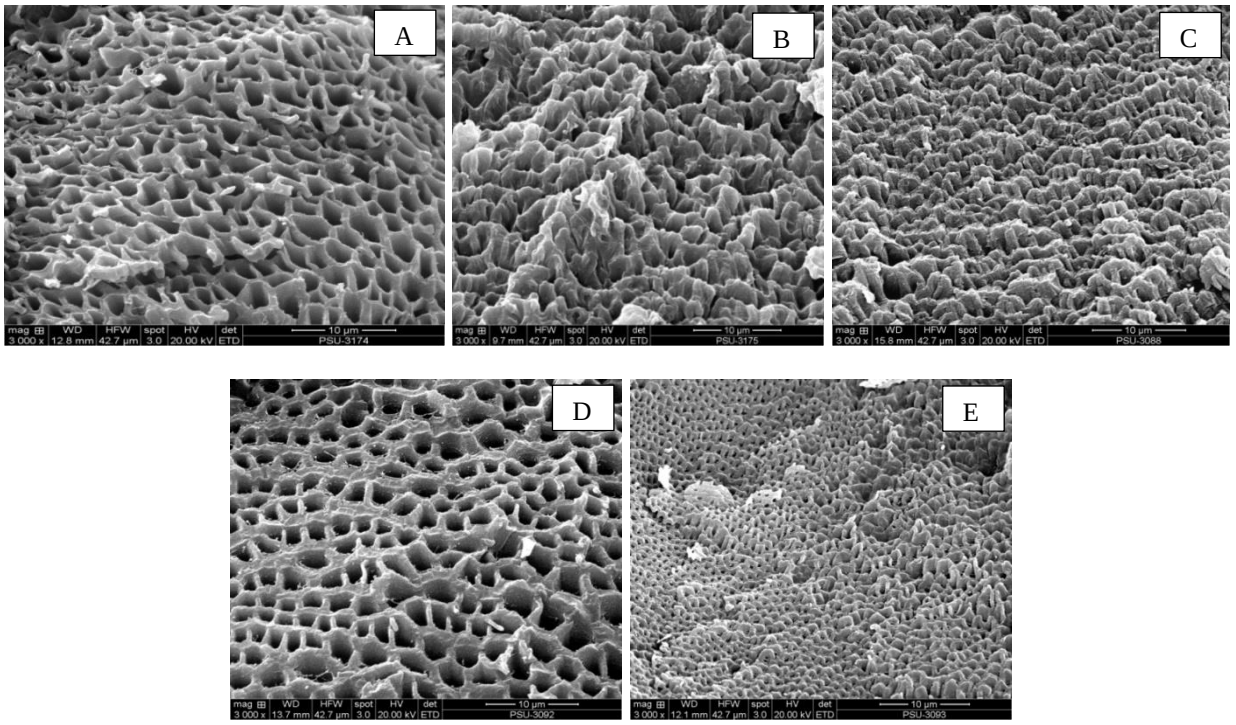

Figure 10 Microstructures of gelatin gel from goat skin pretreated with NaOH at different concentrations for various times. Magnification: 3000x. A, B, C: gelatin from goat skin pretreated with 0.5 M NaOH for 2, 3 and 4 days, respectively and D, E: gelatin from goat skin pretreated with 0.75 M NaOH for 1 and 2 days, respectively**.**

pretreatment time. The coarser gel structure of gelatin from skin pretreated for shorter time was in accordance with the lower gel strength (Table 6). The coarser gel network tended to have the lower gel strength and was easy to disrupt. It is well known that the distribution of α-, β- and γ-chains is an important factor affecting property of gelatin (Sinthusamran *et al.*, 2014). In addition, hydroxyproline is involved in gel formation of nucleation zones via hydrogen bonding through its –OH group (Kittiphattanabawon *et al.*, 2010). Yang *et al.* (2008) reported that the gelatin gel network was governed by pretreatment conditions. Therefore, the arrangement and association of gelatin molecules in the gel matrix directly contributed to gel strength of gelatin from goat skin with alkaline pretreatment.

2.4.7 Color of gelatin gel

Color of gelatin gel from goat skin with different pretreatment conditions expressed as L^*, a^* and b^* are shown in Table 6. Lightness (L^*- values) of gelatin gel decreased, while b^*- value increased when the pretreatment time increased ($p < 0.05$). Nevertheless, no differences in a^*- value were noticeable among all samples ($p > 0.05$). The increases in yellowness (b^*- value) of gelatin gel from skin pretreated for a longer time were in agreement with the shift of Amide A and B to the lower wavenumber (Figure 8). It has been known that Maillard reaction is favored at alkaline condition (Lertittikul *et al.*, 2007). With the longer pretreatment time, the reaction might occur to a higher extent. Among all gelatin samples, that from the skin pretreated at lower NaOH concentration (0.5 M) for the shortest time (2 days) showed the lowest total difference in color value (ΔE^*) (75.85) with the highest lightness (L^*- values). These results showed that the pretreatment conditions affected gel color of gelatin from goat skin.

2.4.8 Amino acid composition

Amino acid compositions of gelatin from goat skin pretreated with 0.75 M NaOH for 2 days, rendering the highest yield and gel strength, are shown in Table 7. Glycine was the major amino acid (329 residues/1000 residues). Gelatin had very low contents of cysteine (1 residues/1000 residues), tyrosine (3 residues/1000

residues) and histidine (5 residues/1000 residues). Glycine generally occurs every third position of α-chain and represents nearly one third of total residues except for 14 amino acids from N-termini and for 10 amino acids from C-termini (Benjakul *et al.*, 2012).

Table 7 Amino acid compositions of gelatin from goat skin pretreated with 0.75 M NaOH for 2 days, compared with those of bovine and porcine gelatins

Amino acids	Residue/1000 residues		
	Goat gelatin	Bovine gelatin*	Porcine gelatin**
Aspartic acid/asparagine	45	44	54
Threonine	18	17	25
Serine	32	29	41
Glutamic acid/glutamine	73	74	81
Glycine	329	341	328
Alanine	110	115	114
Cysteine	1	0	0
Valine	20	21	18
Methionine	6	5	12
Isoleucine	11	11	9
Leucine	25	25	20
Tyrosine	3	1	2
Phenylalanine	13	12	14
Hydroxylysine	6	7	8
Lysine	28	26	22
Histidine	5	5	8
Arginine	49	48	45
Hydroxyproline	99	96	70
Proline	127	123	129
Total	1000	1000	1000
Imino acids	226	219	199

*Jellouli *et al.* (2011); **Tavaklipour (2011)

For imino acids, gelatin had proline and hydroxyproline of 127 and 99 residues/1000 residues, respectively. The imino acid content of gelatin from goat skin (226 residues/1000 residues) was higher than that reported for gelatin from silver carp waste (197 residues/1000 residues) (Tavakolipour, 2011), grey triggerfish skin (176 residues/1000 residues) (Jellouli *et al.*, 2011), salmon skin (166 residues/1000 residues) and cod skin (154 residues/1000 residues) (Arnesen and Gildberg, 2007). Moreover, it was higher than that found in bovine gelatin (219 residues/1000 residues) (Jellouli *et al.*, 2011) and porcine skin gelatin (199 residues/1000 residues) (Tavakolipour, 2011).Gelatin with higher content of hydroxyproline possessed viscoelastic properties of gelatin and ability to develop the strong gel structure (Benjakul *et al.*, 2009). OH groups of hydroxyproline might be involved in hydrogen bondings with adjacent chains (Kittiphattanabawon *et al.*, 2010). This could strengthen gel network of gelatin. Additionally, alanine (110 residues/1000 residues and glutamic acid/glutamine (73 residues/1000 residues) were also found at high content. Therefore, amino acid composition could be another factor determining the properties of gelatin from goat skin.

2.5 Conclusions

The skin of goat could be a promising source of gelatin. Pretreatment condition affected the characteristic and properties of gelatin. Gelatin from goat skin contained α-chains as the major constituent. All gelatins from different pretreatment conditions showed higher gel strength than that of commercial bovine gelatin. The optimal pretreatment condition for gelatin extraction from goat skin was the use of 0.75 M NaOH for 2 days.

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CHAPTER 3

CHARACTERISTICS AND GEL PROPERTIES OF GELATIN FROM GOAT SKIN AS AFFECTED BY PRETREATMENTS USING SODIUM SULFATE AND HYDROGEN PEROXIDE

3.1 Abstract

Pretreatment of skin using Na_2SO_4 (0-1 M) increased the yield of gelatin in a concentration dependent manner. When skins with prior Na_2SO_4 treatment were bleached using H_2O_2 (0-2 M), the resulting gelatin showed the higher yield and gel strength than those without prior Na_2SO_4 treatment. All gelatins had α-chain as major components, followed by β-chain. The degradation induced by H_2O_2 was lower in gelatin with prior Na_2SO_4 treatment. *L**-values increased with increasing H_2O_2 concentrations ($p < 0.05$) due to the bleaching effect of H_2O_2. With Na_2SO_4 and H_2O_2 pretreatments, gelatin gel had finer and more ordered microstructure. The optimal pretreatment condition for gelatin extraction from goat skin included soaking the skin in 0.75 M NaOH, followed by treatment using 0.75 M Na_2SO_4 and subsequent bleaching with 2 M H_2O_2. Gelatin had the imino acid content of 217 residues/1000 residues with gelling and melting temperature of 22.49 and 32.28 °C, respectively.

3.2 Introduction

Gelatin is the water soluble protein obtained by thermal denaturation or partial hydrolysis of collagenous materials (Mohtar *et al.*, 2010). Gelatin is mainly produced from bovine and porcine skins and demineralized bones (Hao *et al.*, 2009). The global demand of gelatin for food and non-food applications was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018 (Sheela, 2014). Nevertheless, the consumption of any pork-related products is prohibited by both Judaism and Islam, while Hindus do not consume cow-related products (Badii and Howell, 2003). In addition, poultry-related products have been also concerned of bird influenza. Moreover, compared with gelatin from aquatic or marine animals, those from land animals are more stable and have better rheological characteristics (Norland, 1990).

Therefore, gelatin from alternative land animals, especially by-products from goat slaughtering, e.g. skin or bone, should be taken into account to serve for increasing demand of gelatin in the world market.

Goats are one of economically important animals raised in Thailand for their meat and milk. The number of goats increased from 130,904 heads in 1998 to 491,779 heads in 2012 and is still gradually increasing (Department of Livestock Development, 2014). The number of goats being reared has increased annually since their meat has become promising for human consumption. When goats are slaughtered, by-product including skin accounts for 64-116 g/kg (based on the body weight) (Warmington and Kirton, 1990). The skin can be used as an alternative raw material for gelatin extraction. Alkaline pretreatment (liming process) is well established for gelatin extraction from bovine skins and bones, which normally takes a few days to four months, depending on the type and concentration of lime used (Schrieber and Gareis, 2007). Alkaline pretreatment plays an important role in breaking cross-links in the skin matrix stabilized by strong bonds, thereby increasing extraction efficiency (John and Courts, 1977). Nevertheless, the skin matrix can be severely solubilized during pretreatment and washing, thereby dramatically lowering the yield (Schrieber and Gareis, 2007). To decrease the loss of gelatin during pretreatment, sodium sulfate (Na_2SO_4) has been reported to prevent over-swelling of the collagen and reduce the loss of yield (Cioca and Siegler, 1981). Additionally, gelatin from mammalian skin is brownish yellow in color, thereby limiting their uses. Hydrogen peroxide (H_2O_2) has been well known as a bleaching agent to improve the whiteness of gelatin from squid and cuttlefish skins (Aewsiri *et al.*, 2009; Nagarajan *et al.*, 2013). Recently, Mad-Ali *et al.* (2015) extracted gelatin from goat skin pretreated with 0.75 M NaOH. However, the loss during pretreatment occurred, caused by over-swelling. Additionally, the gelatin had undesirable brownish. Thus, the use of both anti-over swelling and bleaching agents for gelatin extraction from goat skin could improve both extraction yield and color of gelatin. Therefore, the objective of this study was to investigate the effects of Na_2SO_4 and H_2O_2 solution at various concentrations on yield, color and properties of the resulting gelatin from goat skin obtained from the slaughter house in the southern part of Thailand.

3.3 Materials and Methods

3.3.1 Chemicals

Sodium sulfate (anhydrous) and hydrogen peroxide were procured from Sigma (St Louis, MO, USA). High-molecular-weight markers were obtained from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the bloom strength of 150-250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

3.3.2 Collection and preparation of goat skin

Skins from male Anglo-Nubian goats with the age of approximately 2 years were collected from a local slaughter house in Chana district, Songkhla province, Thailand. Seven kilograms of goat skins were randomly taken from three goats, pooled and used as the composite sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice (a skin/ice ratio of 1:2, w/w) and transported to the Department of Food Technology, Prince of Songkla University, within 2 h. Upon arrival, the skins were cleaned and washed with running water at room temperature (26-28 ˚C). Prepared skins were then cut into small pieces (2.5×2.5 cm^2) using knives, placed in polyethylene bags and stored at -20 ˚C until use. The storage time was not longer than 2 months.

3.3.3 Alkaline pretreatment

Prepared skins were soaked in 0.75 M NaOH solution at a ratio of 1:10 (w/v). During pretreatment of 2 days, the mixtures were left at room temperature (28-30 °C) and stirred manually twice a day. The solution was removed and replaced by the same volume of freshly prepared solution. The skins were then transferred on the perforated screen to remove the solution.

3.3.4 Effect of Na_2SO_4 treatment on yield and characteristics of gelatin

Alkaline pretreated skins were mixed with Na_2SO_4 solution at various concentrations (0, 0.25, 0.5, 0.75 and 1 M) using a skin/solution of 1:10 (w/v). The

mixtures were allowed to stand at room temperature for 24 h. Subsequently, the skins were washed with running water until the pH of wash water became neutral or slightly alkaline. All pretreated skins were subjected to gelatin extraction.

3.3.5 Extraction of gelatin

To extract gelatin, the alkaline pretreated skins without and with subsequent Na_2SO_4 treatment were placed in distilled water at 60 ˚C with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 5 h with a continuous stirring at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The mixtures were then filtered using two layers of cheesecloth. The filtrates were further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea). The resultant filtrates were freeze-dried using a ScanvacModel Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). All gelatins were subjected to analyses.

3.3.6 Analyses

3.3.6.1. Yield and recovery

The yield of gelatin was calculated by the following equations:

$$\text{Yield (\%)} = \frac{\text{Weight of dried gelatin (g)} \times 100}{\text{Weight of initial skin (g)}}$$

$$\text{Recovery (\%)} = [\text{hydroxyproline content of filtrate (g m } L^{-1}) \times \text{volume of filtrate (mL)}]/[\text{hydroxyproline content of initial skin (g } g^{-1}) \times \text{weight of initial skin (g)}] \times 100$$

Hydroxyproline content was determined according to the method of Bergman and Loxley (1963).

3.3.6.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed following the method of Laemmli (1970). Gelatin samples were dissolved in 50 g L^{-1} SDS and the mixtures were incubated at 85 ˚C for 1 h. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris HCl, pH 6.8. containing 40 g L^{-1} SDS and 200 g L^{-1} glycerol). The mixtures were boiled in boiling water for 2 min. Samples (15 µg protein) were loaded onto polyacrylamide gels comprising a 75 g L^{-1} running gel and a 40 g L^{-1} stacking gel and subjected to electrophoresis. Gels were stained with 0.5 g L^{-1} Coomassie blue R-250 in 150 mL L^{-1} methanol and 50 mL L^{-1} acetic acid and destained with 300 mL L^{-1} methanol and 100 mL L^{-1} acetic acid. High MW markers were used for estimation of MW.

3.3.6.3 Determination of gel strength

Gelatin gel was prepared as per the method of Nagarajan *et al.* (2013). Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67%. The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis. Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

3.3.6.4 Determination of color of gelatin gel

The color of gelatin gels (6.67%) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated according to the following equation(Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard (L^* = 93.63, a^* = -0.94 and b^* = 0.40).

Treatment using Na_2SO_4 solution at the concentration rendering the highest yield, recovery and gel strength was selected for further study.

3.3.7 Effect of H_2O_2 on yield and characteristics of gelatin

After washing, alkaline pretreated skins without and with subsequent Na_2SO_4 treatment were soaked in H_2O_2 solution with different concentrations (0, 0.5, 1, 1.5 and 2 M). The mixtures were stirred gently at 4 °C for 24 h. H_2O_2 solutions were changed every 12 h. All skin samples were then washed thoroughly using tap water. The prepared skin was subjected to extraction of gelatin as mentioned above. All gelatins were analyzed as previously described.

Treatment using H_2O_2 solution at the concentration yielding the highest yield, recovery and gel strength as well as whiter color was selected for further characterization.

3.3.8 Characterization of the selected gelatin

Gelatin prepared from goat skin with different pretreatments including 1) skin treated with 0.75 M NaOH, 2) skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4 and 3) skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4 and 2 M H_2O_2. All samples were subjected to following analyses.

3.3.8.1 Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated l-alanine tri-glycine sulphate (DLATGS) detector. The horizontal attenuated total reflectance accessory (HATR) was mounted into the sample compartment. The

internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–650 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean empty cell at 25 °C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

3.3.8.2 Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were determined following the method of Boran *et al.* (2010) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). Gelatin solution (6.67%) was prepared in the same manner as described previously. The solution was pre-heated at 35 °C for 30 min. The measuring geometry used was 3.5 cm parallel plate and the gap was set at 1.0 mm. The measurement was performed at a scan rate of 0.5 °C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 50 to 5 °C and heating from 5 to 50 °C. The gelling and melting temperatures were designated, where tan δ became 1 or δ was 45°.

3.3.8.3 Amino acid analysis

Amino acid composition of the selected gelatin sample was analyzed according to the method of Nagarajan *et al.* (2012) with a slight modification. The sample was hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 2 ml L^{-1} 3-2(2-aminoethyl) indole at 115 °C for 24 h. The hydrolysate was neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

3.3.8.4 Microstructure analysis of gelatin gel

Microstructure of gelatin gel (6.67%, w/v) was visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2-3 mm were

fixed with 25 mL L^{-1} glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 500, 700, 800, 900 and 1000 ml L^{-1}. The samples were subjected to critical point drying. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 15 kV.

3.3.9 Statistical analysis

Completely randomized design was used throughout the study. All experiments were run in triplicate using three different batches of skin. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the Duncan's multiple range test (Steel *et al.*, 1980). For pair comparison, T-test was used. Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3.4 Results and Discussion

3.4.1 Effect of Na_2SO_4 treatment on yield and characteristics of goat gelatin

3.4.1.1 Yield

The yields and recovery of gelatin extracted from goat skin pretreated with 0.75 M NaOH, followed by soaking with Na_2SO_4 at varying concentrations (0, 0.25, 0.5, 0.75 and 1 M) are shown in Table 8. Gelatin from goat skin treated with Na_2SO_4 solution had the increasing yield when Na_2SO_4 concentration was higher than 0.25 M ($p < 0.05$). However, no difference in yield was observed between gelatin extracted from the skins treated with 0.75 M and 1 M Na_2SO_4 ($p > 0.05$). Similar trend was observed for recovery. Increased recovery with increasing Na_2SO_4 concentration suggested that the lower loss of gelatin was achieved as Na_2SO_4 concentration increased. The yields and recoveries of gelatins were 15.7-23.28 % and 33.41-47.58 % (on wet weight basis), respectively. The highest yield from goat skin

Table 8 Yield, recovery, gel strength and gel color of gelatin from alkaline pretreated goat skin, followed by soaking in Na_2SO_4 solution with different concentrations

Na_2SO_4 concentration (M)	Yield (%)	Recovery (%)	Gel strength (g)	Color value			
				*L**	*a**	*b**	Δ*E**
0	15.70 ± 0.21[a]	33.41 ± 0.93[a]	226.34 ± 3.80[a]	21.15 ± 0.23[c]	0.74 ± 0.04[a]	3.57 ± 0.21[b]	71.77 ± 0.24[a]
0.25	16.43 ± 0.43[a]	34.64 ± 0.50[a]	248.29 ± 7.48[b]	18.01 ± 0.34[a]	2.05 ± 0.25[c]	3.38 ± 0.28[b]	74.94 ± 0.35[c]
0.5	19.57 ± 0.48[b]	41.81 ± 0.63[b]	248.15 ± 5.81[b]	18.22 ± 0.46[a]	2.08 ± 0.18[c]	2.94 ± 0.35[a]	74.73 ± 0.46[c]
0.75	23.28 ± 0.77[c]	47.58 ± 1.28[c]	249.45 ± 4.73[b]	19.03 ± 0.27[b]	1.57 ± 0.25[b]	2.88 ± 0.17[a]	73.89 ± 0.26[b]
1	22.30 ± 1.03[c]	47.46 ± 0.97[c]	249.33 ± 4.48[b]	18.77 ± 0.28[b]	1.75 ± 0.13[b]	2.75 ± 0.12[a]	74.16 ± 0.28[b]

Values are presented as mean ± SD (n=3). Different lowercase letters within the same column indicate significant differences ($p < 0.05$).

was obtained when the treatment of skin was carried out using 0.75 M or 1 M Na_2SO_4 ($p < 0.05$). When alkaline pretreatment was implemented, the repulsion of protein molecules in skin was enhanced due to the development of net positive charge on the protein molecules. This was associated with increased swelling, in which some loss of looser collagen localized in skin matrix could take place. With the subsequent Na_2SO_4 treatment, Na_2SO_4 was able to stabilize the skin matrix, plausibly via the water competition on the surface of treated skin. Dehydrated outer skin might induce the formation of condensed surface. As a consequence, collagen was leached out to a lower extent. The interfibrillar bonds of collagen in the corium are stabilized with a solution of Na_2SO_4, in which the degree of swelling is not excessive, thereby reducing the loss of yield during pretreatment (Cioca and Siegler, 1981; Hawkins and Taylor, 1972). The degree of conversion of collagen to gelatin depends on the pretreatment conditions, the processing parameters, and the preservation method of the starting raw material (Karim and Bhat, 2009). The result suggested that treatment of alkaline pretreated skin using Na_2SO_4 solution at an appropriate concentration could increase the yield of gelatin. Therefore, the use of 0.75 M Na_2SO_4 for post-treatment of alkaline pretreated goat skin was effective in lowering the loss of gelatin extracted from goat skin.

3.4.1.2 Protein patterns

Protein patterns of gelatin from goat skin pretreated with alkaline solution, followed by soaking with different Na_2SO_4 concentrations are illustrated in Figure 11. All gelatin samples contained α–chains as the main constituents. MW of α_1- and α_2-chains were estimated to be 120 and 113 kDa, respectively. β- chain was also found in all samples. β- chain, a covalently linked α-chain dimer, had MW of 217 kDa. In general, no notable differences in protein patterns were observed among all gelatin samples. For alkaline pretreatment of channel catfish skin using 1 g L^{-1} ($Ca(OH)_2$) for 76 h, α–chains were more degraded (Liu *et al.*, 2008). Generally, gelatins with higher content of α-chains showed the better functional properties including gelling, emulsifying and foaming properties (Gómez-Guillén *et al.*, 2002). The result indicated that protein components in all gelatins were not affected by subsequent Na_2SO_4 treatment.

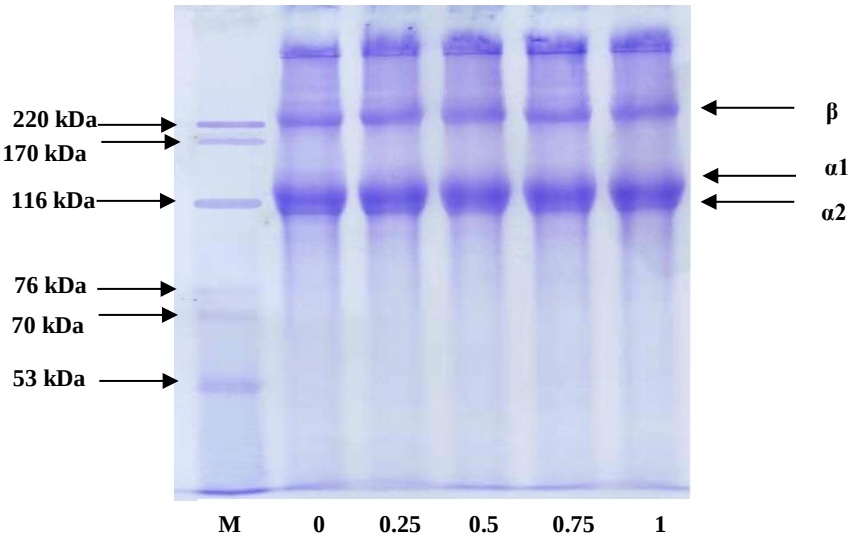

Figure 11 SDS-PAGE patterns of gelatin from alkaline pretreated goat skin followed by soaking in Na_2SO_4 solution at different concentrations. M denotes high molecular weight markers. The numbers denote concentration of Na_2SO_4 (M).

3.4.1.3 Gel strength

Gel strength of gelatin from goat skin pretreated with alkaline solution with subsequent treatment using Na_2SO_4 at various concentrations is shown in Table 8. Gel strength is one of the most crucial functional properties of gelatin. Higher gel strength was found in gelatin from goat skin treated with Na_2SO_4 (248.29-249.45 g), compared to the control (without subsequent Na_2SO_4 treatment) (226.34 g) ($p < 0.05$). Furthermore, gelatin from goat skin also showed the higher gel strength than commercial bovine gelatin (198.63 g) (data not shown) ($p < 0.05$). The result suggested that sulfate groups retained in skin after treatment might be co-extracted along with gelatin. Those sulfate groups might contribute to the enhanced gel strength, via ionic interaction between SO_4^{2-} and $-NH_3^+$ group of protein chains. Ionic interactions between the $-NH_3^+$ group of chitosan-gelatin and the sulfate ion were reported (Ling *et al.*, 2004; Mochizuki *et al.*, 1990). It was noted that post-treatment using Na_2SO_4 at various levels did not affect gel strength of resulting gelatins. Due to

the similar protein pattern, all gelatin samples contained similar components, α- and β- chains. The amount of β- and γ-components of gelatin was one of the factors determining gelation of gelatin (Taheri *et al.*, 2009). Therefore, subsequent treatment of goat skin using Na_2SO_4 not only increased the yield but also enhanced gel strength of resulting gelatin when compared to the control.

3.4.1.4 Color of gelatin gel

Color of gelatin gel from goat skin with the subsequent Na_2SO_4 treatment with various concentrations expressed as L^*, a^* and b^* is shown in Table 8. Slight decreases in L^*- values of gelatin gel were observed when subsequent Na_2SO_4 treatment was implemented. Gelatin gels from skin treated with Na_2SO_4 at 0.75 and 1 M showed slightly higher L^* -value than those of skin with 0.25 and 0.5 M Na_2SO_4 treatment ($p < 0.05$). The decreased L^*-value was concomitant with the higher a^*-value. Lower b^* -value was obtained in gel from gelatin extracted from goat skin with subsequent treatment using Na_2SO_4 at levels higher than 0.25 M ($p < 0.05$). Higher ΔE^* was found in gelatin gel from skin treated with Na_2SO_4 at all levels used, compared with the control gel (without Na_2SO_4 treatment). The increases in a^*- and ΔE^*-values were in accordance with the increased yield. As the outer surface of alkaline pretreated skin was more retained with Na_2SO_4 treatment, the remaining pigments could be co-extracted with gelatin. Ahmad and Benjakul (2011) reported that pretreatment process affected the properties of gelatin in terms of rheological and functional properties as well as color. However, the changes in color did not affect functional properties of gelatin (Kaewruang *et al.*, 2014b). Thus, subsequent treatment of alkaline pretreated goat skin partially affected the color of gels from goat skin.

3.4.2 Effect of H_2O_2 on yield and characteristics of goat gelatin

Gelatin from goat skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4, showing the highest yield of gelatin, were prepared and subjected to bleaching using H_2O_2 at various concentrations. The resulting gelatins obtained had varying yields and characteristics.

3.4.2.1 Yield

The yields of gelatin extracted from goat skin pretreated using 0.75 M NaOH without and with subsequent treatment using 0.75 M Na_2SO_4 were similar ($p > 0.05$), when different H_2O_2 concentrations were used (Table 9). Regardless of H_2O_2 concentration used for bleaching, gelatins from goat skin treated with 0.75 M Na_2SO_4 showed the higher yield (22.91-23.15 %) than those of skin without 0.75 M Na_2SO_4 treatment (14.97-15.45 %). The recovery of gelatin from goat skin treated with various H_2O_2 concentrations were 46.53-47.58 and 31.96-33.41 % when treatment using 0.75 M Na_2SO_4 was included and excluded, respectively. The result suggested that H_2O_2 had no impact on yield and recovery of gelatin. However, Na_2SO_4 treatment played an important role in reducing the loss of gelatin (Table 8). It was reported that H_2O_2 at high concentration (1-8%) could destroy H-bond of collagen cross-linked in the skin of cuttlefish and splendid squid, thereby increasing yield (Aewsiri *et al.*, 2009; Nagarajan *et al.*, 2013). It was suggested that alkaline pretreatment using 0.75 M NaOH was sufficient to swell the skin matrix. Further treatment using H_2O_2 might not facilitate the extraction of gelatin from skin matrix.

3.4.2.2 Protein patterns

Protein patterns of gelatin from goat skin treated without and with 0.75 M Na_2SO_4, followed by bleaching with H_2O_2 at various concentrations are shown in Figure 12. All gelatins extracted from goat skin had α-chains (MW of 116-123 kDa) and β-chain (MW of 217). The slight decrease in both α-chain and β-chain band intensity was observed for gelatin from the skin bleached using 1.5 and 2 M of H_2O_2. Higher degradation of α-chain was also observed for gelatin from cuttlefish and splendid squid when H_2O_2 at the higher concentration was used for bleaching prior to gelatin extraction (Aewsiri *et al.*, 2009; Hoque *et al.*, 2011; Nagarajan *et al.*, 2013). The decomposed H_2O_2 in aqueous solution arises by dissociation and hemolytic cleavage of O–H or O–O bonds, with the formation of highly reactive products: hydroperoxyl anion (HOO^-), and hydroperoxyl ($HOO^\bullet$) and hydroxyl ($OH^\bullet$) radicals (Perkins and Kinsella, 1996). Those radicals were able to react with several substances as well as caused the fragmentation of peptides. Thus, both α- and β-chains

Table 9 Yield, recovery, gel strength and gel color of gelatin from alkaline pretreated goat skin, subjected to bleaching using H_2O_2 at various concentrations without and with prior Na_2SO_4 treatment

Treated skin	H_2O_2 concentrations (M)	Yield (%)	Recovery (%)	Gel strength (g)	Color value			
					*L**	*a**	*b**	Δ*E**
Without 0.75 M Na_2SO_4	0	15.45 ± 0.41^{Aa}	33.41 ± 0.93^{Aa}	224.51 ± 3.74^{Ab}	21.15 ± 0.23^{Ba}	0.74 ± 0.04^{Ab}	3.57 ± 0.21^{Bc}	71.77 ± 0.24^{Ad}
	0.5	15.21 ± 0.26^{Aa}	33.21 ± 0.37^{Aa}	217.15 ± 2.26^{Aa}	25.99 ± 0.35^{Ab}	-0.52 ± 0.09^{Aa}	3.16 ± 0.19^{Abc}	66.90 ± 0.35^{Ac}
	1	15.10 ± 0.30^{Aa}	32.86 ± 0.56^{Aa}	215.69 ± 3.89^{Aa}	26.75 ± 0.25^{Ac}	-0.40 ± 0.07^{Aa}	2.88 ± 0.38^{Ab}	66.02 ± 0.44^{Ab}
	1.5	14.97 ± 0.25^{Aa}	32.14 ± 0.43^{Aa}	214.33 ± 2.08^{Aa}	28.85 ± 0.90^{Bd}	-0.57 ± 0.07^{Aa}	3.44 ± 0.46^{Ac}	64.05 ± 0.88^{Aa}
	2	14.99 ± 0.40^{Aa}	31.96 ± 0.59^{Aa}	212.01 ± 2.92^{Aa}	28.97 ± 0.21^{Bd}	$-0.5\ 2\pm 0.18^{Aa}$	-0.84 ± 0.17^{Aa}	63.87 ± 0.21^{Aa}
With 0.75 M Na_2SO_4	0	23.15 ± 0.27^{Ba}	47.58 ± 1.28^{Ba}	249.67 ± 5.69^{Bb}	19.03 ± 0.27^{Aa}	1.57 ± 0.25^{Bb}	2.88 ± 0.17^{Aab}	73.89 ± 0.26^{Bc}
	0.5	23.01 ± 0.34^{Ba}	47.44 ± 0.83^{Ba}	249.45 ± 6.06^{Bb}	25.93 ± 1.01^{Ab}	-0.47 ± 0.05^{Aa}	4.65 ± 0.55^{Bc}	67.01 ± 1.03^{Ab}
	1	22.89 ± 0.34^{Ba}	46.56 ± 0.87^{Ba}	243.29 ± 1.52^{Bab}	26.77 ± 0.60^{Ab}	-0.52 ± 0.07^{Aa}	4.36 ± 0.43^{Bc}	66.20 ± 0.56^{Ab}
	1.5	22.88 ± 0.38^{Ba}	46.53 ± 0.50^{Ba}	243.00 ± 4.59^{Bab}	26.47 ± 0.87^{Ab}	-0.59 ± 0.03^{Aa}	3.11 ± 0.12^{Ab}	66.41 ± 0.86^{Bb}
	2	22.91 ± 0.36^{Ba}	46.72 ± 0.65^{Ba}	240.33 ± 3.51^{Ba}	27.73 ± 0.46^{Ac}	-0.63 ± 0.07^{Aa}	2.60 ± 0.36^{Ba}	65.13 ± 0.45^{Ba}

Values are presented as mean ± SD (n=3). Different uppercase letters within the same H_2O_2 concentration indicate significant differences ($p < 0.05$). Different lowercase letters within the same pretreated goat skin indicate significant differences ($p < 0.05$).

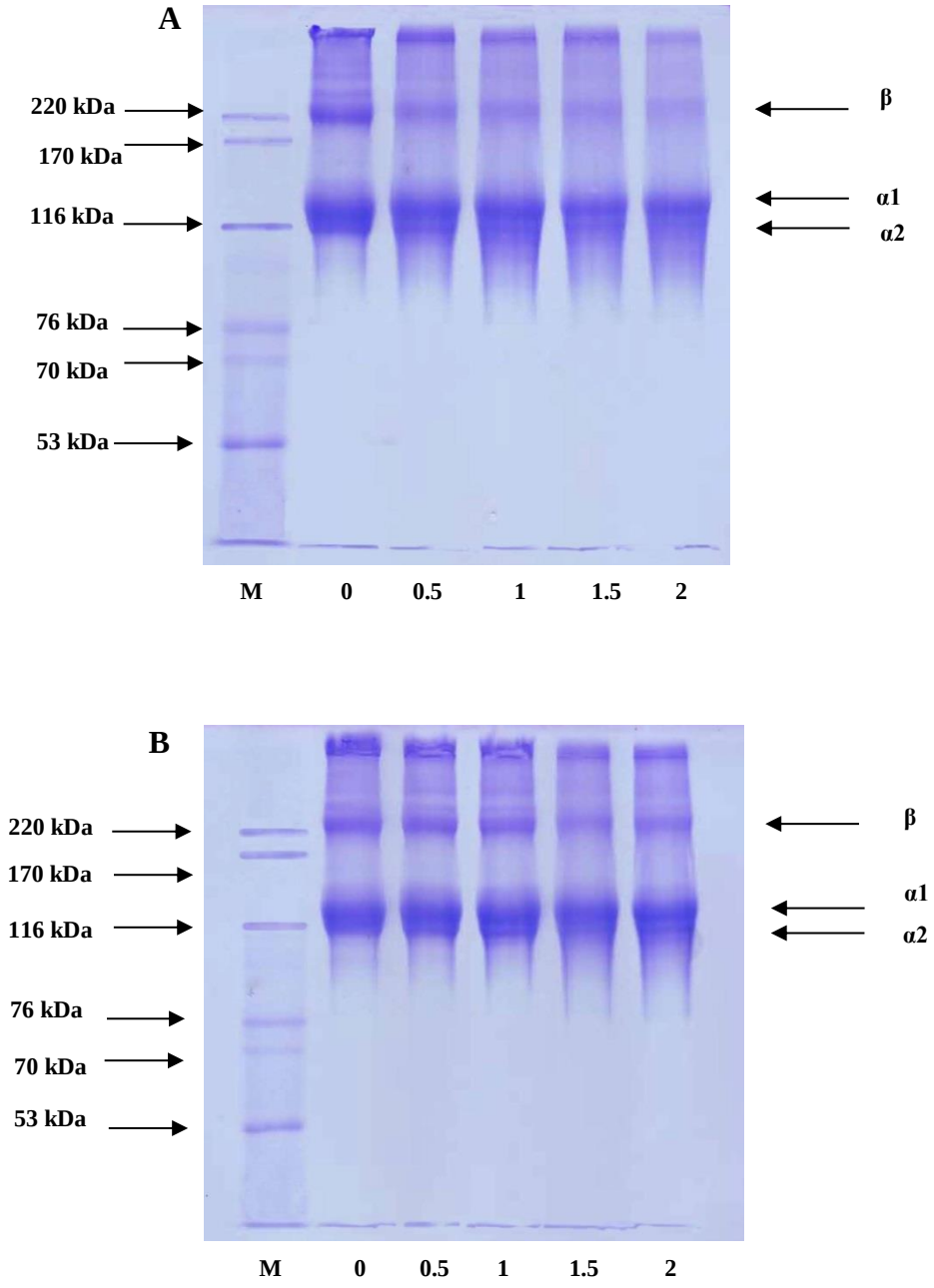

Figure 12 SDS-PAGE patterns of gelatin from alkaline pretreated goat skin, subjected to bleaching using H_2O_2 at various concentrations without (A) and with prior Na_2SO_4 treatment (B). M denotes high molecular weight markers. The numbers denote concentration of H_2O_2 (M).

might be vulnerable to degradation induced by H_2O_2, particularly at high concentrations. Gelatins from goat skin bleached with H_2O_2 without any pretreatment had slightly higher degradation than those of skin pretreated with 0.75 M Na_2SO_4. With Na_2SO_4 pretreatment, the surface of swollen skin could be denser caused by the water removed by Na_2SO_4. As a result, H_2O_2 could penetrate into the swollen skin to a lower extent, and skin was less prone to fragmentation induced by H_2O_2.

3.4.2.3 Gel strength of gelatin gel

Gel strengths of gelatin gel from alkaline pretreated goat skin without and with 0.75 M Na_2SO_4 treatment, followed by bleaching with H_2O_2 at various concentrations are depicted in Table 9. All gelatins had higher gel strength than that of commercial bovine gelatin (data not shown). When comparing gel strength of gelatin from skin without and with Na_2SO_4 treatment, higher values were found for those with Na_2SO_4 treatment, regardless of H_2O_2 concentrations used. The result reconfirmed that the use of 0.75 M Na_2SO_4 for subsequent treatment was able to improve the gel strength of gelatin from the skin of goat. Sulfate groups attached with α-chains or β-chains might undergo ionic interaction with the adjacent protein chains (Ling *et al.*, 2004). Additionally, slightly denser α- and β-chains in gelatin with Na_2SO_4 treatment were found, in comparison with those without Na_2SO_4 treatment (Figure 12A and 12B). Those components might favor the network formation and strengthen the 3-dimentional network of gel. For the samples with Na_2SO_4 treatment, bleaching using H_2O_2 at all concentrations did not affect gel strength of resulting gelatin, except those treated with 2 M H_2O_2, which had the lower gel strength ($p < 0.05$). The poorer gel strength coincided with the higher degradation of α- and β-chains. For gelatin from goat skin without Na_2SO_4 treatment, all resulting gelatin had slightly lower gel strength when H_2O_2 bleaching was implemented, irrespective of H_2O_2 concentrations. The result suggested that H_2O_2 might induce the oxidation of protein with the concomitant formation of carbonyl groups. Those carbonyl groups might undergo Schiff base formation, in which the protein cross-links were formed (Stadtman, 1997). Excessive cross-link of proteins could lead to the poorer gel, in which coarser network was formed. Nagarajan *et al.* (2013) reported that gel strength of gelatin decreased with increasing H_2O_2 concentrations used. Pretreatment condition

and type of raw material have the influence on chemical compositions of gelatin, which directly affect the functional properties, especially gelation (Benjakul *et al.*, 2012). Thus, treatment using Na_2SO_4 prior to bleaching as well as bleaching conditions used affected gel strength of resulting gelatin.

3.4.2.4 Color of gelatin gel

*L**, *a** and *b** -values of gelatin gel from goat skin bleached with H_2O_2 at various concentrations without and with 0.75 M Na_2SO_4 treatment are shown in Table 9. The increases in *L** -value (lightness) of gel were found as H_2O_2 concentration used for bleaching increased ($p < 0.05$). Lower *a** -values were found with all samples with bleaching, compared with the control (without bleaching). However, similar *a**-values were observed among the samples bleached with various H_2O_2 concentrations ($p > 0.05$). Gelatin gels showed varying *b**-values. Gelatin gel had the decreases in ΔE^* when higher levels of H_2O_2 were used for bleaching of skin before extraction. Therefore, bleaching goat skin using 0.5-2 M H_2O_2 solution could improve the color of gelatin gel by increasing *L**-value and decreasing *a** -value and ΔE^*-value. In general, gelatin gel from skin bleached with H_2O_2 at high concentrations (1.5 and 2 M) showed the higher *L** -value but lower ΔE^* when pretreatment using 0.75 M Na_2SO_4 was implemented. When Na_2SO_4 was used for pretreatment, H_2O_2 might not penetrate through the dense surface of swollen skin easily. As a result, bleaching efficacy via oxidation of pigment was lowered. This was evidenced by the lower ΔL^* and higher ΔE^*. Among all samples, gelatin from skin bleached with H_2O_2 at the concentration of 2 M with prior treatment using 0.75 M Na_2SO_4 rendered the gelatin gel with the lowest ΔE^*. Oxidizing agents, obtained from the decomposition of H_2O_2, were capable of destroying the chromophore. $OH^{\bullet}$ is a strong nucleophile, which is able to break the chemical bonds that make up the chromophore. Consequently, chromatophores could be destroyed or did not absorb visible light (Aewsiri *et al.*, 2009). The results indicated that H_2O_2, especially at high concentration, could improve the color of gelatin from goat skin.

3.4.3 Characterization of the selected goat gelatin

Gelatin extracted from skin with only alkaline pretreatment (G1) was characterized in comparison with those from skin with alkaline pretreatment, followed by Na_2SO_4 treatment (G2) and those from skin with alkaline and Na_2SO_4 treatments, followed by bleaching (G3).

3.4.3.1 Fourier transform infrared (FTIR) spectra

FTIR spectra of different gelatins including G1 G2 and G3 are illustrated in Figure 13. FTIR spectroscopy is used to monitor the functional groups and secondary structure of gelatin (Muyonga *et al.*, 2004). All gelatin samples possessed the major peaks in amide region and showed the amide-I band at the wavenumber of 1631.92, 1631.37 and 1634.17 cm^{-1} for G1, G2 and G3, respectively. Amide-I vibration mode is primarily C=O stretching vibration coupled with the C-N stretch and C-C-N deformation (Bandekar, 1992). The characteristic absorption bands of G1, G2 and G3 in amide-II region were found at the wavenumbers of 1543.97, 1533.87 and 1549.25, respectively. The amide-II vibration mode is attributed to combination of the N-H in plane bend and the C-N stretching vibration with smaller contributions from the C=O in plane bend and the C-C and N-C stretching vibrations (Jackson *et al.*, 1995). Additionally, amide-III was detected around the wavenumber of 1237.32, 1234.33 and 1239.33 cm^{-1} for G1, G2 and G3, respectively. The amide-III represents the combination peaks between C-N stretching vibrations and N-H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains (Jackson *et al.*, 1995). The wavenumbers of amide-I, amide-II and amide-III peaks were lower for unbleached skin (G1 and G2), compared to gelatin extracted from skin bleached with 2 M H_2O_2 (G3). Higher wavenumbers of bleached samples (G3) might be associated with the formation of carbonyl group during bleaching, caused by protein oxidation (Nagarajan *et al.*, 2013). This was coincidental with the higher amplitude of G3 for all amides peaks. When comparing between gelatin without

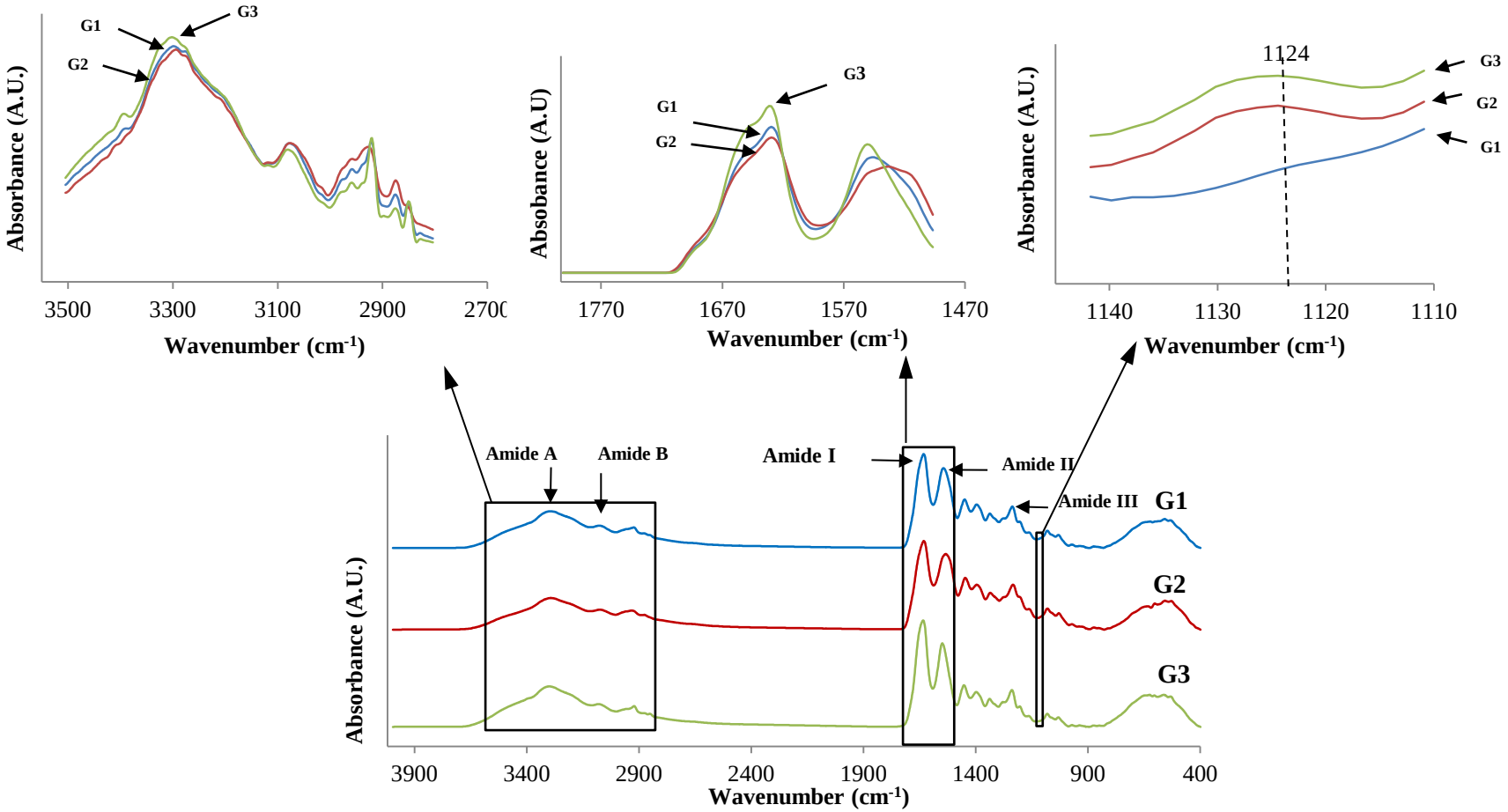

Figure 13 FTIR spectra of gelatin from goat skin with different pretreatment conditions. G1: gelatin from goat skin treated with 0.75 NaOH, G2: gelatin from goat skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4, G3: gelatin from goat skin treated with, 0.75 M NaOH, followed by 0.75 M Na_2SO_4, and bleached with 2 M H_2O_2

bleaching, the wavenumber of amide-II and III for G2 was shifted to lower wavenumber, compared with G1. The lower amplitude was also found in G2, particularly for amide-II. The result suggested that sulfates retained in G1 might interact with those functional groups in the amide regions tested.

Amide-A band, arising from the stretching vibrations of the N-H group, was detected at 3296.44, 3293.16 and 3300.17 cm^{-1} for G1, G2 and G3, respectively. Amide-A represents N-H stretching coupled with hydrogen bonding (Muyonga *et al.*, 2004). When the N-H group of a peptide is involved in an hydrogen bond, the peak position shifts to lower wavenumbers (Doyle *et al.*, 1975). In amide-A region, the highest wavenumber was found in G3, suggesting higher N-H groups available. H_2O_2 used for bleaching was able to induce fragmentation of protein chains with the formation of NH_2 group. This was related with the high degradation of proteins in G3 (Figure 12B). The amide-B was noticeable at wavenumber of 3077.90, 3076.15 and 3081.37 cm^{-1} for G1, G2 and G3, respectively. Amide-B represents asymmetrical stretch of CH_2 (Doyle *et al.*, 1975). This peak is involved with hydrogen bonding between free N-H stretch coupled with hydrogen in polypeptide chain (Matmaroh *et al.*, 2011). In this study, the highest wavenumber of amide-B were found in G3, indicating that the less interaction between protein chains, particularly via hydrogen bonding. With higher degradation, protein chains could not align properly, in which strong gel might not be developed as indicated by lower gel strength of G3, compared with G2 (Figure 14A). It was noted that G2 and G3 had the small peak at wavenumber of 1124 cm^{-1}. However, there was no peak at the same wavenumber in G1. This peak was likely due to sulfate group attached to gelatin, during treatment. Lane (2007) reported that sulfate absorption region was at 1050-1250 cm^{-1}. Therefore, the secondary structure and functional group of gelatins from goat skin were affected by Na_2SO_4 and H_2O_2 treatment.

3.4.3.2 Gelling and melting temperatures

The changes in the phase angle (δ) of gelatin solutions during cooling (50 to 5 °C) and subsequent heating (5 to 50 °C) are illustrated in Figure 14B and 14C, respectively. All gelatin samples with different pretreatment conditions formed a

gel at the temperatures of 23.62, 24.02 and 22.49 °C for G1, G2 and G3, respectively. This was regarded as the sharp decrease and rapid transition in phase angle during cooling. Higher gelling temperature was found in G2, compared to others ($p < 0.05$). This was coincidental with the higher gel strength of G2 (Figure 14A). The lower gelling temperature of G3 might be caused by slight fragmentation of both α - and β - chains induced by H_2O_2. With the shorter chains, the lower temperature was more likely required to form the gel. As a result, the junction zones could be initiated and the gel matrix could be developed. When Na_2SO_4 treatment was implemented, the resulting gelatin (G2) showed higher gelling temperature, compared with G1 (without Na_2SO_4 treatment). SO_4^{2-} group incorporated into gelatin might favor the ionic interaction between protein molecules, in which gel could be formed easily. The gelling temperature of gelatin from goat skin was higher than those of gelatins from fish skin including bigeye snapper (10.0 °C) (Binsi *et al.*, 2009) and silver carp (18.7 °C) (Boran *et al.*, 2010). Furthermore, gelling temperature was slightly higher than that of bovine (21.7 °C) (Kasankala *et al.*, 2007). Karim and Bhat (2009) reported that melting point and gelling point are governed mainly by the amino acid composition (pro + hyp content), molecular weight distribution and also the ratio of α/β chains in the gelatin.

Melting points of gelatins from goat skin with various pretreatments were 33.63, 34.10 and 32.28 °C for G1, G2 and G3, respectively, which were higher than those reported for gelatin from bigeye snapper skin (16.8 °C) (Binsi *et al.*, 2009), silver skin (27.1 °C) (Boran *et al.*, 2010), porcine (31.5 °C) and bovine (30 °C) (Kasankala *et al.*, 2007). The high melting point in this study reflected the strong gel network of goat gelatin. The proline plays a critical role in enhancing the formation of polyproline II helix (Benjakul *et al.*, 2012) Thermal stability of gelatin gel is directly related with proline-rich regions in gelatin molecules (Gómez-Guillén *et al.*, 2002). Apart from imino content, the melting point of gelatin also increases with increasing MW of components (Jamilah and Harvinder, 2002). With higher melting temperature, gel could be maintained for a longer time, thereby providing the better mouth feel when consumed. In general, high melting points of all gelatins were in accordance with high gelling point. The results indicated that treatments, using both Na_2SO_4 and

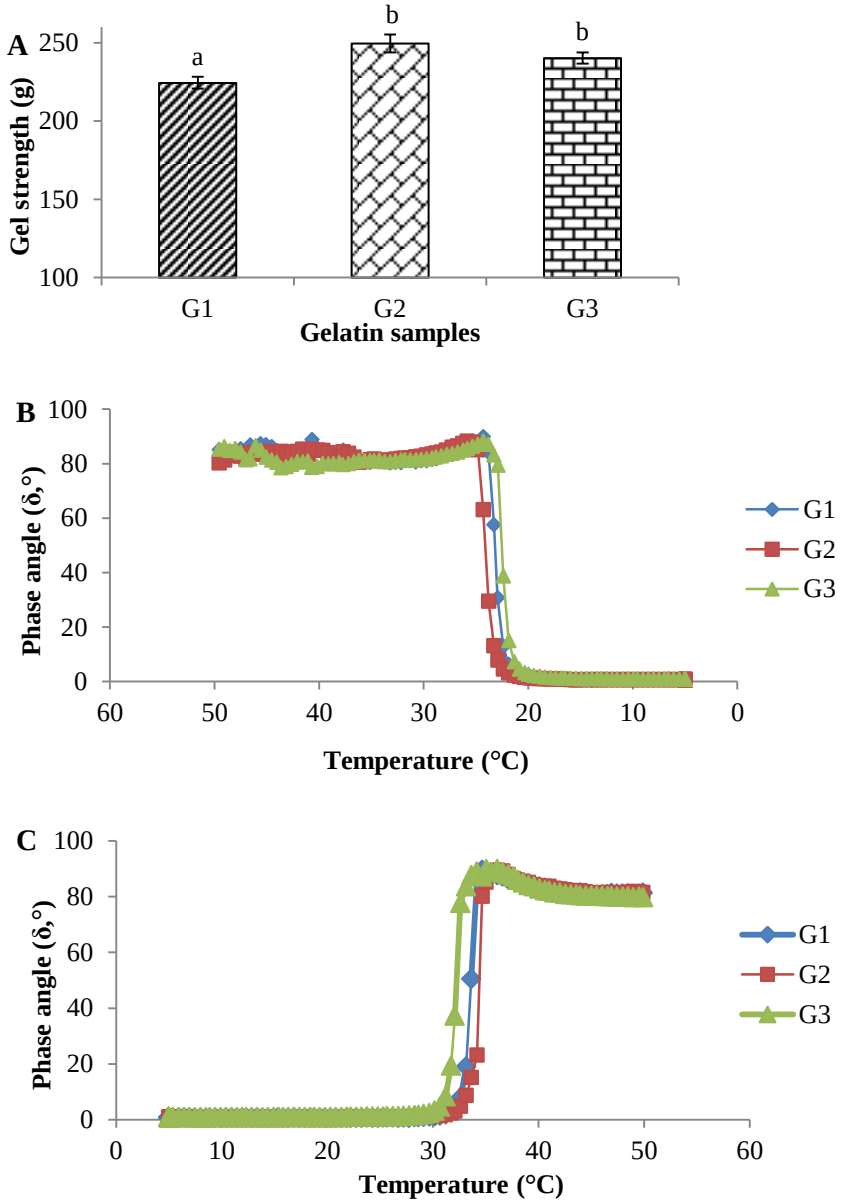

Figure 14 Gel strength (A) and changes in phase angle (δ, °) of gelatin solution (6.67%, w/v) from goat skin with different pretreatment conditions during cooling (B) and subsequent heating (C). G1: gelatin from goat skin treated with 0.75 NaOH, G2: gelatin from goat skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4, G3: gelatin from goat skin treated with, 0.75 M NaOH, followed by 0.75 M Na_2SO_4, and bleached with 2 M H_2O_2

H_2O_2, had the influence on the gelling and melting characteristics of goat skin gelatin.

3.4.3.3 Amino acid composition

Amino acid compositions of gelatin from goat skin pretreated with three different conditions are shown in Table 10. All gelatins showed similar amino acid compositions, in which glycine was the major amino acid (324-331residues/1000 residues), followed by alanine (110-112 residues/1000 residues). Low contents of cysteine (1 residue/1000 residues), tyrosine (2-4 residues/1000 residues), histidine (4-5 residues/1000 residues) and hydroxylysine (6-7 residues/1000 residues) were found in all gelatin samples. Higher content of glycine was found in G1 (331 residues/1000 residues), followed by G2 (327 residues/1000 residues) and G3 (324 residues/1000 residues), respectively. Glycine generally occurs after every third position of α-chain and represents nearly one third of total residues except for 14 amino acids from N-terminal and for 10 amino acids from C-terminal (Benjakul *et al.*, 2012). For imino acid, all gelatins contained proline and hydroxyproline of 121-124 and 96-99 residues/1000 residues, respectively. The imino acid content of G1 (223 residues/1000 residues) was slightly higher than that observed in G2 (221 residues/1000 residues) and G3 (217 residues/1000 residues), respectively. Giménez *et al.* (2005) reported that gelatin from dover sole skin pretreated with different conditions had different proline and hydroxyproline contents. It was noted that the imino acid contents of gelatin from goat skin were higher than those reported for gelatin from silver carp waste (197 residues/1000 residues) (Tavakolipour, 2011), grey triggerfish skin (176 residues/1000 residues) (Jellouli *et al.*, 2011), bigeye snapper skins (186.29-187.42 residues/1000 residues) (Benjakul *et al.*, 2009), salmon skin (166 residues/1000 residues) and cod skin (154 residues/1000 residues) (Arnesen and Gildberg, 2007). Moreover, they were also higher than that found in bovine gelatin (219 residues/1000 residues) and porcine skin gelatin (199 residues/1000 residues) (Tavakolipour, 2011). Gelatin with higher content of hydroxyproline possessed the ability to develop the strong gel structure (Benjakul *et al.*, 2009). OH groups of hydroxyproline might be involved in hydrogen bondings with adjacent chains. This could strengthen gel network of gelatin. Additionally, alanine might contribute to hydrophobic interaction,

Table 10 Amino acid compositions of gelatin from goat skin with different pretreatment conditions

Amino acids	Residues/1000 residues		
	G1	G2	G3
Aspartic acid/asparagine	47	45	46
Threonine	19	19	19
Serine	32	33	33
Glutamic acid/glutamine	75	75	76
Glycine	331	327	324
Alanine	112	111	110
Cysteine	1	1	1
Valine	21	21	22
Methionine	5	6	6
Isoleucine	11	11	12
Leucine	25	26	27
Tyrosine	2	3	4
Phenylalanine	12	13	13
Hydroxylysine	6	7	7
Lysine	27	28	29
Histidine	4	5	5
Arginine	48	48	48
Hydroxyproline	99	96	96
Proline	124	125	121
Total	1000	1000	1000
Imino acids	223	221	217

Note
G1: gelatin from goat skin treated with 0.75 M NaOH
G2: gelatin from goat skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4
G3: gelatin from goat skin treated with, 0.75 M NaOH, followed by 0.75 M Na_2SO_4, and bleached with 2 M H_2O_2

while glutamic acid/glutamine (75-76 residues/1000 residues) could be involved in ionic interaction between chains. The results indicated that treatment conditions had no profound effect on the amino acids compositions of gelatin from goat skin.

3.4.3.4 Microstructures of gelatin gels

The microstructures of gelatin gels from goat skin with different pretreatments are illustrated in Figure 15. The conformation and chain length of proteins in gel matrix directly governed the gel strength of gelatin (Benjakul *et al.*, 2012). Generally, gelatin gels were sponge or coral-like in structure. Among all samples, gelatin gel from G2 and G3 showed the finer and ordered network with very small voids than that of G1. SO_4^{2-} in both G2 and G3 might contribute to the enhanced cross-linking via ionic interaction. The finer and denser structure of gel network could be more resistant to external force applied. Conversely, the larger voids were found in

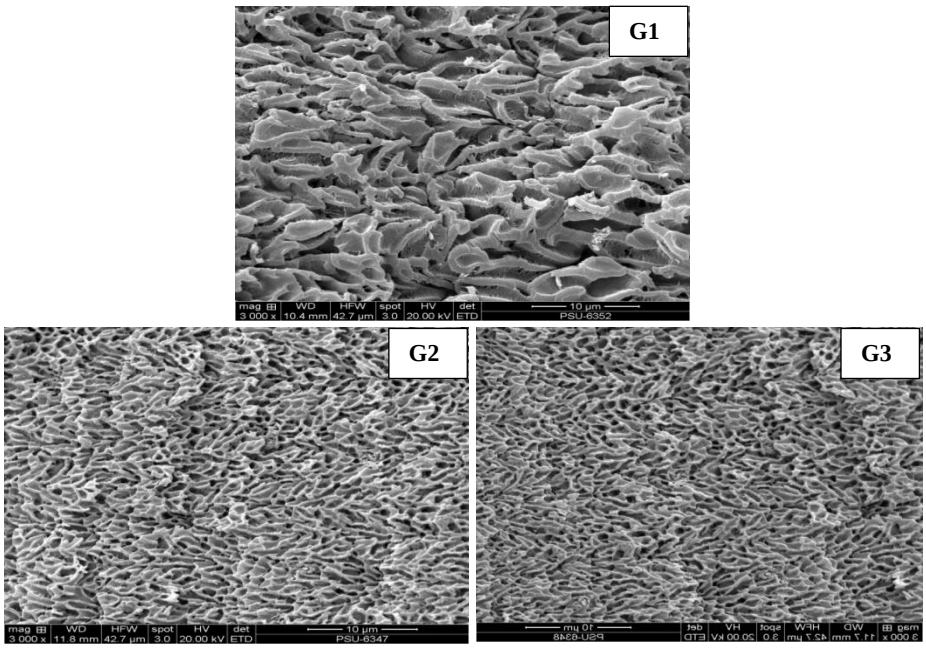

Figure 15 Microstructures of gelatin gel from goat skin with different pretreatment conditions. Magnification: 3000x. G1: gelatin from goat skin treated with 0.75 NaOH, G2: gelatin from goat skin treated with 0.75 M NaOH, followed by 0.75 M Na_2SO_4, G3: gelatin from goat skin treated with, 0.75 M NaOH, followed by 0.75 M Na_2SO_4, and bleached with 2 M H_2O_2

G1. The coarser gel network was inclined to have the lower gel strength and was easy to be disrupted. It is well known that the distribution of α-, β- and γ-chains is a substantial factor affecting property of gelatin (Sinthusamran *et al.*, 2014). Hydroxyproline is also involved in gel formation of nucleation zones via hydrogen bonding through its –OH group (Kittiphattanabawon *et al.*, 2010). In addition, pretreatment process governs the properties of gelatin gel (Yang *et al.*, 2008). Kaewruang *et al.* (2014a) found that phosphates attached to gelatin via phosphorylation might be involved in network formation, in which an ordered structure with finer strands could be developed. Therefore, the arrangement and association of gelatin molecules in the gel matrix directly contributed to gel strength of gelatin from goat skin and those were determined by pretreatments used.

3.5 Conclusions

The skin from goat could be a promising source of gelatin. Pretreatment conditions affected the characteristic and properties of gelatin. Gelatin from goat skin contained α-chains as the major constituent. All gelatins from different pretreatment conditions showed higher gel strength than that of commercial bovine gelatin. The optimal pretreatment condition for gelatin extraction from goat skin included the pretreatment of skin using 0.75 M NaOH, followed by treatment using 0.75 M Na_2SO_4 and subsequent bleaching with 2 M H_2O_2.

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CHAPTER 4

CHARACTERISTICS AND GEL PROPERTIES OF GELATIN FROM GOAT SKIN AS AFFECTED BY EXTRACTION CONDITIONS

4.1 Abstract

Characteristics and gel properties of gelatin from goat skin, as influenced by extraction conditions, were studied. Yield of gelatin was 22.1-23.1% (wet weight basis). All gelatins contained α-chains as the predominant components, followed by β-chain. However, no β-chain was retained when extracted at 70 °C. Gelatin extracted at 50 °C for 2.5 h exhibited the highest gel strength (267 g) ($p < 0.05$). Gelling and melting temperatures for goat skin gelatin were in the ranges of 21.18-25.17 and 30.69-34.12 °C, respectively. Gel strength, gelling and melting temperatures of gelatin gels generally decreased with increasing extraction temperatures and times. Gels of gelatin extracted at a higher temperature for longer time had larger strands and voids. Gelatin extracted at 50 °C for 2.5 h contained imino acids of 225 residues/1000 residues and showed higher gel strength than bovine gelatin. Thus, it could be used as a potential replacer for commercial gelatins.

4.2 Introduction

Gelatin is the fibrous protein obtained by thermal denaturation or partial hydrolysis of collagenous materials (Mohtar *et al.*, 2010). It has many applications in food and non-food industries. In food industry, gelatin is one of the water soluble polymers that can be used to improve stability and consistency of food. For medical and pharmaceutical industries, it can be used to produce soft and hard capsules, wound dressing and adsorbent pads (Widyasari and Rawdkuen, 2014). Gelatin is mainly produced from bovine and porcine skins and demineralized bones (Mohtar *et al.*, 2010). The global demand of gelatin for food and non-food applications was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018 (Sheela, 2014). Different types and sources of materials determine properties of obtained gelatin such as melting and gelling temperatures and gel strength, etc.

(Norziah *et al.*, 2009). Compared with gelatin from aquatic or marine animals, those from land animals are more stable and have better rheological characteristics (Norland, 1990). Therefore, gelatin from alternative land animals, especially by-products from goat slaughtering, e.g. skin or bone, should be taken into account to serve for increasing demand of gelatin in the world market.

Gelatin extraction from mammal skin generally required the appropriate pretreatment to bring about high yield and desirable properties (Hinterwaldner, 1977). There are two types of gelatin with different characteristics including type-A and type B, which can be obtained from acid-pretreated and alkaline-pretreated collagenous materials, respectively (Benjakul *et al.*, 2012). Acid pretreatment is widely implemented for less fully cross-linked collagens found in pig or fish skins, whereas alkaline pretreatment is suitable for skin containing more complex cross-linked collagens found in bovine hide (Gómez-Guillén *et al.*, 2002). Alkaline solution plays an important role in breaking cross-links in the skin matrix stabilized by strong bonds, thereby increasing extraction efficiency (John and Courts, 1977). Although gelatin has a wide range of applications, the pessimism and strong concerns still persist among consumers, mainly due to religious sentiments (Asher, 1999). Both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume bovine products (Badii and Howell, 2003). Poultry-related product has been also concerned in terms of avian influenza. Therefore, gelatin from alternative sources has been searched.

Goat is one of economically important animals raised in Thailand for their meat and milk. The number of goats increased annually since their meat has become promising for consumption, especially for Muslims. When goats are slaughtered, by-products including skin account for 6.4-11.6% (based on the body weight) (Warmington and Kirton, 1990). The skin can be used as an alternative raw material for gelatin extraction. Recently, Mad-Ali *et al.* (2015) reported that alkaline pretreatment under the appropriate condition was necessary for gelatin extraction from goat skin. However, no information concerning the influence of extraction conditions especially temperatures and times on gelatin from goat skin has been reported. Therefore, this study aimed to determine yield, physicochemical

characteristics and gelling properties of gelatin from goat skin as affected by different extraction temperatures and times.

4.3 Materials and Methods

4.3.1 Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulfate (SDS), Coomassie blue R-250 and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were obtained from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the bloom strength of 150-250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

4.3.2 Collection and preparation of goat skin

Skins from Anglo-Nubian goats with the age of approximately 2 years were collected from a local slaughter house in Chana district, Songkhla province, Thailand. Seven kilograms of goat skins were randomly taken from three goats, pooled and used as the composite sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice (a skin/ice ratio of 1:2, w/w) and transported to the Department of Food Technology, Prince of Songkla University, within 2 h. Upon arrival, the skins were cleaned and washed with running water (26-28 °C). Prepared skins were then cut into small pieces (2.5×2.5 cm^2) using knives, placed in polyethylene bags and stored at -20 °C until use. The storage time was not longer than 2 months.

4.3.3 Pretreatment of goat skin

Prepared skins were soaked in 0.75 M NaOH solution at a ratio of 1:10 (w/v). During pretreatment of 2 days, the mixtures were left at room temperature (28-30 °C) and stirred manually twice a day. The solution was removed and replaced by the same volume of freshly prepared solution every day. The skins were then transferred on the perforated screen to remove the solution.

Alkaline pretreated skins were mixed with 10 volumes of 0.75 M Na_2SO_4 solution. The mixtures were allowed to stand at room temperature for 24 h. Subsequently, the skins were washed with running water until the pH of wash water became neutral or slightly alkaline. After washing, the obtained skins were soaked in 2 M H_2O_2 solution. The mixture was allowed to stand at 4 °C for 24 h. During soaking, H_2O_2 solution was changed every 12 h. The skin samples were then washed thoroughly three times with 10 volumes of tap water. The prepared skins were subjected to gelatin extraction.

4.3.4 Extraction of gelatin

To extract gelatin, the prepared skins were placed in distilled water at 50, 60 and 70 °C with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 2.5 and 5 h with a continuous stirring at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The mixtures were then filtered using two layers of cheesecloth. The filtrates were further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea). The resultant filtrates were freeze-dried using a ScanvacModel Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). All gelatins were subjected to analyses.

4.3.5 Analyses

4.3.5.1Yield and recovery

The yield of gelatin was calculated by the following equations:

$$\text{Yield (\%)} = \frac{\text{Weight of dried gelatin (g)} \times 100}{\text{Weight of initial skin (g)}}$$

$$\text{Recovery (\%)} = [\text{hydroxyproline content of filtrate (g/ml)} \times \text{volume of filtrate (ml)}]/[\text{hydroxyproline content of initial skin (g/g)} \times \text{weight of initial skin (g)}] \times 100$$

Hydroxyproline content was determined according to the method of (Bergman and Loxley, 1963).

4.3.5.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the method of Laemmli (1970). Gelatin samples were dissolved in 5% SDS and the mixtures were incubated at 85 °C for 1 h. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris HCl, pH 6.8. containing 4% SDS and 20% glycerol). The mixtures were boiled in boiling water for 2 min. Samples (15 µg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis. Gels were stained with 0.05% (w/v) Coomassie blue R-250 in 15 % (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% acetic acid. High MW markers were used for estimation of MW.

4.3.5.3 Determination of gel strength

Gelatin gel was prepared as per the method of Kittiphattanabawon *et al.* (2010). Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis.

Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

4.3.5.4 Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated

l-alanine tri-glycine sulfate (DLATGS) detector. The horizontal attenuated total reflectance accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–650 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean empty cell at 25 C. Deconvolution was performed on the average spectra for the amide I bands, using a resolution enhancement factor of 1.8 and full height band width of 13 cm^{-1}. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

4.3.5.5 Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of Boran *et al.* (2010) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). Gelatin solution (6.67%, w/v) was prepared in the same manner as described previously. The solution was preheated at 35 °C for 30 min. The measuring geometry used was 3.5 cm parallel plate with the gap of 1.0 mm. The measurement was performed at a scan rate of 0.5 °C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 50 to 5 °C and heating from 5 to 50 °C. The gelling and melting temperatures were designated, where tan δ became 1 or δ was 45°.

4.3.5.6 Microstructure analysis of gelatin gel

Microstructure of gelatin gel (6.67%, w/v) was visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100 % (v/v). The samples were subjected to critical point drying. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were

observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 15 kV.

4.3.5.7 Determination of color of gelatin gel

The color of gelatin gels (6.67% w/v) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated according to the following equation (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard (L^* = 93.63, a^* = -0.94 and b^* = 0.40).

4.3.5.8 Amino acid analysis

Amino acid composition of the selected gelatin sample was analyzed according to the method of Nagarajan *et al.* (2012) with a slight modification. The sample was hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115 °C for 24 h. The hydrolysate was neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

4.3.6 Statistical analysis

All experiments were run in triplicate using three different lots of skins. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the Duncan's multiple range test (Steel *et al.*, 1980). Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

4.4 Results and Discussion

4.4.1 Yield and recovery

Yield and recovery of gelatin extracted from pretreated goat skin under different extraction conditions are shown in Table 11. Yields of 22.1-23.1% and 22.5-22.9% (on wet weight basis) were found for gelatin extracted for 2.5 and 5 h, respectively, when extraction temperatures of 50-70 °C was used. The recovery of gelatin extracted at 50-70 °C for 2.5 and 5 h were 45.98-47.02% and 46.18-46.73%, respectively. There were no differences in yield and recovery of gelatin obtained from all extraction conditions used ($p > 0.05$). Therefore, temperatures and times used in the present study provided the sufficient energy to destabilize the bondings between α-chains or β- chains in the native mother collagen. As a consequence, similar amount of gelatin was extracted from the prepared skin. With appropriate pretreatment, warm water could penetrate into loosen or swollen skin. Therefore, the triple helix structure became amorphous and could be extracted into the medium with ease. Based on the low energy and time consumption, the extraction at 50 °C for 2.5 h was recommended as the proper extraction condition. Further extraction using higher temperatures and times could not facilitate the extraction yield of gelatin. However, the increases in yield with increasing extraction temperatures and times were reported for gelatin extracted from the skin of sea bass (Sinthusamran *et al.*, 2014), unicorn leatherjacket (Kaewruang *et al.*, 2013) and shark (Kittiphattanabawon *et al.*, 2010). The yields of gelatin are therefore associated with the type of raw material and gelatin extraction process, including the pretreatment process (Kittiphattanabawon *et al.*, 2010; Nagarajan *et al.*, 2012).

4.4.2 Protein patterns

Protein patterns of gelatin extracted from goat skin at different temperatures for various times are shown in Figure 16. All gelatin samples contained α–chains as the major components. β-chain was also found in gelatins extracted at 50 and 60 °C, however, it was not found in those extracted at 70 °C, regardless of

extraction times. MWs of α_1- and α_2-chains were estimated to be 131 and 124 kDa, respectively. β-chain, a dimer, had MW of 236 kDa. It was noted that bands of α- and

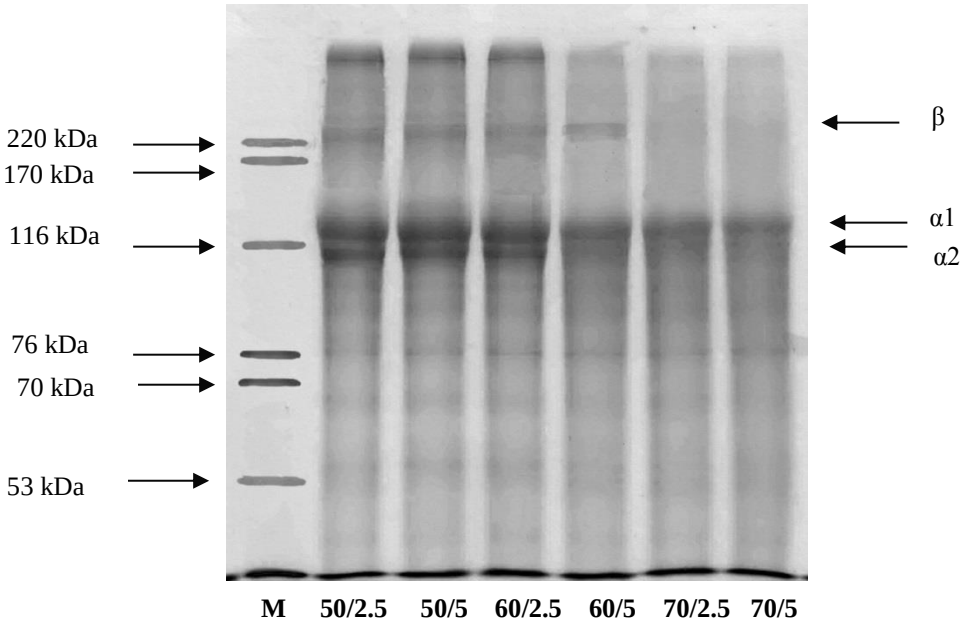

Figure 16 SDS-PAGE patterns of gelatin from goat skin extracted at different temperatures for various times. M denotes high-molecular-weight markers. The numbers before and after ‘‘/’’ denote extraction temperature (°C) and extraction time (h), respectively.

β-chains were still retained when extraction temperatures was conducted at 60 °C for 2.5 h. Nevertheless, the α-chains were more degraded when gelatin was extracted at 60 °C for 5 h. The disappearance of both α- and β- chains were more likely owing to thermal degradation. The results indicated that the degradation of α- and β- chains was more pronounced when extraction temperature and time increased. Generally, gelatin with high content of α- and β-components were reported to exhibit the better functional properties including gelling, emulsifying and foaming properties (Gómez-Guillén *et al.*, 2002). Thermal degradation of gelatins from the bamboo shark, blacktip and splendid squid skin was reported to increase with increasing extraction temperatures (Kittiphattanabawon *et al.*, 2010; Nagarajan *et al.*, 2012). Thus, extraction temperatures and times had the impact on components of gelatin from goat skin.

Table 11 Extraction yield (% wet weight basis), recovery and gel color of gelatin from goat skin extracted at different temperatures for various times

Times (h)	Temperature (°C)	Yield (%)	Recovery (%)	Color value			
				*L**	*a**	*b**	Δ*E**
2.5	50	22.06 ± 0.50^{Aa}	46.15 ± 0.26^{Aa}	40.24 ± 0.11^{Aa}	-2.53 ± 0.04^{Bb}	-6.75 ± 0.14^{Bc}	53.86 ± 0.11^{Bc}
	60	22.51 ± 0.34^{Aa}	45.98 ± 0.39^{Aa}	39.17 ± 0.21^{Ab}	-2.57 ± 0.05^{Bb}	-3.87 ± 0.12^{Ab}	54.63 ± 0.21^{Bb}
	70	23.06 ± 0.42^{Aa}	47.02 ± 0.31^{Aa}	33.75 ± 0.16^{Ac}	-1.29 ± 0.08^{Ba}	-1.82 ± 0.08^{Ba}	59.89 ± 0.16^{Ba}
5	50	22.54 ± 0.40^{Aa}	46.73 ± 0.29^{Aa}	38.40 ± 0.19^{Ba}	-1.68 ± 0.06^{Ac}	-3.11 ± 0.12^{Ac}	55.32 ± 0.18^{Ac}
	60	22.94 ± 0.37^{Aa}	46.18 ± 0.18^{Aa}	37.33 ± 0.16^{Bb}	-1.57 ± 0.04^{Ab}	-3.78 ± 0.07^{Ab}	56.43 ± 0.16^{Ab}
	70	22.68 ± 0.56^{Aa}	46.26 ± 0.22^{Aa}	30.25 ± 1.06^{Ac}	-0.81 ± 0.08^{Aa}	-0.58 ± 0.15^{Aa}	63.36 ± 1.06^{Aa}

Values are presented as mean ± SD (n = 3).
Different uppercase letters within the same column under the same temperature indicate significant differences ($p < 0.05$).
Different lowercase letters within the same column under the same extraction time indicate significant differences ($p < 0.05$).

4.4.3 Gel strength

Gel strengths of gelatin extracted from goat skin under varying conditions are shown in Figure 17. The gel strength is one of the most important functional properties of gelatins. The highest gel strength (267 g) was found in gelatin extracted at 50 °C for 2.5 h ($p < 0.05$). At the same extraction temperature, the gel strength of gelatin from goat skin decreased as the extraction time increased ($p < 0.05$). The lower gel strength of gelatin was observed with increasing extraction temperatures when the same extraction time was used ($p < 0.05$). This result was in accordance with the report of Sinthusamran *et al.* (2014) and Kittiphattanabawon *et al.* (2010). The decrease in gel strength was generally in agreement with the decreases in α- and β- chains band intensity (Figure 16). The amount of α- and β-components and the amino acid composition of gelatin were reported as the factors determining gelation of gelatin (Benjakul *et al.*, 2012). It was found that the lowest gel strength was observed in gelatin extracted at higher temperature, particularly at 70 °C. The shorter chains of gelatin extracted at 70 °C could not align properly and the junction

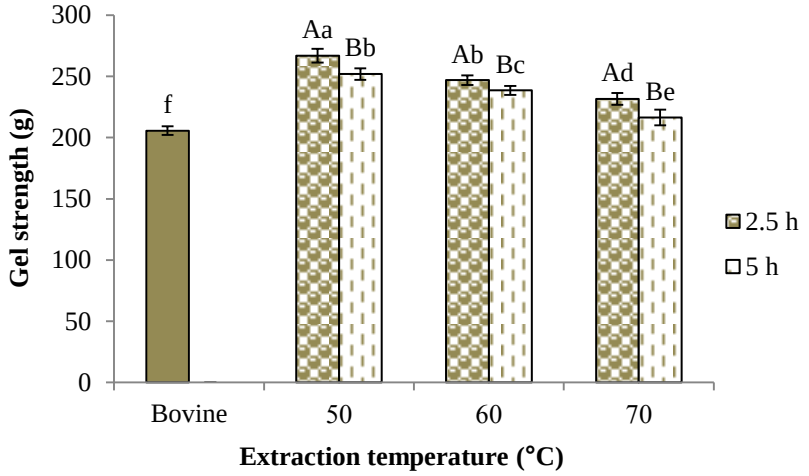

Figure 17 Gel strength of gelatin from goat skin extracted at different temperatures for various times. Bars represent the standard deviation (n = 3). Different uppercase letters on the bars within the same extraction temperature indicate significant differences ($p < 0.05$). Different lowercase letters on the bars indicate significant differences ($p < 0.05$).

zone could not be formed to a higher degree. As a consequence, the strong gel network was not developed as indicated by the lower gel strength. The result suggested that extraction condition directly affected the gel strength of resulting gelatin from goat skin.

4.4.4 Fourier transform infrared (FTIR) spectra

FTIR spectra of gelatin from the skins of goat extracted at different extraction temperatures for 2.5 and 5 h are depicted in Figure 18. In general, all gelatins showed similar spectra. The amide I band of all gelatin samples appeared at 1629–1633 cm^{-1}. The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the C-N stretch, C-C-N deformation and in-plane NH bending modes (Bandekar, 1992). The absorption in the amide I region is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins (Sinthusamran *et al.*, 2014). The amide-I peak of gelatin extracted for a longer time appeared at higher wavenumber, compared with those with the shorter time. This indicated the greater loss of triple helix due to the enhanced disruption of inter-chain interaction caused by longer extraction time. Additionally, gelatins extracted at lower temperature for a shorter time showed the lower amplitude, compared with those extracted at higher temperature for a longer time, particularly at 70 °C. The result suggested that the greater disruption of intra-molecular bonding and more exposure of C=O when extraction was carried out at higher temperature for longer time. Lower amplitude of amide I observed and slightly lower wavenumber was associated with molecular order due to the interaction of C=O with adjacent chains via hydrogen bond (Sinthusamran *et al.*, 2014). Overall, extraction at higher temperature with extended time led to the pronounced destruction of triple helix between α-chains. The disorder of molecular structure owing to transformation of an α-helical to a random coil structure occurred during heating (Kaewruang *et al.*, 2013). In addition, gelatin extracted under different conditions exhibited the amide II bands at the wavenumbers of 1538–1544 cm^{-1}. It was noted that the amide II bands of gelatins extracted at higher temperatures for a longer times were shifted to the higher wavenumber. The amide II band resulted from an out-of-phase combination of a C-N stretch and in-plane N-H deformation modes of the peptide group (Bandekar, 1992).

Furthermore, the amide III bands of all gelatin samples were observed at wavenumbers of 1236–1237 cm^{-1}, which indicated disorder in the gelatin molecules and were more likely associated with loss of triple helix state (Friess and Lee, 1996). During extraction at high temperature, hydrogen bonds stabilizing triple helix were destroyed, leading to amorphous structure. The amide III band represents the combination peaks between C-N stretching vibrations and N-H deformation from the amide linkages as well as the absorptions arising from wagging vibrations of CH_2 groups in the glycine backbone and proline side-chains (Jackson *et al.*, 1995). However, no remarkable differences were observed in amide III among all samples.

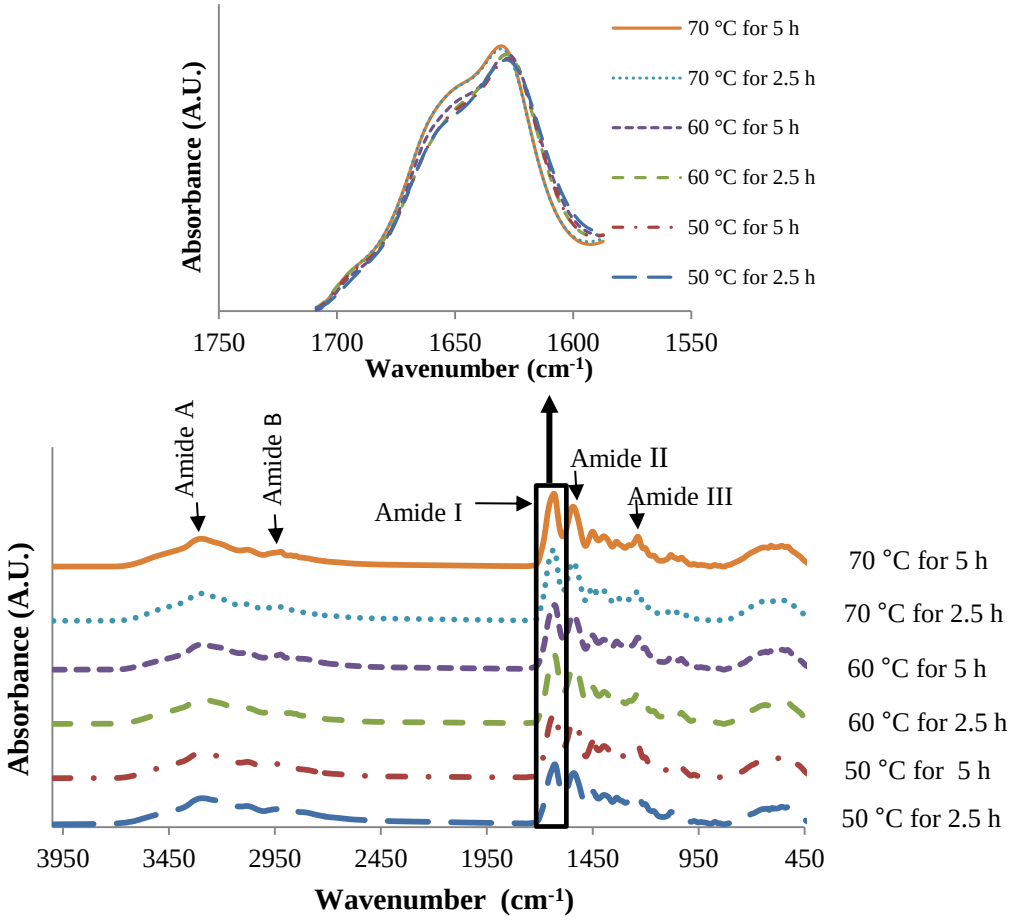

Figure 18 FTIR spectra of gelatin from goat skin extracted at different temperatures for various times

The amide-A band, arising from the stretching vibrations of N–H group, were observed at 3291-3297 cm^{-1}. The amide-A band is associated with the N–H stretching vibration and shows the existence of H-bonds (Sinthusamran *et al.*, 2014). Normally, a free N–H stretching vibration occurs in the range of 3400– 3440 cm^{-1}. When the N–H group of a peptide is involved in a H-bond, the position is shifted to lower wavenumbers or frequencies (Doyle *et al.*, 1975). The amide-A also tends to join with the CH_2 stretch peak when carboxylic acid groups exist in a dimeric inter-molecular interaction (Doyle *et al.*, 1975). The shift to the lower wavenumber of the amide-A was observed in gelatin extracted at higher temperature for a longer time was plausibly associated with higher interaction of free amino group released from the degraded gelatins via Maillard reaction. This could enhance the yellow color of gelatin extracted at higher temperature for longer time (Table 11). The amide B band was observed at 3079-3083 cm^{-1}, corresponding to the asymmetric stretching vibration of =C–H as well as NH^{3+}. Among all samples, gelatin extracted for a longer time had the lowest wavenumber for the amide-B peak, suggesting the interaction of NH_3 group between peptide chains (Nagarajan *et al.*, 2012). Thus, the secondary structure and functional group of gelatins obtained from the goat skin were affected by extraction temperatures and times.

4.4.5 Gelling and melting temperatures

The changes in the phase angle (δ) of gelatin solutions during cooling (50 to 5 °C) and subsequent heating (5 to 50 °C) are depicted in Figure 19A and 19B, respectively. All gelatin samples with different extraction conditions formed a gel in the range of 21.18-25.17 °C. Sharp decrease and rapid transition in phase angle during cooling were regarded as the increase in amount of energy that is elastically stored in storage modulus (*G'*) (Kasankala *et al.*, 2007). It was found that gelling temperature of gelatins decreased significantly with increasing extraction times ($p < 0.05$). At the same extraction time, gelling temperature of gelatin gels decreased when the extraction temperature increased ($p < 0.05$). The decrease in gelling point was in agreement with poorer gel strength of gelatin (Figure 17). Nagarajan *et al.* (2012) reported that gelatin extraction conditions had the impact on the physico-chemical properties of gelatin, such as molecular weight distribution, the amount of β- and γ-

components and the free amino acid group. Among all gelatin samples, that extracted at 50 °C for 2.5 h had the highest gelling point (25.17 °C), which was higher than those of fish gelatins from skin of bigeye snapper (10.0 °C) (Binsi *et al.*, 2009) and silver carp (18.7 °C) (Boran *et al.*, 2010). Moreover, gelling temperature of goat skin gelatin was also higher than that of bovine gelatin (21.7 °C) (Kasankala *et al.*, 2007). The result suggested that goat skin gelatin was able to form gel at higher temperature than some mammalian gelatins. Karim and Bhat (2009) reported that melting point and gelling point are governed mainly by imino acid (proline + hydroxyproline) content, molecular weight distribution and also the ratio of α/β chains in the gelatin.

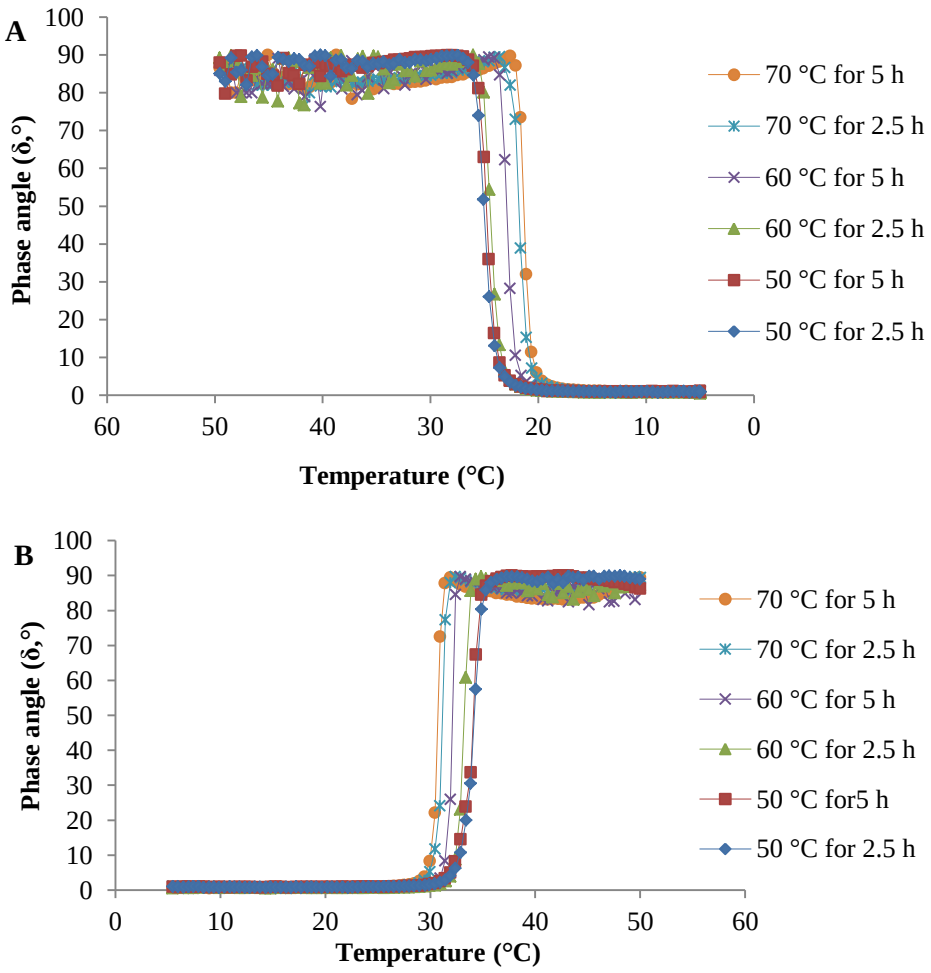

Figure 19 Changes in phase angle (δ, °) of gelatin solution (6.67%, w/v) from goat skin extracted at different temperatures for various times during cooling (A) and subsequent heating (B)

Melting points of gelatins from goat skin extracted with various conditions were found in the range of 30.69-34.12 °C. The decrease in melting points of gelatin gels was found when extraction temperatures and times increased ($p < 0.05$). Gelatin extracted at 50 °C for 2.5 h showed the highest melting point ($p < 0.05$) (34.12 °C). Melting points of goat skin gelatin were higher than those reported for gelatin from bigeye snapper skin (16.8 °C) (Binsi *et al.*, 2009), silver skin (27.1 °C), porcine (31.5 °C) and bovine (30 °C) (Kasankala *et al.*, 2007). Proline plays a crucial role in boosting the formation of polyproline II helix (Ross-Murphy, 1992). Thermal stability of gelatin gel is directly related with pro-rich regions in gelatin molecules (Gómez-Guillén *et al.*, 2002). Apart from imino acid content, the melting point of gelatin also increases with increasing MW (Jamilah and Harvinder, 2002). In the present study, gelatin extracted at higher temperature with lower melting points contained a larger proportion of low MW peptide chains. With higher melting temperature, gel could be retained for a longer time, thereby rendering the better mouth feel when consumed. The gelling and melting temperatures of gelatin depend on species used as raw material, which may have different living environments and habitat temperatures (Gómez-Guillén *et al.*, 2002). Age of animal in conjunction with cross-link formation is another important factor affecting the gelling and melting behavior of gelatin (Hinterwaldner, 1977). Thus, extraction conditions affected gelling and melting temperature of gelatin gel from goat skin.

4.4.6 Microstructures of gelatin gels

The microstructures of gelatin gels from goat skin with different extraction conditions are illustrated in Figure 20. Generally, the conformation and chain length of the proteins in gel matrix determine gelation of gelatin (Benjakul *et al.*, 2009). All gelatin gels were sponge or coral-like in structure. Gelatin extracted at 50 °C for 2.5 h showed the finest gel network with high connectivity of protein strands. Conversely, the larger strands with bigger voids were found in the gelatin gel extracted at higher temperature for an extended time. The coarser network of the gel might be easier to disrupt by the force applied. The fine gel structure of gelatin extracted at lower temperature for shorter time coincided with the higher gel strength (Figure 17). Gelatin extracted at lower temperature for shorter time had longer chain

length due to the lower degradation (Figure 16). As a result, junction zones could be formed to a greater extent. This led to the high interconnectivity between strands in the network. It is well known that the distribution of α-, β- and γ-components is a key factor affecting property of gelatin. The finer gel network was related with the uniform distribution of water (Kittiphattanabawon *et al.*, 2010). The result revealed that the extraction condition had an impact on the molecular weight distribution of proteins or peptides in gelatin, thereby affecting arrangement and interaction of proteins in the gel network.

4.4.7 Color of gelatin gel

Color of the gelatin gel from goat skin with different extraction temperatures and times expressed as L^*, a^* and b^* is shown in Table 11. The lighter color (L^*-values) was observed for gelatin gels extracted for 2.5 h, compared with those of gelatin extracted for 5 h ($p < 0.05$) when the same extraction temperature was used. At the same extraction times, the L^*-values of gelatin gels decreased with increasing extraction temperatures ($p < 0.05$). On the other hand, the increases in both a^*- and b^*- values were observed when the extraction temperatures and times increased ($p < 0.05$). This might be owing to non-enzymatic browning reaction arisen at higher temperature, especially when extraction time increased (Ajandouz and Puigserver, 1999). The increases in yellowness (b^*- value) of gelatin gel from skin extracted at higher temperature for a longer time were in accordance with the shift of Amide A and B to the lower wavenumber (Figure 18). Among all gelatin samples, those extracted at a lowest temperature (50 °C) for a shorter time (2.5 h) had the lowest total difference in color value (ΔE^*) (53.86). These result showed that the extraction conditions affected the gel color of gelatin extracted from goat skin.

4.4.8 Amino acid composition

Amino acid compositions of gelatin from goat skin extracted at 50 °C for 2.5 h, rendering the highest gel strength, are shown in Table 12. Glycine was the

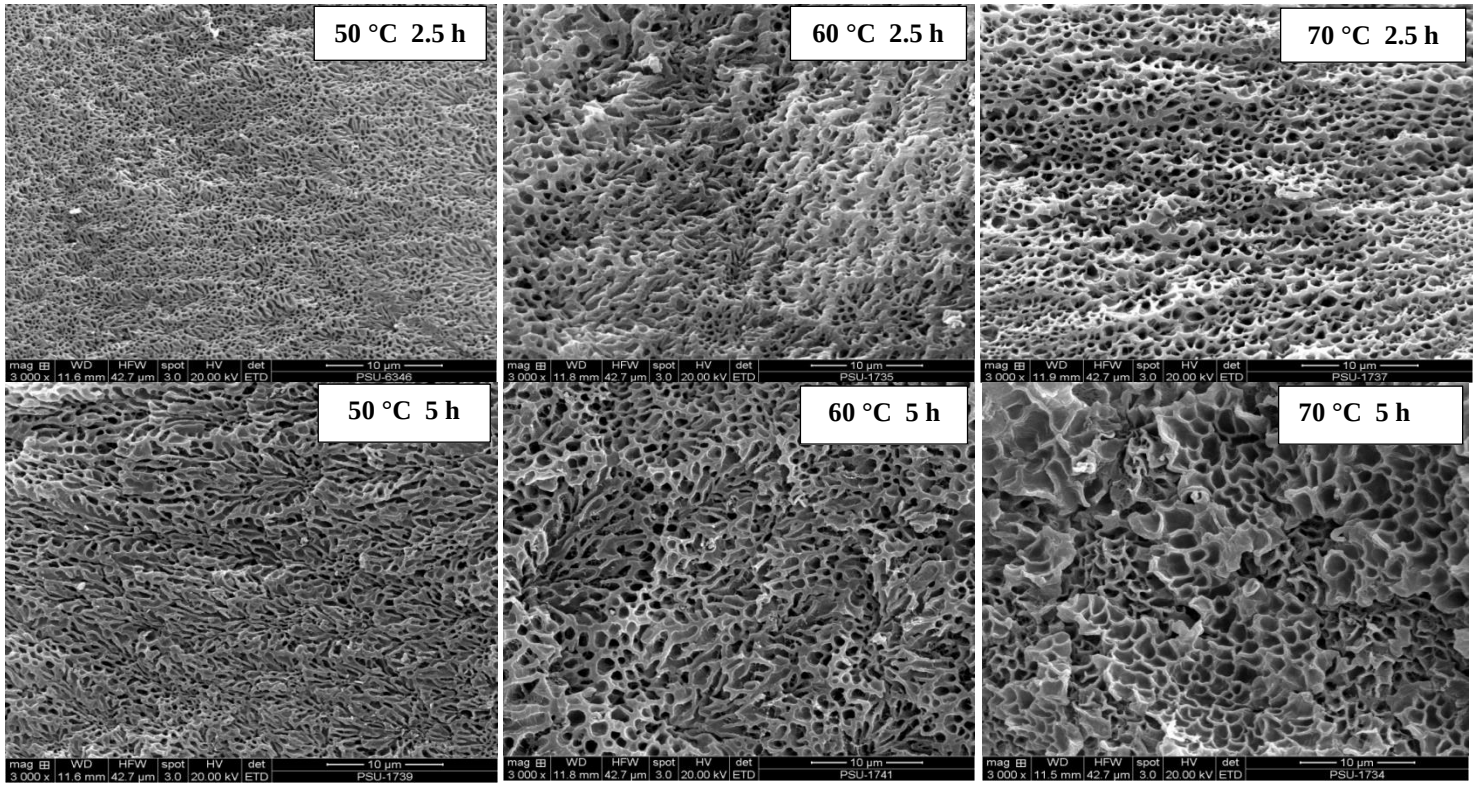

Figure 20 Microstructures of gelatin gel from goat skin extracted at different temperatures for various times. Magnification: 3000x.

major amino acid (330 residues/1000 residues). Gelatin had very low contents of cysteine (1 residues/1000 residues), tyrosine (2 residues/1000 residues) and histidine (4 residues/1000 residues). Glycine generally occurs every third position of α-chain and represents nearly one third of total residues except for 14 amino acids from N-termini and for 10 amino acids from C-termini (Benjakul *et al.*, 2012). For imino acids, gelatin had proline and hydroxyproline of 125 and 100 residues/1000 residues, respectively. The imino acid content of gelatin from goat skin (225 residues/1000 residues) was higher than that reported for gelatin from silver carp waste (197 residues/1000 residues) (Tavakolipour, 2011), grey triggerfish skin (176 residues/1000 residues) (Jellouli *et al.*, 2011), salmon skin (166 residues/1000 residues) and cod skin (154 residues/1000 residues) (Arnesen and Gildberg, 2007). Moreover, it was higher than that found in bovine gelatin (219 residues/1000 residues) (Jellouli *et al.*, 2011) and porcine skin gelatin (199 residues/1000 residues) (Tavakolipour, 2011) (Table 12). Gelatin with higher content of hydroxyproline showed the ability to develop the strong gel structure (Benjakul *et al.*, 2009). OH groups of hydroxyproline might be involved in hydrogen bondings with adjacent chains (Kittiphattanabawon *et al.*, 2010). This could strengthen gel network of gelatin. Additionally, alanine (110 residues/1000 residues and glutamic acid/glutamine (76 residues/1000 residues) were also found at high content. Those amino acids were in the similar range with those found in bovine and porcine gelatin. Therefore, amino acid composition could be another factor determining the properties of gelatin from goat skin.

4.5 Conclusions

Both extraction temperature and time affected the characteristic and properties of gelatin from goat skin. Gelatin from goat skin contained α-chain as the major constituent but both α- and β- chains decreased with increasing extraction temperature and time. The highest gel strength was observed when gelatin was extracted at 50 °C for 2.5 h. Goat skin gelatin had higher gel strength than commercial bovine gelatin.

Table 12 Amino acid compositions of gelatin from goat skin extracted at 50 °C for 2.5 h, compared with those of bovine and porcine gelatins

Amino acids	Residue/1000 residues		
	Goat gelatin	Bovine gelatin*	Porcine gelatin**
Aspartic acid/asparagine	47	44	54
Threonine	19	17	25
Serine	32	29	41
Glutamic acid/glutamine	76	74	81
Glycine	330	341	328
Alanine	110	115	114
Cysteine	1	0	0
Valine	19	21	18
Methionine	4	5	12
Isoleucine	11	11	9
Leucine	25	25	20
Tyrosine	2	1	2
Phenylalanine	12	12	14
Hydroxylysine	6	7	8
Lysine	27	26	22
Histidine	4	5	8
Arginine	49	48	45
Hydroxyproline	100	96	70
Proline	125	123	129
Total	1000	1000	1000
Imino acids	225	219	199

* Jellouli *et al.* (2011)
** Tavakolipour *et al.* (2011)

4.6 References

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CHAPTER 5

CHARACTERISTICS AND GEL PROPERTIES OF GELATIN FROM GOAT SKIN AS AFFECTED BY SPRAY DRYING

5.1 Abstract

Gelatin powder from goat skin prepared by spray drying at various inlet temperatures (160-200 °C) was characterized. Predominant particle sizes were in the range of 4.65-5.14 µm. Gelatin powder was mostly concave in shape with varying sizes, depending on inlet temperatures used. All gelatin powders were creamy whitish. Powder generally became more yellowish as the inlet temperature of spray drying increased ($p < 0.05$). All gelatins contained α- chain as the dominant constituent. Nevertheless, α- chain of gelatin spray-dried at 200 °C almost disappeared. Gel strength of gelatin decreased as the inlet temperature for spray drying increased ($p < 0.05$). Gelatin with inlet temperature of 160 °C had the highest gel strength (260 g) ($p < 0.05$), which was comparable to the freeze-dried counterpart (268 g) ($p > 0.05$). Goat skin gelatin spray-dried with inlet temperatures of 160 or 180 °C had higher gel strength than commercial bovine gelatin ($p < 0.05$). All gelatins had solubility greater than 90% in the wide pH ranges (1-10). Therefore, spray drying with appropriate inlet temperature could be an effective means for production of gelatin powder from goat skin, exhibiting the properties equivalent to commercial bovine gelatin.

5.2 Introduction

Gelatin is the fibrous protein obtained by thermal denaturation or partial hydrolysis of collagenous materials. It has many applications in food and non-food industries (Sae-leaw *et al.*, 2016). Gelatin is mainly produced from bovine and porcine skins and demineralized bones (Mohtar *et al.*, 2010). The global demand of gelatin was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018 (Sheela, 2014). Sources of materials generally determine properties of gelatin such as melting and gelling temperatures and gel strength, etc. (Benjakul *et al.*, 2012). Compared with gelatin from aquatic or marine animals, gelatins from land animals have the better gelling property with superior rheological characteristics (Norland,

1990). Although gelatin has a wide range of applications, the pessimism and strong concerns still persist among consumers, mainly due to religious sentiments (Asher, 1999). Porcine gelatin cannot be used in Kosher and Halal foods, while Hindus do not consume bovine gelatin (Kaewruang *et al.*, 2013). Poultry gelatin has been also concerned, due to avian influenza. Therefore, gelatin from alternative land animals, especially by-products from goat slaughtering, e.g. skin or bone, should be taken into account to serve for increasing demand of gelatin in the world market.

Goat is one of economically important animals raised in Thailand for their meat and milk. The number of goats increases annually since their meat and milk have become promising for consumption, especially for Muslims. When goats are slaughtered, skin generated as by-product including skin accounts for 6.4-11.6% (based on the body weight) (Warmington and Kirton, 1990). The skin can be used as an alternative raw material for gelatin production. Recently, Mad-Ali *et al.* (2015) reported that alkaline pretreatment under the appropriate condition was necessary for gelatin extraction from goat skin.

Drying is a process used for food preservation and several drying methods have been implemented throughout the world. Compared with spray drying, freeze drying is time-consuming and costly. Freeze-drying process is 4–5 times more expensive than spray drying, and approximately 9 times more expensive than a single-stage evaporation process (Hammami and René, 1997). Spray drying has been widely applied in the food industry due to many advantages of the powders gained, especially good quality and low water activity (Ferrari *et al.*, 2012). Additionally, spray drying technology can be a means to remove undesirable odor from gelatin (Sae-leaw *et al.*, 2016). Gelatin hydrolysate from the skin of unicorn leatherjacket *(Aluterus monoceros*) with reduced off-odor was prepared using spray drying (Sai-Ut *et al.*, 2014). During the transformation of liquid feed into dry powder at high temperature, volatile odorous compounds can be eliminated to high extent (Sae-leaw *et al.*, 2016).

However, no information regarding the influence of spray drying conditions on characteristics and properties of gelatin powder from goat skin exists.

Therefore, this study aimed to determine the effect of spray drying at various inlet temperatures on characteristics and properties of gelatin from goat skin.

5.3 Materials and Methods

5.3.1 Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulfate (SDS), Coomassie blue R-250 and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were obtained from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the bloom strength of 150-250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

5.3.2 Collection and preparation of goat skin

Skins from Anglo-Nubian goats with the age of approximately 2 years were collected from a local slaughter house in Chana district, Songkhla province, Thailand. Seven kilograms of goat skins were randomly taken from three goats, pooled and used as the composite sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice (a skin/ice ratio of 1:2, w/w) and transported to the Department of Food Technology, Prince of Songkla University, within 2 h. Upon arrival, the skins were cleaned and washed with running water (26-28 ˚C). Prepared skins were then cut into small pieces (2.5×2.5 cm^2) using knives, placed in polyethylene bags and stored at -20 ˚C until use. The storage time was not longer than 2 months.

5.3.3 Pretreatment of goat skin

Prepared skins were soaked in 0.75 M NaOH solution at a ratio of 1:10 (w/v). During pretreatment of 2 days, the mixtures were left at room temperature (28-30 °C) and stirred manually twice a day. The solution was removed and replaced by the same volume of freshly prepared solution every day. The skins were then transferred on the perforated screen to remove the solution.

Alkali pretreated skins were mixed with 10 volumes of 0.75 M Na_2SO_4 solution. The mixtures were allowed to stand at room temperature for 24 h. Subsequently, the skins were washed with running water until the pH of wash water became neutral or slightly alkaline. After washing, the obtained skins were soaked in 2 M H_2O_2 solution at a ratio of 1:10 (w/v). The mixture was left at 4 °C for 24 h. During soaking, H_2O_2 solution was changed every 12 h. The skin samples were then washed thoroughly three times with 10 volumes of tap water. The prepared skins were subjected to gelatin extraction.

5.3.4 Extraction of gelatin

To extract gelatin, the pretreated skins were placed in distilled water at 50 ˚C with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 2.5 h with a continuous stirring at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The mixture was then filtered using two layers of cheesecloth. The filtrate was further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea).

To clarify the gelatin, the resulting filtrate was mixed with (0.5%, w/v) diatomaceous earth and stirred using an overhead stirrer at a speed of 100 rpm for 30 min. The mixture was then centrifuged at 8000×g at 28 °C using a centrifuge model Avanti J-E (Beckman Coulter, Inc., Palo Alto, CA, USA) for 15 min to remove the debris. The supernatant was subsequently mixed with (0.3%, w/v) activated carbon. The mixture was stirred at room temperature using an overhead stirrer at a speed of 100 rpm for 30 min. The mixture was then centrifuged at 12,000×g. The supernatant with 1.25% solid content (w/v) was collected and subjected to drying.

5.3.5 Drying of gelatin

For spray-drying, gelatin solution (1.25% solid) was dried using a spray dryer (LabPlant SD-06 Basic, North Yorkshire, England) equipped with a spry-drying chamber having 500 mm height and 210 mm diameter and a two-liquid-nozzle

spray nozzle (0.5 mm in size). A cyclone separator, a hot-air blower, and an exhaust blower were equipped. The gelatin solution was fed by a peristaltic pump at 485 mL h^{-1} into the chamber. The drying air (air velocity of 2 ms^{-1}) from the blower in a downward current flow mode was used with the following process conditions: inlet temperatures of 160, 180, and 200 °C, and being atomized by pressure air (2.8 bars). The corresponding outlet temperatures were 80, 91 and 98 °C, respectively.

For freeze-drying, gelatin solution was frozen at -40 °C for 6 h. The frozen samples were subjected to sublimation using a freeze dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark) at -50 °C for 72 h.

Freeze-dried gelatin and gelatin spray-dried at inlet temperatures of 160, 180 and 200 °C had moisture content of 6.04, 4.52, 4.38, and 4.02%, respectively. The gelatin powders were transferred into a ziplock bag and kept in a plastic vacuum box prior to storage at -40 °C. The storage time was not longer than one month. Gelatin samples were subjected to analyses.

5.3.6 Analyses

5.3.6.1 Determination of mean particle size and particle size distribution as well as morphology

Mean particle size and particle size distribution of spray-dried gelatin was measured using a laser light diffraction instrument (Laser Scattering Spectrometer Mastersizer model MAM 5005, Malvern Instruments Ltd., Worcestershire, UK). The powder sample was dispersed in 99.5% ethanol and the particle distribution was monitored during five successive readings. The particle size was expressed as the volume-weighted mean particle diameter (d_{43}), which is the mean diameter of a sphere with the same volume, and is generally used to characterize the particles (Takeungwongtrakul and Benjakul, 2015).

The particle morphology of gelatin powders was visualized using a scanning electron microscopy (SEM). The powders were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA).

The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an accelerating voltage of 20 kV with magnification of 2500×.

5.3.6.2 Determination of color

Color of gelatin powder was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. Chroma (C^*), representing intensity of saturation, and hue angle ($h°$) were calculated using the following equations:

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

$$h° = \tan^{-1}(b^*/a^*)$$

The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was also calculated according to the following equation (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard (L^* = 93.63, a^* = -0.94 and b^* = 0.40).

5.3.6.3 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the method of Laemmli (1970) as modified by Mad-Ali *et al.* (2015). Gelatin samples were dissolved in 5% SDS and the mixtures were incubated at 85 ˚C for 1 h. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris HCl, pH 6.8. containing 4% SDS and 20% glycerol). The mixtures were boiled in boiling water for 2 min. Samples (15 µg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis. Gels were stained with 0.05% (w/v) Coomassie blue R-

250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% acetic acid. High MW markers were used for estimation of MW.

5.3.6.4 Determination of gel strength

Gelatin gel was prepared as per the method of Kittiphattanabawon *et al.* (2010). Gelatin powder was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis.

Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

5.3.6.5 Determination of solubility

Solubility of gelatin powder at different pHs was determined by the method of Ahmad and Benjakul (2011) with a slight modification. The gelatin samples were dissolved in distilled water at 60 °C to obtain a final concentration of 1% and the mixture was stirred at room temperature (26–28 °C) until gelatin powder was completely solubilized. The gelatin solution (8 mL) was added to a centrifuge tube and the pH was adjusted with either 6 M NaOH or 6 M HCl to obtain a final pH ranging from 1 to 10. The volume of solutions was made up to 10 mL by distilled water previously adjusted to the same pH of gelatin solution. The solution was centrifuged at 8,500×g at room temperature for 10 min using a microcentrifuge (MIKRO20, Hettich Zentrifugan, Germany). Protein content in the supernatant was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as a standard. Protein solubility was calculated in comparison with that obtained at the pH giving the highest solubility. The solubility was then expressed as percentages.

5.3.7 Statistical analysis

All experiments were run in triplicate using three different lots of skins. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the Duncan's multiple range test. Statistical analysis was performed using the statistical Package for Social Sciences (SPSS 11.0 for windows: SPSS Inc., Chicago, IL, USA).

5.4 Results and Discussion

5.4.1 Particle size and morphology of gelatin powder

Particle sizes of the gelatin powder prepared using spray drying with different inlet temperatures were expressed as the volume-weighted mean particle diameter (d_{43}) as shown in Table 13 and Figure 21. Gelatin powder with inlet temperatures of 160, 180 and 200 °C had the particle diameter in the range of 0.65-15.65, 0.72-18.86 and 0.79-17.18 µm, respectively. For gelatin spray-dried at 160 °C, it showed a monomodal distribution, indicating homogenous particle, in which mean diameter was 4.8 µm. Nevertheless, a bimodal distribution was observed for gelatin spray-dried at higher temperatures and two peaks, representing two different ranges of particle sizes were noticeable (Figure 21). The major peak, corresponding to the main population, had the lower particle diameters, having mean diameters of 4.6 and 5.1 µm for gelatin spray-dried at 180 and 200 °C, respectively. The minor peaks showed the larger particle size, approximately 36.2-43.6 µm. One of the most important physical parameters of powders is particle size. Particle size has the influence on the flow out of storage bins, the blending of different components, and compaction and segregation of a mixture, where smaller particles remain at the bottom and larger particles are localized at the top. In addition, this property significantly influences the properties of food products, such as aroma, texture and appearance (O'Hagan *et al.*, 2005). According to Rodríguez-Díaz *et al.* (2014), the faster drying rates associated with the higher temperature gradients, produced larger particles than drying with slower rate. Furthermore, very fast drying set up a structure early and did not allow the particles to shrink during drying. Nevertheless, there was no difference in mean

particle size of spray-dried gelatin using different inlet temperatures ($p > 0.05$). For spray drying, the size of the dried particles generally depends on the size of the atomized droplets. The droplet size is affected by the atomization model (rotary or nozzle atomizer), physical properties of the feed solution and feed solid concentration (Kurozawa *et al.*, 2009). In the present study, the same nozzle was used for all samples. Thus, atomized particles played an important role in determining the size of resulting gelatin particles, irrespective of inlet temperatures.

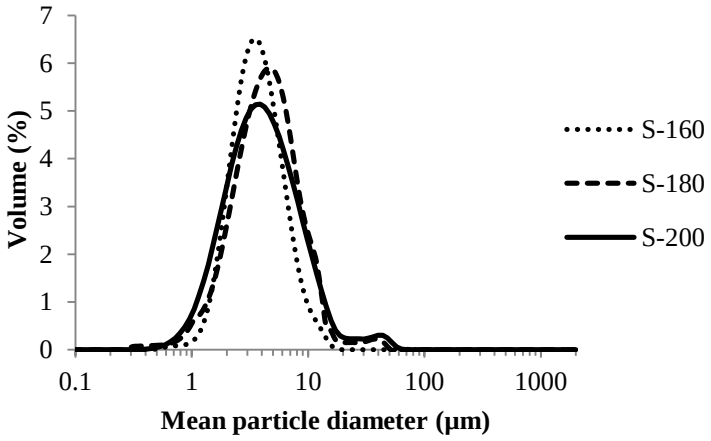

Figure 21 Particle size distribution for the gelatin powders from goat skin prepared by spray-drying at different inlet temperatures. S-160: goat skin gelatin spray-dried at 160 °C, S-180: goat skin gelatin spray-dried at 180 °C, S-200: goat skin gelatin spray-dried at 200 °C

Morphology of gelatin powder obtained from spray drying at different inlet temperatures is displayed in Figure 22. All gelatin powders possessed various sizes and shapes. Gelatin spray-dried at the lowest temperature (160 °C) showed a large proportion of particles having the concavities in shape, which was the characteristic of atomized products after spray drying (Favaro-Trindade *et al.*, 2010). Gelatin samples spray-dried at higher temperature (180 and 200 °C) exhibited a wider distribution of particle size. When drying temperature increased, some particles were spherical in shape with smooth surface. In general, a vacuole (a vapor bubble) could be formed within a particle after a skin was developed on the surface. The surface could inflate (due to the low gas permeability of the surrounding skin) when the

particle temperature exceeded the boiling point and the vapor pressure within the vacuole rose above the local ambient pressure (Hassan and Mumford, 1993). Similar trends were reported for skim milk powder (Nijdam and Langrish, 2005) and whey protein powders (Anandharamakrishnan *et al.*, 2007) obtained from spray drying process. When drying temperature is sufficiently high, moisture is evaporated very quickly and the skin becomes dry and hard. Hollow particle cannot deflate when vapor condenses within the vacuole as the particles move into cooler regions of the dryer (Suhimi and Mohammad, 2011). On the other hand, when drying temperature is lower, the skin remains moist. As a result, the hollow particles can deflate and shrivel as it cools (Nijdam and Langrish, 2005). According to Favaro-Trindade *et al.* (2010), the solvent at the surface is evaporated quickly during spray drying, leading to the formation of a tough shell of solid polymer. The solvent diffusion occurs at a much slower rate than the transfer of heat to the interior of the droplets. Hence, the pressure is built up within the shell, causing the expansion of the droplet. In addition, chemical

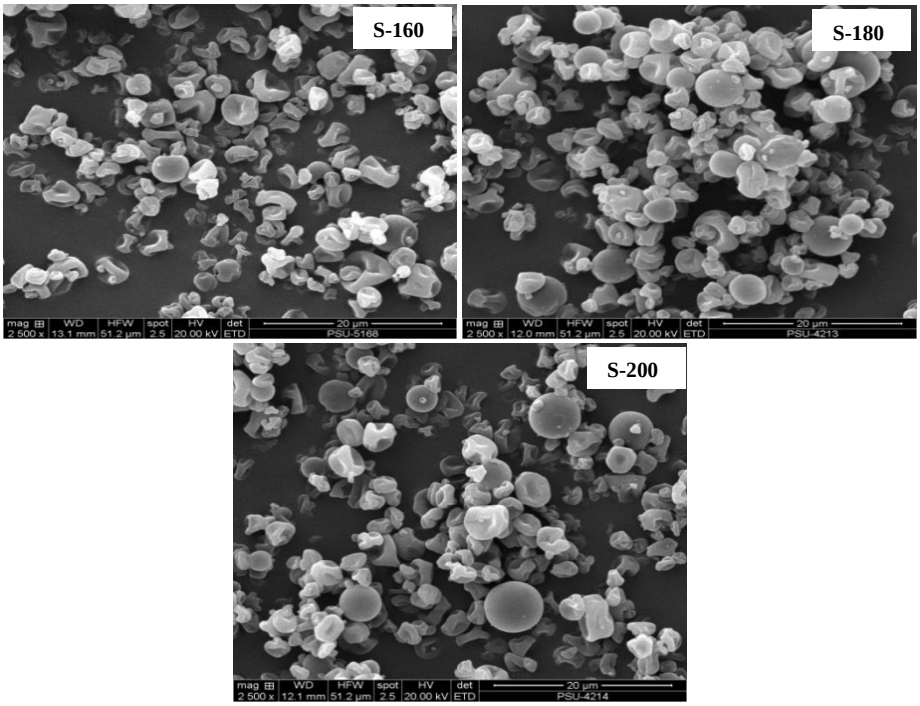

Figure 22 Microstructures of gelatin powders from goat skin prepared by spray-drying at different inlet temperatures. Magnification: 2500×. S-160: goat skin gelatin spray-dried at 160 °C, S-180: goat skin gelatin spray-dried at 180 °C, S-200: goat skin gelatin spray-dried at 200 °C.

compositions might be one of factors influencing the formation of powder structure. Kim *et al.* (2002) reported that fat content was associated with the formation of the shallow dents of whey protein concentrate powder. Dried milk powder with high lactose content was reported to have a highly wrinkled surface (Mistry *et al.*, 1992). The morphology of spray-dried protein particles was also affected by protein formulation. According to Maa *et al.* (1997), spray-dried particles exhibited a smooth surface regardless of the protein-to-lactose ratio, whereas roughness was observed when mannitol was present at >30 % of total solids, due to recrystallization. Protein particles containing trehalose at concentrations >50% were highly agglomerated. The presence of surfactant resulted in noticeably smoother and more spherical particles. Large food particles generally affect the texture and have poor dispersibility. The presence of large particles may be undesirable in most foods, including sweetened condensed milk, ice cream, and chocolate (Favaro-Trindade *et al.*, 2010). Spherical particles are considered to have a higher bulk density, which is preferable for shipping due to the lower transport and packaging costs (Bhandari *et al.*, 2008). For the spray-dried gelatin, some wrinkled particles were formed. The formation of wrinkled surface is a common phenomenon in spray dried powders when a rapid skin or crust formation takes place for droplets during the early stages of drying. The increased flexibility of wall might allow the shrinkage of particle during drying without rupturing the skin, thereby enhancing the wrinkles and roughness of particles (Wang and Langrish, 2010). Drying conditions therefore had an impact on morphology of gelatin powder obtained from goat skin.

5.4.2 Color of gelatin powder

The color of gelatin powder from goat skin spray dried at various inlet temperatures expressed as L^*, a^* and b^* is shown in Table 13. All samples had creamy whitish color. L^*- value (lightness) of gelatin powder decreased but b^*-value (yellowness) increased when inlet temperatures increased ($p < 0.05$). However, no differences in a^*-value were found among all samples ($p > 0.05$). C^*-value (chroma), reflecting the intensity of color saturation, increased with increasing inlet temperatures used ($p < 0.05$). It generally had the positive correlation with b^*-value.

Table 13 Mean particle size and color of gelatin powders obtained from spray drying at various inlet temperatures

Inlet temperatures (°C)	Mean particle size (d_{43}, μm)	Color values					
		*L**	*a**	*b**	*C**	*h°*	Δ*E*
160	4.83 ± 0.67[a]	92.14 ± 0.24[b]	-0.32 ± 0.05[a]	4.71 ± 0.38[a]	4.72 ± 0.37[a]	93.87 ± 0.30[b]	4.33 ± 0.37[a]
180	4.65 ± 0.78[a]	91.70 ± 0.53[b]	-0.30 ± 0.05[a]	5.71 ± 0.17[b]	5.72 ± 0.17[b]	93.04 ± 0.22[ab]	5.38 ± 0.23[b]
200	5.14 ± 1.03[a]	89.90 ± 0.69[a]	-0.30 ± 0.06[a]	7.03 ± 0.28[c]	7.04 ± 0.28[c]	92.39 ± 0.27[a]	7.19 ± 0.09[c]

Values are expressed as mean ± SD (n =3).
Different lowercase letters within the same column indicate significant difference ($p < 0.05$).
Outlet temperatures were 80, 91 and 98 ºC, respectively.

The lower $h°$ (hue angle) of gelatin was observed when the inlet temperature of 200 °C was used, in comparison to that of gelatin with inlet temperature of 160 °C ($p < 0.05$). $h°$ is related with the changes of a^* and b^*, in which a $h°$ of 90° represents pure yellow and a $h°$ of 0° represents pure red (McGuire, 1992). During spray drying at higher temperature, non-enzymatic browning reaction might take place to some extent (Sae-leaw *et al.*, 2016). This was also affirmed by more yellowish hue. Among all gelatin samples, that with inlet temperature of 200 °C showed the highest total difference in color value (ΔE^*) (7.19). These result showed that the spray drying conditions directly affected the color of gelatin powder from goat skin.

5.4.3 Protein patterns

Protein patterns of gelatin powder with different inlet temperatures in comparison with commercial bovine gelatin and freeze-dried gelatin are shown in Figure 23. All gelatin samples contained α-chain with a MW of 130–122 kDa as the major constituent, except gelatin powder prepared using the inlet temperature of 200 °C, in which α-chains almost disappeared. β-component, a covalently cross-liked dimer of α-chain with a MW of 236 kDa, was also found at a lower level in all samples. α-chain and its dimer (β-chain) generally have the MW in the range of 80-130 and 160-250 kDa, respectively(Imeson, 2012). Commercial bovine gelatin consisted of α- and β-chains. The protein patterns of gelatin were similar to those found in gelatin from goat skin reported by Mad-Ali *et al.* (2015). In general, similar protein patterns were found between gelatin powder spray-dried at 160 and 180 °C. Additionally, both samples showed similar pattern to that of freeze-dried gelatin. When gelatin was exposed to the hot air used for spray drying at high temperature, protein degradation more likely took place. Spray-dried gelatin generally had the temperature near to outlet temperatures. At higher outlet temperature, gelatin was more prone to thermal degradation. This was evidenced by the marked disappearance of all components in gelatin spray-dried at 200 °C. Sae-leaw *et al.* (2016) also reported that the degradation peptides with the MW lower than α2-chain were noticeable in gelatin from sea bass skin, subjected to spray drying. The result revealed that the drying conditions had an impact on protein components of the resulting

gelatin, in which the pronounced degradation occurred when gelatin was spray-dried at high temperature.

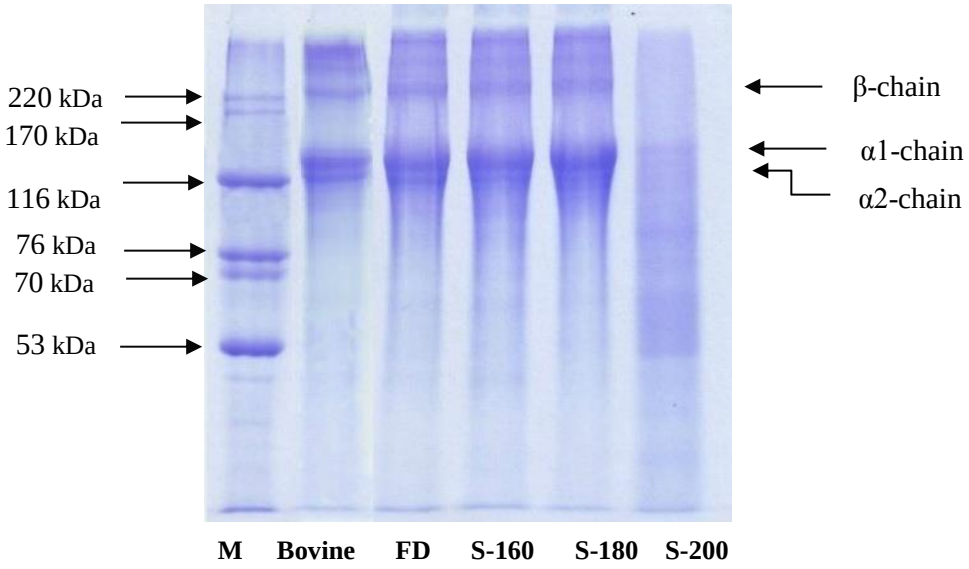

Figure 23 Protein pattern of gelatin powders from goat skin prepared by spray-drying at different inlet temperatures. M denotes high-molecular-weight markers. S-160: goat skin gelatin spray-dried at 160 °C, S-180: goat skin gelatin spray-dried at 180 °C, S-200: goat skin gelatin spray-dried at 200 °C, Bovine: Commercial bovine gelatin, FD: Freeze-dried goat skin gelatin.

5.4.4 Gel strength

Gel strength of gelatin powder obtained from spray drying process at different inlet temperatures is depicted in Figure 24. Gel strength is one of the most important functional properties of gelatin. Gelatin spray-dried at 160 °C had a higher gel strength than those spray-dried at 180 and 200 °C ($p < 0.05$). However, there was no difference in gel strength between gelatin spray-dried at 160°C and freeze-dried counterpart ($p > 0.05$). Gelatins from goat skin, except that spray-dried at 200 °C, had higher gel strength than commercial bovine gelatin ($p < 0.05$). Gel strength of spray-dried gelatin generally decreased as inlet temperature increased ($p < 0.05$). The drastic decrease in gel strength was observed when the inlet temperature of 200 °C was implemented ($p < 0.05$). Higher drying temperature most likely caused protein degradation, thereby producing protein fragments with lowered gelling ability. A

weak gelatin gel was associated with the formation of small fragments (Sae-leaw *et al.*, 2016). Freeze-dried gelatin from shark cartilage and seabass skin exhibited a higher gel strength than spray-dried gelatin (Kwak *et al.*, 2009; Sae-leaw *et al.*, 2016). The marked decrease in gel strength was in accordance with the drastic decreases in α-, β- chains band intensity of gelatin spray-dried at high temperatures, particularly at 200 °C (Figure 23). The amount of α-, β- and γ-components and the amino acid composition of gelatin were reported as the essential factors governing gelation of gelatin (Taheri *et al.*, 2009). Short chains of gelatin have less ability to anneal each other to form junction zone during gelling, thereby hindering the growth of the existing nucleation sites (Benjakul *et al.*, 2012). The difference in gel strength could be due to the differences in intrinsic characteristics, such as amino acid composition and the ratio of α/β chains present in the gelatin (Badii and Howell, 2006). The location of imino acids in the peptide chain also affects gel formation (Benjakul *et al.*, 2012). Thus, spray drying at 160 °C yielded gelatin powder with gelling ability equivalent to freeze-dried gelatin.

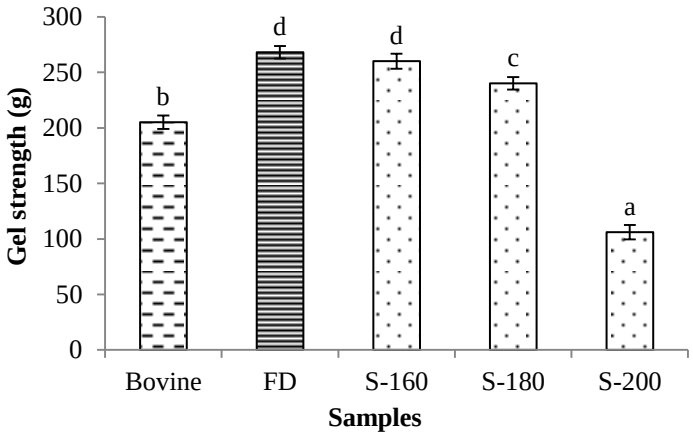

Figure 24 Gel strength of gelatin powders from goat skin prepared by spray-drying at different inlet temperatures. Bars represent the standard deviation (n = 3). Different lowercase letters on the bars indicate significant differences ($p < 0.05$). S-160: goat skin gelatin spray-dried at 160 °C, S-180: goat skin gelatin spray-dried at 180 °C, S-200: goat skin gelatin spray-dried at 200 °C, Bovine: Commercial bovine gelatin, FD: Freeze-dried goat skin gelatin.

5.4.5 Protein solubility

The solubility of gelatin powders as affected by inlet temperatures of spray drying is presented in Figure 25. Solubility of freeze-dried gelatin and commercial bovine gelatin was also examined. All gelatins showed high solubility, in which more than 90% solubility was obtained at all pH (1-10) values tested. Among all samples, the solubility of gelatin prepared by freeze drying process was higher than that obtained from spray drying at 160 and 180 °C as well as commercial bovine gelatin ($p < 0.05$). During drying, proteins might be aligned and aggregated to some extent. This led to the decreased solubility of gelatin powder. Nonetheless, the highest solubility was observed for gelatin spray-dried at 200 °C. Gelatin spray-dried at the extremely high inlet temperatures was degraded into smaller peptides (Figure 23) with

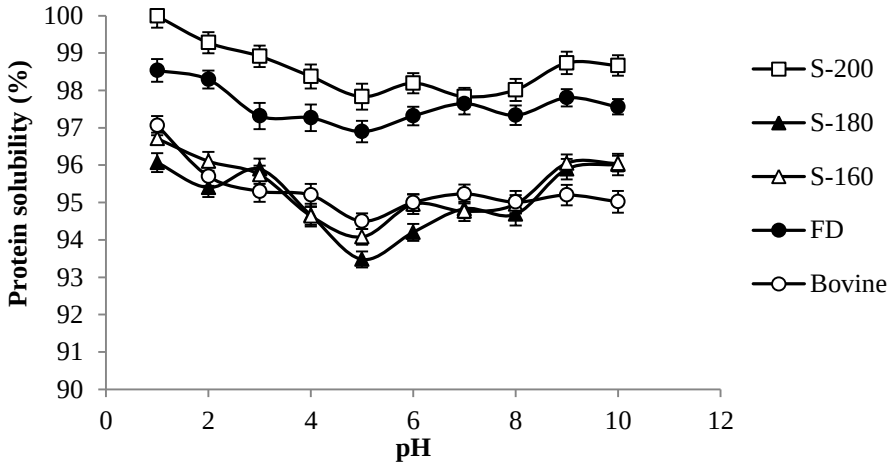

Figure 25 Protein solubility of gelatin powder from goat skin prepared by spray-drying at different inlet temperatures. Bars represent the standard deviation (n = 3). S-160: goat skin gelatin spray-dried at 160 °C, S-180: goat skin gelatin spray-dried at 180 °C, S-200: goat skin gelatin spray-dried at 200 °C, Bovine: Commercial bovine gelatin, FD: Freeze-dried goat skin gelatin.

higher hydrophilicity. When the proteins were hydrolyzed, the carboxyl and amino groups at termini were exposed, leading to the higher hydrophilicity (Kittiphattanabawon *et al.*, 2012). This result was in agreement with Sae-leaw *et al.* (2016) who reported that the solubility of gelatin from seabass skin obtained by spray

drying at higher inlet temperatures was generally higher than that of gelatin spray-dried at lower temperatures. The lowest solubility of all gelatin samples was observed at pH about 5. Normally, the minimum solubility occurs at the isoelectric point (pI) of proteins due to the lack of electrostatic repulsion, which promotes aggregation and precipitation. In general, gelatin type-B or alkaline-processed gelatin has pI of approximately 5 (Benjakul *et al.*, 2009). During alkaline pretreatment of skin, some glutamine and asparagine can be converted to their acidic forms, i.e. glutamic acid and aspartic acid, respectively, via desamidation (Radhika and Sehgal, 1997). In the present study, goat skin was alkali-treated prior to extraction (Mad-Ali *et al.*, 2015). Bovine bone gelatin had the lowest solubility at pH 5 (90.6%). Bovine gelatin has been identified as type-B gelatin with pI of approximately 4.88 (Zhang *et al.*, 2006). The difference in solubility of different gelatins might result from the differences in molecular weight and the content of polar and non-polar groups in amino acids (Ahmad and Benjakul, 2011). Due to high solubility of all gelatin samples in wide pH range, they can be used widely and effectively since solubility is a prerequisite for most functionalities of food proteins.

5.5 Conclusions

Spray drying with higher inlet temperatures (200 ºC) negatively affected the characteristics and properties of gelatin from goat skin, except it yielded gelatin with increased solubility. Gelatin from goat skin contained α- chain as the major constituent but the component decreased with increasing drying temperature. The highest gel strength was observed when gelatin was spray-dried at an inlet temperature of 160 °C. The obtained gelatin powder had higher gel strength than commercial bovine gelatin and its gel strength was comparable to freeze-dried counterpart.

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CHAPTER 6

CHARACTERISTICS AND FUNCTIONAL PROPERTIES OF GELATIN FROM GOAT SKIN AS AFFECTED BY DRYING METHODS

6.1 Abstract

Characteristics and functional properties of spray-dried goat skin gelatin (SDGG) and freeze-dried counterpart (FDGG) were determined, in comparison with commercial bovine gelatin (BG). SDGG had the highest surface hydrophobicity, compared with others ($p < 0.05$). SDGG gel showed slightly higher a^* and b^* values as well as the higher solution turbidity than those of FDGG ($p < 0.05$). Both SDGG and FDGG solutions could set at room temperature (25-28 °C) within 18.52-19.30 min and showed the gelling and melting temperatures of 25.14-25.23 and 34.09-34.18 °C, respectively. Gels from SDGG and FDGG had the denser structure with smaller voids than those from BG. Foaming properties of all gelatins increased with increasing concentrations (1-3%). SDGG had a higher foam expansion and stability than FDGG. Emulsion containing SDGG had the higher droplet size (d_{32}, d_{43}) and flocculation factor than that stabilized by FDGG ($p < 0.05$). The former also showed the lower stability as indicated by the higher coalescence index with lower negative charge after 10 days of storage. Thus, drying methods affected the characteristics and functional properties of goat skin gelatin.

6.2 Introduction

Gelatin is the fibrous protein obtained by thermal denaturation or partial hydrolysis of collagenous materials such as bovine and porcine skins as well as demineralized bones (Mohtar *et al.*, 2010). Gelatin has many applications in food and non-food industries (Sinthusamran *et al.*, 2014). Gelatin has been used as an wetting, foaming and emulsifying agents in food, pharmaceutical, medical and technical applications due to its surface-active properties (Balti *et al.*, 2011). Generally, the properties of gelatin are governed by several factors, such as raw material and the intrinsic parameters, including chemical composition, molecular weight distribution

as well as amino acid composition (Benjakul *et al.*, 2012; Regenstein and Zhou, 2007). Functional properties of gelatin are also affected by pretreatment and extraction condition (Kołodziejska *et al.*, 2004). The global demand of gelatin was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018 (Sheela, 2014). Compared with gelatin from aquatic or marine animals, gelatins from land animals have the better gelling property with superior rheological characteristics (Norland, 1990). Although gelatin has a wide range of applications, the pessimism and strong concerns still persist among consumers, mainly due to religious sentiments (Asher, 1999). Porcine gelatin cannot be used in Kosher and Halal foods, while bovine gelatin is prohibited for Hindus (Kaewruang *et al.*, 2013). Poultry gelatin has been also concerned, due to avian influenza. Thus, gelatin from alternative land animals, especially by-products from goat slaughtering, e.g. skin or bone, should be taken into account to serve for increasing demand of gelatin in the world market. Goat is one of economically important animals raised in Thailand for their meat and milk, especially for Muslims. When goats are slaughtered, skin generated as by-product accounts for 6.4-11.6% (based on the body weight) (Warmington and Kirton, 1990). Goat skin can be used as an alternative raw material for gelatin production, in which the appropriate alkaline pretreatment is required (Mad-Ali *et al.*, 2015).

Spray drying has been widely applied in the food industry due to good quality and low water activity of powder gained (Ferrari *et al.*, 2012). After gelatin is extracted, drying is one important process to bring about the long shelf-life gelatin. Compared with spray drying, freeze drying is time-consuming and costly. Freeze-drying process is 4–5 times more expensive than spray drying (Hammami and René, 1997). Spray drying can be a means to remove undesirable odor from gelatin (Sae-leaw *et al.*, 2016; Sai-Ut *et al.*, 2014). Nevertheless, Sae-leaw *et al.* (2016) reported that drying conditions influenced the properties of gelatin from sea bass skin, especially gelling properties. Drying methods can affect the characteristics and properties of gelatin from goat skin, however such an information has not been reported. Thus, the present study aimed to characterize and determine functional properties of gelatin from goat skin as affected by different drying methods.

6.3 Materials and Methods

6.3.1 Chemicals/gelatin

All chemicals were of analytical grade. 1-anilinonaphthalene-8-sulfonic acid (ANS) and glutaraldehyde were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Food grade bovine bone gelatin with the bloom strength of 150-250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

6.3.2 Collection and preparation of goat skins

Skins from Anglo-Nubian goats with the age of approximately 2 years were collected from a local slaughter house in Chana district, Songkhla province, Thailand. Seven kilograms of goat skins were randomly taken from three goats, pooled and used as the composite sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice (a skin/ice ratio of 1:2, w/w) and transported to the Department of Food Technology, Prince of Songkla University, within 2 h. Upon arrival, the skins were cleaned and washed with running water (26-28 ˚C). Prepared skins were then cut into small pieces (2.5×2.5 cm^2) using knives, placed in polyethylene bags and stored at -20 ˚C until use. The storage time was not longer than 2 months. Before use, the frozen skins were thawed using a tap water (26-28 °C) for 15 min.

6.3.3 Pretreatment of goat skins

Prepared skins were pretreated with 0.75 M NaOH solution at a ratio of 1:10 (w/v) at room temperature (25-28 °C). The mixture was stirred manually twice a day. Alkaline solution was removed and replaced by the same volume of freshly prepared solution every day. The skins were then transferred on the perforated screen to remove the solution.

Alkali-pretreated skins were subsequently mixed with 10 volumes of 0.75 M Na_2SO_4 solution. The mixture was allowed to stand at room temperature for 24 h. Subsequently, the skins were washed with running water until the pH of wash

water became neutral or slightly alkaline. After washing, the obtained skins were soaked in 2 M H_2O_2 solution at a ratio of 1:10 (w/v). The mixture was left at 4 °C for 24 h. During soaking, H_2O_2 solution was changed every 12 h. The skin samples were then washed thoroughly three times with 10 volumes of tap water. The obtained skins were subjected to gelatin extraction.

6.3.4 Extraction of gelatins

To extract gelatin, the pretreated skins were placed in distilled water (50 ˚C) with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 2.5 h with a continuous stirring at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The mixture was then filtered using two layers of cheesecloth. The filtrate was further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea).

To clarify the gelatin, the resulting filtrate was mixed with diatomaceous earth (0.5%, w/v) and stirred using an overhead stirrer at a speed of 100 rpm for 30 min. The mixture was then centrifuged at 8000×g at 28 °C using a centrifuge model Avanti J-E (Beckman Coulter, Inc., Palo Alto, CA, USA) for 15 min to remove the debris. The supernatant was subsequently mixed with activated carbon (0.3%, w/v). The mixture was stirred at room temperature using an overhead stirrer at a speed of 100 rpm for 30 min. The mixture was then centrifuged at 12,000×g. The supernatant with 1.25% solid content (w/v) was collected and subjected to drying.

6.3.5 Drying of gelatin

Clarified gelatin solution was separated into two portions. The first portion was dried using a spray dryer (LabPlant SD-06 Basic, North Yorkshire, England) equipped with a spry-drying chamber having 500 mm height and 210 mm diameter and a two-liquid-nozzle spray nozzle (0.5 mm in size). A cyclone separator, a hot-air blower, and an exhaust blower were equipped. The gelatin solution was fed by a peristaltic pump at 485 mL/h into the chamber, and atomized by hot air (air

velocity of 2 m/s) from the blower in a downward current flow mode, using an inlet temperature of 160 °C, and an atomizing pressure of 2.8 bars. The second portion was subjected to freeze-drying using a freeze dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark) at -50 °C for 72 h.

The obtained gelatins were transferred into a ziplock bag and placed in a plastic vacuum box until used for analyses.

6.3.6 Analyses

6.3.6.1 Proximate analysis

Moisture, ash and fat contents of the dried gelatin samples were determined according to the AOAC method (AOAC, 2000). Protein content was measured by the Kjeldahl method (AOAC, 2000) and a nitrogen conversion factor of 5.4 was used for calculation of protein content (Eastoe and Eastoe, 1952). Hydroxyproline content was determined according to the method of Bergman and Loxley (1963).

6.3.6.2 Determination of protein surface hydrophobicity

Protein surface hydrophobicity was determined by the method of Benjakul *et al.* (1997). Gelatin was dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 mM NaCl to obtain a final protein concentration of 5 g/L. The gelatin solution was diluted to 0.125, 0.25, 0.5, and g/L using the same buffer. The diluted gelatin solutions (4 mL) were well mixed with 20 μL of 8 mM 1- anilinonaphthalene-8-sulfonic acid (ANS) in 0.1 mM phosphate buffer, pH 7.0. The relative fluorescence intensity of ANS-protein conjugates was measured using a spectrofluorometer (RF-15001, Shimadzu, Kyoto, Japan) at the excitation wavelength of 374 nm and the emission wavelength of 485 nm. Protein surface hydrophobicity was calculated from initial slopes of plots of relative fluorescence intensity versus protein concentration (g/L) using a linear regression analysis. The initial slope was referred to as S_0ANS.

6.3.6.3 Determination of ζ-potential

Gelatin samples were dissolved in distilled water at a concentration of 0.5 mg/mL. The mixtures were stirred at room temperature for 6 h. The ζ-potential of each sample (20 mL) was measured using a zeta potential analyzer (ZetaPALS, Brookhaven Instruments Co., Holtsville, NY, USA). ζ-Potential of samples adjusted to different pHs with 1.0 M nitric acid or 1.0 M KOH using an autotitrator (BIZTU, Brookhaven Instruments Co., Holtsville, New York, USA) was determined. The pI was estimated from pH rendering ζ-potential of zero.

6.3.6.4 Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated l-alanine tri-glycine sulfate (DLATGS) detector. The horizontal attenuated total reflectance (HATR) accessory was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–450 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were rationed against a background spectrum recorded from the clean empty cell at 25 °C. Deconvolution was performed on the average spectra for the amide-A, amide-B, amide-I and amide II bands using a resolution enhancement factor of 1.8 and full height band width of 13 cm^{-1}. Analysis of spectral data was carried out using the OPUS 3.0 data collection software program (Bruker, Ettlingen, Germany).

6.3.6.5 Determination of gelling properties

6.3.6.5.1 Gel strength

Gelatin gel was prepared as per the method of Kittiphattanabawon *et al.* (2010). Gelatin sample was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely. Gelatin solution was transferred to a cylindrical mold with 3 cm diameter

and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis.

Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

6.3.6.5.2 Color of gel

The color of gelatin gels (6.67% w/v) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated as described by Mad-Ali *et al.* (2015).

6.3.6.5.3 Turbidity of gelatin solution

The turbidity of gelatin solution (6.67%, w/v) was determined according to the method of Fernández-Díaz *et al.* (2001). The turbidity of gelatin solutions was measured by reading the absorbance at 360 nm using a double-beam spectrophotometer (model UV-1601, Shimadzu, Kyoto, Japan).

6.3.6.5.4 Setting time for gel formation

The setting time of gelatin solution was determined at 4 °C and 25 °C, according to the method of Sinthusamran *et al.* (2014). The gelatin solution (6.67%, w/v) was prepared in the same manner as described previously. The solution (2 mL) was transferred to thin wall test tube (diameter of 12 mm and length of 75 mm) (PYREX®, Corning, NY, USA) and preheated at 60 °C for 10 min, followed by incubation in a water bath with temperatures of 4 and 25 °C. An aluminum needle with the diameter and length of 0.1 and 25 cm, respectively, was inserted manually into the gelatin solution and raised every 10 s. The time at which the needle could not

detach from the gelatin sample was recorded as the setting time. The setting time was expressed in min.

6.3.6.5.5 Gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of Mad-Ali *et al.* (2015) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). Gelatin solution (6.67%, w/v) was preheated at 35 °C for 30 min. The measuring geometry used was 3.5 cm parallel plate with the gap of 1.0 mm. The measurement was performed at a scan rate of 0.5 °C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 50 to 5 °C and heating from 5 to 50 °C. The gelling and melting temperatures were designated, where tan δ became 1 or δ was 45°.

6.3.6.5.6 Microstructure of gelatin gel

Microstructure of gelatin gel (6.67%, w/v) was visualized using a scanning electron microscopy (SEM). Gelatin gel having a thickness of 2-3 mm was fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100 % (v/v). The samples were subjected to critical point drying. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 15 kV.

6.3.6.6 Determination of foaming properties

Foam expansion (FE) and foam stability (FS) of gelatin solutions were determined as described by Shahidi *et al.* (1995) with a slight modification. Gelatin solutions (1%, 2% and 3%, w/v) were transferred into 100 mL cylinders and homogenized at 13,400 rpm using an IKA Labortechnik homogenizer (Selangor, Malaysia) for 1 min at room temperature (25-26 °C). The sample was allowed to stand for 0 and 60 min. FE and FS were then calculated using the following equations:

$$FE\ (\%) = (V_T/V_0) \times 100$$

$$FS\ (\%) = (V_t/V_0) \times 100$$

where V_T is total volume after whipping; V_0 is the original volume before whipping and V_t is the total volume after standing at room temperature for 60 min.

6.3.6.7 Determination of emulsifying properties

Oil-in-water emulsions were prepared as described by Aewsiri *et al.* (2013). Gelatins were dissolved in distilled water to obtain a final concentration of 2% (w/v). Emulsions were prepared by homogenizing the mixture of sunflower oil and solution of gelatin at a ratio of 1:9 (v/v) at a speed of 10,000 rpm for 2 min. The coarse emulsions were then passed through a two stage high-pressure homogenizer (M-110P, Microfluidizer, Newton, MA, USA) at 3,000 psi for 3 passes. NaN_3 (0.02%, w/w) was added to the emulsions as an antimicrobial agent. All emulsions were then stored at room temperature (25-28 °C) for 10 days. The samples were taken at day 0, 5 and 10 for analyses.

6.3.6.7.1 Oil droplet size

Droplet size distribution of emulsions was determined using a ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). Prior to analysis, emulsion was 100-fold diluted with 1% (w/v) sodium dodecyl sulfate (SDS) solution in order to dissociate flocculated droplets. The surface-weighted mean (d_{32}) and the volume-weighted mean particle diameters (d_{43}) of the emulsion droplets were measured using the following equations:

$$d_{32} = \frac{\sum n_i d_i^3}{\sum nidi^2}$$

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i\, d_i^3}$$

where n_i and d_i are the number of droplets of a determined size range and the droplet diameter, respectively.

6.3.6.7.2 Flocculation factor and coalescence index

To determine flocculation factor (F_f) and coalescence index (C_i), the emulsions were diluted with distilled water in the presence and absence of 1% (w/v) SDS. F_f and C_i were calculated using the following equations:

$$F_f = \frac{d_{43-SDS}}{d_{43+SDS}}$$

$$C_i = \frac{d_{43+SDS,t} - d_{43+SDS,in}}{d_{43+SDS,in}} \times 100$$

where d_{43+SDS} and d_{43-SDS} are the volume weight distribution of the emulsion droplets in the presence and absence of 1% SDS, respectively. $d_{43+SDS,in}$ is the initial volume weight distribution of the emulsion droplets in the presence of 1% SDS; $d_{43+SDS,t}$ is the volume weight distribution of the emulsion droplets in the presence of 1% SDS at the designated storage time.

6.3.6.7.3 ζ-Potential analysis

The electrical charge (ζ-potential) of oil droplets in the emulsions was determined using a ZetaPlus zeta potential analyzer (Model ZetaPALS, Brookhaven Instruments, Co., Holtsville, NY, USA) at room temperature. The oil-in-water emulsions were diluted 250-fold prior to measurements. The diluted emulsions were mixed thoroughly and then injected into the measurement chamber of the instrument. The ζ-potential was then recorded.

6.3.6.8 Sensory evaluation

Gelatin gel (6.67%, w/v) with 3 cm diameter and 2.5 cm height were incubated at 4 °C for 18 h. They were coded with 3-digit random numbers. Gel samples (8-10 °C) were served on the white paper dishes at room temperature under the fluorescent daylight-type illumination. Thirty non-trained panelists (aged between 20 and 40) were the students and staffs at the Department of Food Technology, who were acquainted with gelatin products. The panelists were asked to evaluate for appearance, color, odor, flavor, texture and overall liking of gel samples using 9-point

hedonic scale (1, extremely dislike; 2, very much dislike; 3, moderately dislike; 4, slightly dislike; 5, neither like nor dislike; 6, slightly like; 7, moderately like; 8, very much like; 9, extremely like) as per the method of Meilgaard *et al.* (2006). Between the samples, the panelists were asked to rinse their mouth with distilled water.

6.3.7 Statistical analysis

Experiments were run in triplicate using three different lots of samples. The data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 11.0 for windows: SPSS Inc., Chicago, IL, USA).

6.4 Results and Discussion

6.4.1 Proximate composition

SDGG and FDGG had the moisture contents of 4.52 and 6.04%, respectively. Moisture content of BG was 8.52% (Table 14). Based on dry weight, gelatin samples had high protein content (> 95%) with low fat (0.23-0.81%) and ash (0.32-0.49%) contents. The fat in the skins might be leached out to some extent during extraction, thereby being contaminated in the resulting gelatin. BG showed the

Table 14 Proximate composition and hydroxyproline content of gelatin from goat skin with different drying methods

Samples	Moisture (%)	Fat (%)*	Protein (%)*	Ash (%)*	Hydroxyproline content (mg/g)*
SDGG	4.52 ± 0.46^{c}	0.81 ± 0.23^{a}	98.49 ± 0.50^{a}	0.49 ± 0.08^{a}	81.82 ± 0.20^{a}
FDGG	6.04 ± 0.37^{b}	0.78 ± 0.11^{a}	98.57 ± 0.46^{a}	0.44 ± 0.11^{a}	83.66 ± 0.14^{a}
BG	8.52 ± 0.41^{a}	0.23 ± 0.12^{b}	96.76 ± 0.73^{b}	0.32 ± 0.09^{b}	78.67 ± 0.16^{b}

Values are presented as mean ± SD (n=3).
*Dry weight basis
Different lowercase letters in the same column indicate significant difference ($p < 0.05$). SDGG, FDGG and BG represent spray-dried goat gelatin, freeze-dried goat gelatin and commercial bovine gelatin, respectively.

lower protein and ash contents, compared with SDGG and FDGG ($p < 0.05$). Conversely, higher fat content was found in gelatin from goat skin ($p < 0.05$). Nevertheless, no differences in all components were observed between SDGG and FDGG ($p > 0.05$). Different raw materials and process used, especially clarification, might lead to varying chemical compositions in resulting gelatins. It was noted that all gelatins had the lower ash content than the recommended maximum value (2.6%) (Jones, 1977) and the limit given for edible gelatin (2%) (GME, 2005). Higher hydroxyproline content was found for gelatin from goat skin than that of BG ($p < 0.05$). This was in accordance with higher protein content in the former. The result suggested that gelatin from goat skin showed higher collagenous substances than BG.

6.4.2 Protein surface hydrophobicity

Surface hydrophobicity expressed as S_0ANS of SDGG and FDGG in comparison with BG samples is shown in Table 15. Among all gelatins, BG showed the lowest S_0ANS ($p < 0.05$). SDGG had a higher S_0ANS than FDGG ($p < 0.05$). When gelatin was spray-dried at high temperature, the hydrophobic domains might be exposed and more likely interacted with ANS probe. The increased S_0ANS indicates an exposure of the interior of molecules (Mutilangi *et al.*, 1996). ANS, a fluorescence probe, has been found to bind to hydrophobic amino acids containing an aromatic ring, such as tyrosine, phenylalanine and tryptophan, and can be used to indicate the surface hydrophobicity of proteins (Benjakul *et al.*, 1997). The difference in S_0ANS between gelatins from different species might be due to the differences in amino acid compositions and sequence, as well as chain length of peptide (Kittiphattanabawon *et al.*, 2012). Thus, drying method had the impact on conformational changes of gelatin from goat skin.

6.4.3 ζ-Potentail

The ζ-potential values of different gelatin samples as a function of pH are shown in Figure 26. All gelatin samples were positively charged at acidic pH ranges and became negatively charged under alkaline conditions. Net charge of zero was obtained at pH 4.83, 4.88 and 4.72 for SDGG, FDGG and BG, respectively.

Table 15 Gel strength, surface hydrophobicity, solution turbidity and gel color of gelatin from goat skin with different drying methods

Samples	Gel strength (g)	Surface Hydrophobicity (S_0ANS)	Turbidity (A_{360})	Color			
				*L**	*a**	*b**	Δ*E**
SDGG	258 ± 4.38[a]	3.49 ± 0.10[a]	2.01 ± 0.06[a]	27.92 ± 0.47[b]	-0.15 ± 0.59[a]	-1.35 ± 0.16[b]	64.95 ± 0.53[a]
FDGG	260 ± 4.57[a]	2.35± 0.14[b]	1.81 ± 0.05[b]	27.39 ± 0.17[b]	-0.27 ± 0.03[b]	-2.08 ± 0.15[c]	65.50 ± 0.15[a]
BG	207 ± 3.37[b]	1.78 ±0.16[c]	0.50 ± 0.07[c]	30.55 ± 2.67[a]	-0.15 ± 1.70[a]	14.05 ± 1.70[a]	63.76 ± 1.98[b]

Values are presented as mean ± SD (n=3).
Different lowercase letters in the same column indicate significant difference ($p < 0.05$).
SDGG, FDGG and BG represent spray-dried goat gelatin, freeze-dried goat gelatin and commercial bovine gelatin, respectively.

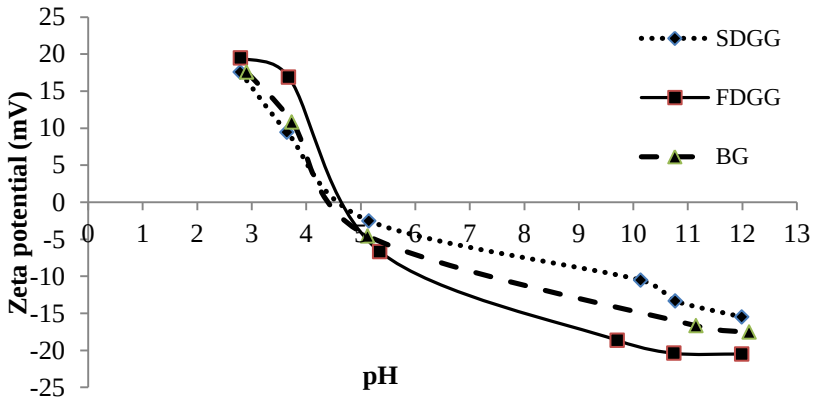

Figure 26 Zeta potential of gelatins from goat skin with different drying methods. SDGG, FDGG and BG represent spray-dried goat skin gelatin, freeze-dried goat skin gelatin and commercial bovine gelatin, respectively.

Those pHs were presumably isoelectric points (pI) of those gelatin samples. Protein molecules in an aqueous system have zero net charge at their pI, in which the positive charges are balanced out by the negative charges (Nagarajan *et al.*, 2013). Due to the higher amount of acidic amino acids (glutamic acid and aspartic acid) than that of basic amino acids (lysine and arginine) in gelatin from skin of goat (Mad-Ali *et al.*, 2015) and bovine (Gómez-Estaca *et al.*, 2009), pI were found in acidic pH range. During alkaline pretreatment of skin, some glutamine and asparagine can be converted to their acidic forms, i.e. glutamic acid and aspartic acid, respectively, via desamidation (Radhika and Sehgal, 1997). This also led to the increase in acidic amino acids associated with the decrease in pI. Differences in charge characteristics or distributions more likely determined functional properties of gelatins obtained from various sources. However, drying methods had no pronounced effect on pI of gelatin from goat skin.

6.4.4 Fourier transform infrared (FTIR) spectra

FTIR spectra of SDGG and FDGG in comparison with BG are depicted in Figure 27. The amide I band of SDGG, FDGG and BG appeared at 1634.17, 1632.73 and 1629.48 cm^{-1}, respectively, which was in agreement with Yakimets *et al.* (2005) who stated that the absorption peak at 1633 cm^{-1} was characteristic of the

coiled structure of gelatin. The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the C-N stretch, C-C-N deformation and in-plane NH bending modes (Bandekar, 1992). The spectral differences in amide I of different gelatin samples were largely attributed to different conformation of polypeptide chains as well as hydrogen bonding in protein (Uriarte-Montoya *et al.*, 2011). Generally, the amide I peak of SDGG showed slightly higher wavenumber, compared with that of FDGG. This suggested the loss of triple helix due to the enhanced disruption of inter-chain interaction caused by spray drying at high temperature. Nevertheless, no marked difference in intensity of amide I peak between SDGG and FDGG was found. When comparing the amide I between goat skin gelatin and BG, the latter had a lower wavenumber. Additionally, lower amplitude was also observed in BG, suggesting that the changes in molecular order due to the interaction of C=O with adjacent chains (Sinthusamran *et al.*, 2014). The decrease in peak intensity and narrowing of peak area might reflect the association of peptide fragments (Prystupa and Donald, 1996).

The characteristic absorption bands of SDGG, FDGG and BG in the amide II bands were found at the wavenumbers of 1544.25, 1526.39 and 1531.37 cm^{-1}, respectively. The amide II band resulted from an out-of-phase combination of a C-N stretch and in-plane NH deformation modes of the peptide group (Bandekar, 1992). The frequency range of 1550-1520 cm^{-1} is due to amide II with α-helical structure between 1550-1540 cm^{-1} and β-sheets at 1525–1520 cm^{-1} (Hashim *et al.*, 2010). It was noted that the amide II band of FDGG was shifted to the lower wavenumber, compared with SDGG. A shift of the amide II peak to lower wavenumber is associated with the existence of hydrogen bonds (Sinthusamran *et al.*, 2013). During spray drying, hydrogen bonds were destroyed and N-H bonds might be disrupted. Hashim *et al.* (2010) reported that the amide II vibration is caused by deformation of the N–H bonds. Lower amplitude was found in FDGG, compared to SDGG, indicating that N-H was more involved in bonding with the adjacent α-chains (Ahmad and Benjakul, 2011). In the present study, BG showed the lowest amplitude with the lowest wavenumber.

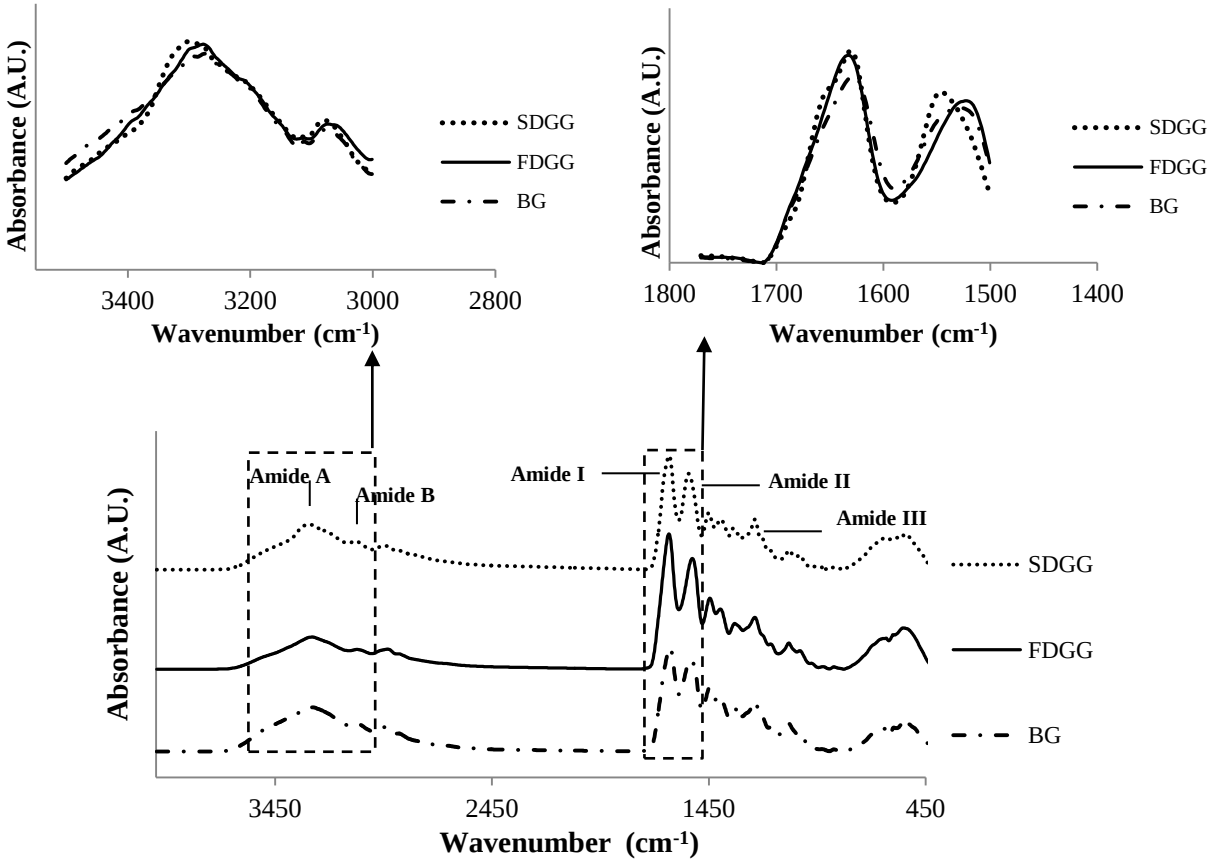

Figure 27 FTIR spectra of gelatin from goat skin with different drying methods. SDGG, FDGG and BG represent spray-dried goat skin gelatin, freeze-dried goat skin gelatin and commercial bovine gelatin, respectively.

Amide III was detected around the wavenumber of 1237.45, 1238.14, 1237.74, cm^{-1} for SDGG, FDGG and BG, respectively. The amide III represented the combination peaks between C–N stretching vibrations and N–H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains (Jackson *et al.*, 1995). No remarkable differences were observed in amide III among all samples.

The amide A band, arising from the stretching vibrations of N–H group, appeared at 3297.05, 3279.66 and 3276.66 cm^{-1} for SDGG, FDGG and BG, respectively. Normally, a free N–H stretching vibration occurs at the wavenumbers of 3400–3440 cm^{-1}. When the N–H group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequencies (Doyle *et al.*, 1975). Lower wavenumber with the slightly lower amplitude was found in FDGG, compared with SDGG. FDGG might form inter-molecular interaction during freeze-drying, particularly via hydrogen bonding. Additionally, the lowest wavenumber with the lowest amplitude was found in BG, compared with the others. Ahmad and Benjakul (2011) reported that the lower amplitude as well as the lower wavenumber at amide A region indicated N-H group of shorter peptide fragments in gelatin sample was involved in hydrogen bonding. The amide B was observed at 3080.38, 3073.26 and 3076.59 cm^{-1} for SDGG, FDGG and BG, respectively, corresponding to asymmetric stretch vibration of =C-H as well as $-NH_3^+$ (Ahmad and Benjakul, 2011). Among all samples, BG showed the lowest wavenumber with the lowest amplitude of amide B peak. FDGG had the lower wavenumber than SDGG, suggesting the higher interaction of $-NH_3^+$ group between peptide chains, occurring during freeze-drying. Therefore, the secondary structure of gelatins was affected by drying methods and condition used. Furthermore, pretreatment process and type of raw material also had the influence on structure and functional groups of gelatin.

6.4.5 Gelling properties

6.4.5.1 Gel strength

Gel strength of different samples is presented in Table 15. SDGG, FDGG and BG had the gel strength of 258, 260 and 207 g, respectively. There was no difference in gel strength between SDGG and FDGG ($p > 0.05$). Lower gel strength of BG might be due to lower imino acid content, especially hydroxyproline content (Table 14). Imino acids are considered to be associated with the stability of the triple-helix of collagen and gel structure through hydrogen bonding between free water molecules and the hydroxyl group of the hydroxyproline in gelatin (Fernández-Díaz *et al.*, 2001). Gelatin from bovine skin was reported to contain a lower content of imino acids than that from goat skin (Gómez-Estaca *et al.*, 2009; Mad-Ali *et al.*, 2015). Additionally, the difference in gel strength could be due to the differences in intrinsic characteristics, such as molecular weight distribution and amino acid composition. Protein degradation fragments may reduce the ability of α-chains to anneal correctly by hindering the growth of the existing nucleation sites (Ledward, 1986). Due to the high gel strength observed for both SDGG and FDGG ($p > 0.05$), the negligible degradation was presumed. Pretreatment condition and type of raw material have the influence on chemical compositions of gelatin, which directly affect the functional properties, especially gelation (Benjakul *et al.*, 2012). This was evidenced by the lower gel strength of BG, compared with goat skin gelatin, regardless of drying methods used.

6.4.5.2 Color of gelatin gel

The color of various gelatin gels expressed as $L*$, a^* and b^* is shown in Table 15. Similar L^*-values (lightness) were observed between both SDGG and FDGG gels ($p > 0.05$), while the higher a^*- (redness) and b^*-values (yellowness) were found for SDGG gel, compared with FDGG counterpart ($p < 0.05$). During spray drying, non-enzymatic browning reaction might arise at high temperature (Sae-leaw *et al.*, 2016). This could enhance the yellow color of the gel. However, the markedly lower b^*-values of both gelatins from goat skin were observed, compared

with BG. Gels of SDGG and FDGG showed the higher ΔE^* with the lower L^*, than that of BG gel. Thus, type of raw material and process of gelatin extraction, especially drying methods, could impact the color of gelatin gel.

6.4.5.3 Turbidity of gelatin solution

Solution turbidity of SDGG and FDGG in comparison with BG is shown in Table 15. The turbidity and dark color of gelatin is commonly caused by inorganic, protein and mucosubstance contaminants, which are not removed during the extraction (Zarai *et al.*, 2012). Both SDGG and FDGG solutions showed the higher turbidity than BG solution ($p < 0.05$). Gelatin manufacture generally has a good process to clarify the impurities from the gelatin solution, such as chemical clarification and filtration processes. When comparing solution turbidity between SDGG and FDGG, the former exhibited a higher turbidity than the latter ($p < 0.05$). When gelatin was spray-dried at high temperature, gelatin molecules from SDGG might undergo conformation changes, which favored the aggregation to some degree, especially via the exposed hydrophobic reactive groups. The highest turbidity of SDGG coincided with the highest surface hydrophobicity (Table 15). When protein is exposed to a high temperature, aggregation is generally induced, contributing to increased turbidity (Johnson and Zabik, 1981). Formation of a coagulum- or a translucent-type gel is related to the molecular properties of protein molecules such as average hydrophobicity and net charge (Damodaran, 1996). Therefore, drying methods affected the turbidity of gelatin solution from goat skin.

6.4.5.4 Setting time for gel formation

The setting times required for the gel formation of different gelatins are presented in Figure 28. The setting time of gelatin solutions at 4 °C was in the ranges of 0.40-0.50 min. The setting time at 4 °C of both SDGG and FDGG were shorter than BG ($p < 0.05$). Nevertheless, no difference in setting time at 4 °C between SDGG and FDGG was found ($p > 0.05$). For the setting time at room temperature (25 °C), all gelatin samples were set within 18.52-34.72 min. A similar result was observed, in comparision with setting at 4 °C. However, a longer setting time was

required at 25 °C. In general, gelatin with low molecular weight peptides yield a longer setting time (Kittiphattanabawon *et al.*, 2010; Sinthusamran *et al.*, 2014). Amino acid composition, especially imino content was also reported to affect functional properties of gelatin (Aewsiri *et al.*, 2008; Benjakul *et al.*, 2009). Shorter setting time was coincidental with higher hydroxyproline content (Table 14) and higher gel strength (Table 15). It was noted that both SDGG and FDGG were able to set at 25 °C and their setting times were 2 fold shorter than that of BG. The nucleated polypeptides of gelatin from goat skin were more likely generated during gelation. Subsequent network formation could be augmented. When comparing gelatin samples between SDGG and FDGG, no differences in setting times were found for both temperatures tested ($p > 0.05$). The results suggested that drying methods had no effect on the time used for network development of gelatin from goat skin.

6.4.5.5 Gelling and melting temperature

The changes in the phase angle (δ) of SDGG and FDGG in comparison with BG during cooling (from 50 to 5 °C) and subsequent heating (from 5 to 50 °C) are depicted in Figure 28B and 28C, respectively. All gelatin samples formed a gel in the temperature range of 22.4-25.2 °C. Sharp decrease and rapid transition in phase angle during cooling were regarded as the increase in amount of energy that is elastically stored in storage modulus (*G'*) (Kasankala *et al.*, 2007). SDGG and FDGG showed higher gelling points than BG ($p < 0.05$). However, no difference in gelling point between SDGG and FDGG was found ($p > 0.05$). Lower gelling point of BG was in agreement with lower hydroxyproline content (Table 14) and poorer gel strength (Table 15) as well as the longer setting time (Figure 28). Gelling point is governed mainly by imino acid content, molecular weight distribution and also the ratio of α/β chains in the gelatin (Karim and Bhat, 2009). Gelatin from goat skin contains a higher content of imino acids than that from bovine skin (Gómez-Estaca *et al.*, 2009; Mad-Ali *et al.*, 2015). Mad-Ali *et al.* (2015) also reported that pretreatment conditions prior to gelatin extraction had an influence on gelling point of resulting gelatin from goat skin.

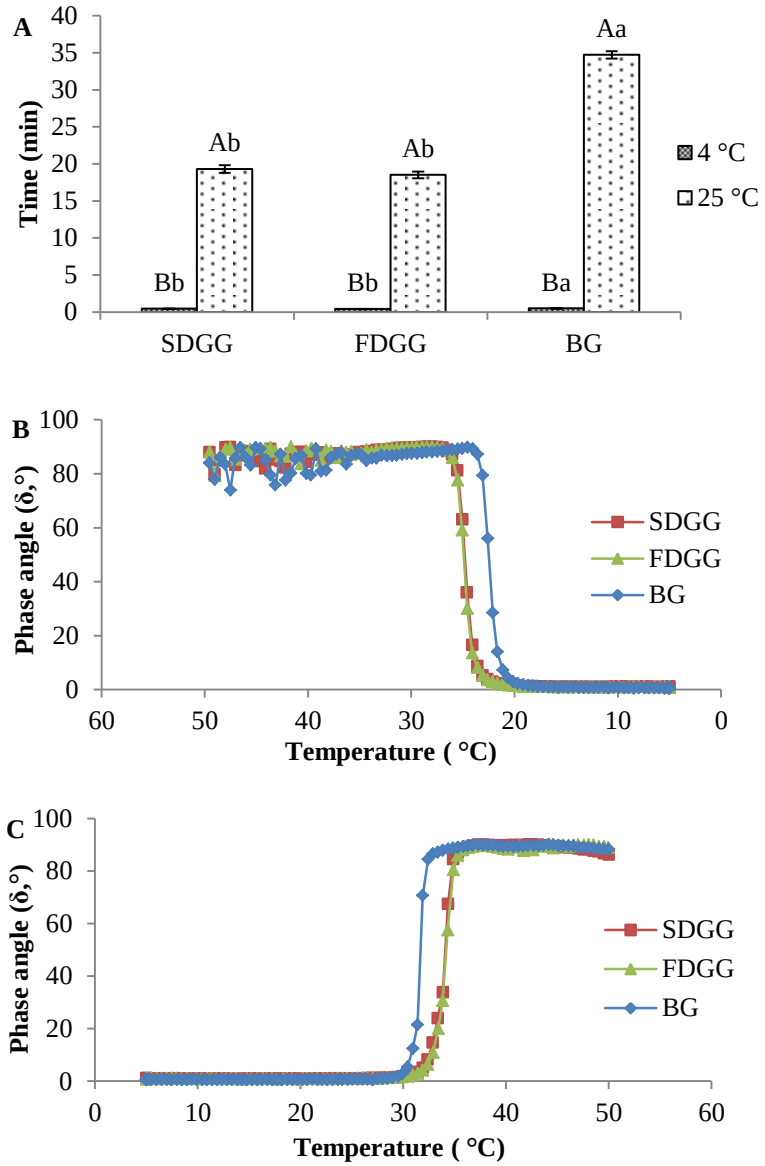

Figure 28 Setting time (A) and changes in phase angle (δ, °) during cooling (B) and subsequent heating (°C) of gelatin solution (6.67%, w/v) from goat skin with different drying methods. SDGG, FDGG and BG represent spray-dried goat skin gelatin, freeze-dried goat skin gelatin and commercial bovine gelatin, respectively. Bars represent the standard deviation (n = 3). Different lowercase letters within the same samples indicate significant differences ($p < 0.05$). Different uppercase letters within the same setting temperature indicate significant differences ($p < 0.05$).

Melting points of all gelatins were in the temperature range of 31.7-34.2 °C. Melting points of goat skin gelatin were higher than that of BG. Thermal stability of gelatin gel is directly related with proline-rich regions in gelatin molecules (Gómez-Guillén *et al.*, 2002). Proline plays a crucial role in promoting the formation of polyproline II helix (Ross-Murphy, 1992). Apart from imino content, the melting point of gelatin also increases with increasing MW (Jamilah and Harvinder, 2002). With higher melting temperature, gel could be retained for a longer time, thereby rendering the better mouth feel when consumed. The gelling and melting temperatures of gelatin depend on species used as raw material, which may have different living environments and habitat temperatures (Gómez-Guillén *et al.*, 2002). Nevertheless, drying methods had no effect on melting point of gelatin from goat skin.

6.4.5.6 Microstructure of gelatin gels

Gel microstructures of goat skin gelatin prepared by spray and freeze drying methods and BG are illustrated in Figure 29. All gelatin gels were sponge or coral-like in structure. Both SDGG and FDGG showed the fine and dense gel network with high connectivity of protein strands. The fine and ordered structure of goat skin gelatin gel was in agreement with high gel strength (Table 15). Spray drying might not show the negative effect on the chain length of protein. On the other hand, the larger strands with bigger voids were found in the gel of BG. The coarser network of the gel might be easier to disrupt by the force applied. The coarser gel structure of BG was coincidental with the lower gel strength (Table 15). It is well known that the distribution of α-, β- and γ-chains is an important factor affecting property of gelatin (Sinthusamran *et al.*, 2014). In addition, hydroxyproline is associated with gel formation via initiation of nucleation zones via hydrogen bonding through its –OH group (Kittiphattanabawon *et al.*, 2010). Coarser gel structure of BG coincided with lower hydroxyproline content, compared with the others (Table14). The formation of gel network also depended on pretreatment and extraction conditions (Yang *et al.*, 2008) as well as the types of raw material (Benjakul *et al.*, 2012). Therefore, the arrangement and association of molecules in the gel matrix of gelatin from goat skin was not affected by drying methods. However, types of raw material directly contributed to gel network of gelatins.

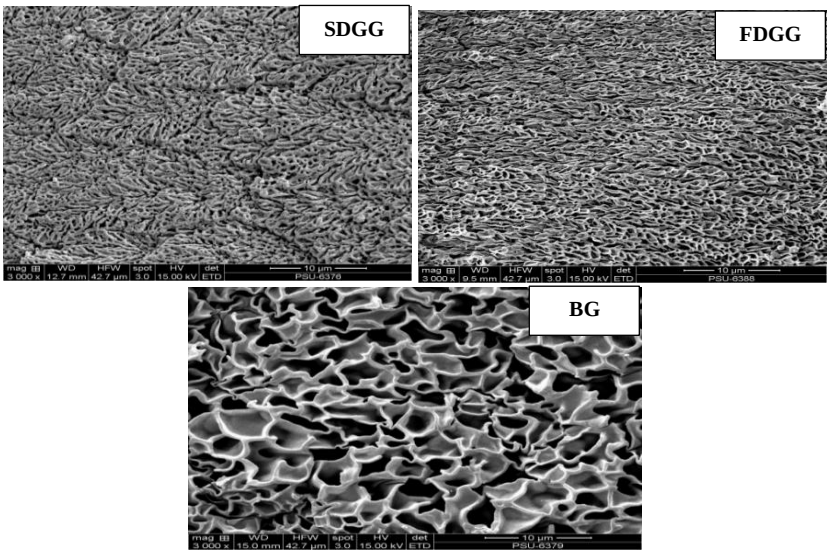

Figure 29 Microstructures of gelatin gel from goat skin with different drying methods. SDGG, FDGG and BG represent spray-dried goat skin gelatin, freeze-dried goat skin gelatin and commercial bovine gelatin, respectively.

6.4.6 Foaming property

Foam expansion (FE) and foam stability (FS) of SDGG and FDGG in comparison with BG at different concentration (1-3%) are shown in Figure 30. FE of all gelatins increased as the concentration of gelatin increased ($p < 0.05$). Foam with higher protein concentration have the denser and more stable films because of an increased thickness at the interface (Zayas, 1997). The foam forming ability of proteins is generally related to their film-forming ability at air-water interface. Proteins, which rapidly adsorb at the newly-created air-liquid interface during bubbling and undergo unfolding and molecular rearrangement at the interface, exhibit better foaming ability than proteins that adsorb slowly and resist unfolding at the interface (Damodaran, 1997). At the same concentrations used, SDGG showed a higher FE than FDGG samples ($p < 0.05$). The higher foaming ability of the former was in agreement with the higher S_0ANS (Table 15). The positive correlation between surface hydrophobicity of unfolded proteins and foaming characteristics was reported by Townsend and Nakai (1983). The foaming capacity of protein was promoted by exposing more hydrophobic residues and by increasing its ability to decrease surface tension (Mutilangi *et al.*, 1996). For the better adsorption at the air–water interface,

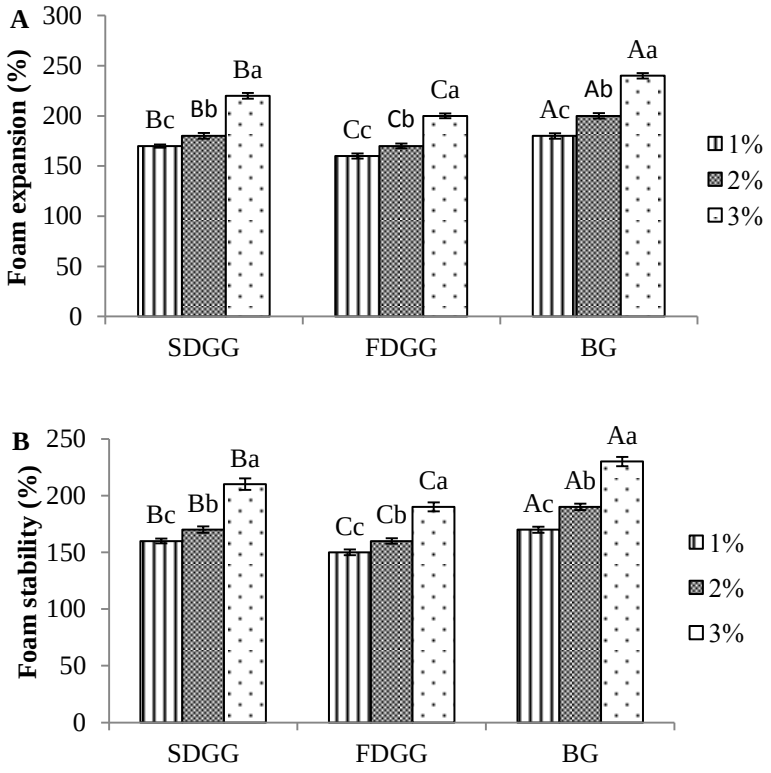

Figure 30 Foam expansion and foam stability of gelatin from goat skin with different drying methods. SDGG, FDGG and BG represent spray-dried goat skin gelatin, freeze-dried goat skin gelatin and commercial bovine gelatin, respectively. Bars represent the standard deviation (n=3). Different lowercase letters within the same samples indicate significant differences ($p < 0.05$). Different uppercase letters within the same gelatin concentration indicate significant differences ($p < 0.05$).

molecules should contain hydrophobic regions (Mutilangi *et al.*, 1996). FS of all gelatin samples increased with increasing protein concentrations ($p < 0.05$). FS is directly impacted by protein concentration, which affects the thickness, mechanical strength and cohesiveness of film (Zayas, 1997). Additionally, foam stability depends on the nature of the film and indicates the extent of protein-protein interaction within the matrix (Mutilangi *et al.*, 1996) and it is also generally positively correlated with molecular weight of peptides. SDGG showed a higher FS than FDGG at the same levels of protein concentrations used ($p < 0.05$). FS was generally in accordance with FE. The stability of foams depends on different parameters, such as the rate of attaining equilibrium surface tension, bulk and surface viscosities, steric stabilization,

and electrical repulsion between the two sides of the foam lamella (Liu *et al.*, 2003). It was noted that BG sample showed higher FS than both SDGG and FDGG at all concentrations used ($p < 0.05$). The result suggested that film of BG developed at the interface was more stable than those of SDGG and FDGG. Furthermore, drying methods played a role in film forming ability and foam stability of gelatin from goat skin.

6.4.7 Emulsifying property

6.4.7.1 Oil droplet size distribution

Particle sizes of oil droplets in emulsion containing SDGG and FDGG, in comparison with those of BG sample, expressed as the surface-weighted mean (d_{32}) and the volume-weighted mean particle diameter (d_{43}), are shown in Table 16. Emulsion stabilized by BG generally had lower d_{32} and d_{43}, compared with those containing SDGG and FDGG ($p < 0.05$). SDGG and FDGG might migrate and adsorb at the surface of the newly formed oil droplets more slowly and less effectively than BG. When comparing droplet size in emulsion stabilized by SDGG and FDGG, emulsion containing SDGG had the higher d_{32} and d_{43} than those containing FDGG during 5-10 days of storage ($p < 0.05$). The d_{32} is inversely related to specific surface area. The smaller d_{32} indicates the higher specific surface area, offering the increase in protein loads at interface of emulsions (Hebishy *et al.*, 2013). The d_{43} can be used as the index of coalescence and flocculation (Hebishy *et al.*, 2013). The increase in d_{43} reflects the assembly of individual droplets into larger flocs (Intarasirisawat *et al.*, 2014). The increases in d_{32} and d_{43} were pronounced for all samples when the storage time was increased up to 10 days ($p < 0.05$). This indicated the instability of emulsion, where the emulsion was collapsed via coalescence as well as the Ostwarld ripening phenomenon or assembly of individual droplets by flocculation, was increased as storage time increased. With longer storage time, oil droplets were prone to align themselves closely. This led to the enhanced flocculation and coalescence. The results suggested that drying methods had the influence on emulsion stability of goat skin gelatin.

Table 16 Particle size, flocculation factor, coalescence index and ζ-potential of oil droplets in emulsions containing gelatin from goat skin with different drying methods

Samples	Storage time (day)	d_{32} (μm)	d_{43} (μm)	Flocculation factor (F_f)	Coalescence index (C_i)	ζ-Potential (mV)
SDGG	0	0.74 ± 0.03^{Ac}	0.74 ± 0.03^{Ac}	1.03 ± 0.03^{Ac}	-	-21.45 ± 0.75^{Ac}
	5	0.89 ± 0.01^{Ab}	0.90 ± 0.02^{Ab}	1.37 ± 0.03^{Ab}	21.62 ± 1.57^{Ab}	-15.38 ± 0.32^{Ab}
	10	1.01 ± 0.01^{Aa}	1.01 ± 0.01^{Aa}	1.56 ± 0.04^{Aa}	36.49 ± 1.45^{Aa}	-14.40 ± 0.12^{Aa}
FDGG	0	0.74 ± 0.01^{Ac}	0.75 ± 0.01^{Ac}	1.03 ± 0.05^{Ac}	-	-20.12 ± 0.61^{Ac}
	5	0.87 ± 0.03^{Bb}	0.87 ± 0.02^{Bb}	1.29 ± 0.02^{Bb}	16.00 ± 1.25^{Bb}	-17.28 ± 1.06^{Bb}
	10	0.97 ± 0.03^{Ba}	0.98 ± 0.03^{Ba}	1.44 ± 0.02^{Ba}	30.67 ± 1.32^{Ba}	-16.05 ± 1.03^{Ba}
BG	0	0.68 ± 0.02^{Bc}	0.68 ± 0.02^{Bc}	1.01 ± 0.03^{Ac}	-	-20.52 ± 0.39^{Ac}
	5	0.74 ± 0.02^{Cb}	0.76 ± 0.02^{Cb}	1.20 ± 0.02^{Cb}	11.19 ± 1.36^{Cb}	-18.22 ± 0.28^{Cb}
	10	0.81 ± 0.01^{Ca}	0.86 ± 0.01^{Ca}	1.31 ± 0.03^{Ca}	25.82 ± 1.72^{Ca}	-17.67 ± 0.92^{Ca}

Values are presented as mean ± SD (n=3).
Different lowercase letters in the same column with in the same sample indicate significant difference ($p < 0.05$).
Different uppercase letters in the same column with in the same storage time indicate significant difference ($p < 0.05$).
SDGG, FDGG and BG represent spray-dried goat gelatin, freeze-dried goat gelatin and commercial bovine gelatin, respectively.

6.4.7.2 Flocculation and coalescence

Flocculation factor (F_f) and coalescence index (C_i) of emulsion throughout 10 days of storage at room temperature are shown in Table 16. During storage, all emulsion samples had the increase in F_f and C_i ($p < 0.05$). This result was coincidental with the increases in d_{32} and d_{43}. FDGG showed the lower F_f and C_i, compared with SDGG ($p < 0.05$). *Ff* and C_i of emulsion containing goat skin gelatin were higher than that of BG at all storage times tested ($p < 0.05$). When the repulsive forces between droplets were not sufficiently strong, emulsion more likely underwent flocculation. In addition, when adhesion energies were large enough, the adhesion could be boosted (Takeungwongtrakul *et al.*, 2014). Coalescence takes place when two or more oil droplets approach together and join together to form a larger one after the interfacial membrane is ruptured. The process is irreversible and contribute to the instability of emulsion (Takeungwongtrakul *et al.*, 2014). In the present study, the higher C_i was found in emulsion containing SDGG for all storage times, in comparison with that of FDGG stabilized emulsion ($p < 0.05$). The result reconfirmed the impact of drying methods on emulsion stability of gelatin from goat skin.

6.4.7.3 ζ-Potential

ζ-Potential of emulsions containing different gelatins as a function of storage time is presented in Table 16. ζ-potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed droplet. This value is related to the stability of emulsion (Takeungwongtrakul *et al.*, 2014). All emulsion samples had ζ-potential values higher than −20 mV at 0 day of storage. Negatively charged residues on oil droplet regulated by gelatins surrounding the oil droplets mostly contributed to repulsion between droplets, thereby lowering coalescence. With increasing storage time, ζ-potential of all samples decreased. The layers of protein surrounding droplets might undergo aggregation via ionic interaction during the extended storage as indicated by the change in ζ- potential. The insufficient electrostatic repulsion might result in the development of flocculation and coalescence. After 10 days of storage, emulsion containing SDGG had the lowest

negative charge ($p < 0.05$). Emulsion containing BG had the highest ζ- potential after 10 days of storage, in which the highest repulsion between droplets was postulated. This resulted in the lowest C_i. Thus, the stability of emulsion were governed by ζ-potential surrounding droplets, which was more likely associated with the charge of protein films at the interface. Drying methods therefore showed the impact on the stability of emulsion stabilized by goat skin gelatin.

6.4.8 Likeness score

Likeness score of gel from SDGG and FDGG, in comparison with BG is shown in Table 17. Gels from goat skin generally had the lower likeness scores for all attributes than those from BG ($p < 0.05$), regardless of drying methods, except for texture likeness. The higher texture likeness of FDGG was observed, compared with BG ($p < 0.05$). This result was in accordance with the higher gel strength (Table 15). Freeze drying at low temperature might not induce the conformational change or aggregation of gelatin. Thus, the stronger gel was developed and higher likeness score was noted. For gels from goat skin gelatin, no differences in all attributes were observed between SDGG and FDGG ($p > 0.05$). In the present study, clarification using diatomaceous earth, followed by activated carbon was carried out. Pigments and off-flavor compounds might be eliminated to some extent. The spray drying process rendered gelatin from goat skin yielding the gel with overall likeness equivalent to freeze-dried gelatin. Therefore, drying methods had no impact on sensory properties of gelatin from goat skin.

Table 17 Likeness score of gelatin gel from goat skin with different drying methods

Samples	Appearance	Color	Odor	Flavor	Texture	Overall
SDGG	6.78 ± 1.04^{b}	6.96 ± 1.02^{b}	6.48 ± 1.27^{b}	6.18 ± 1.18^{b}	7.05 ± 0.88^{ab}	6.73 ± 0.70^{b}
FDGG	6.92 ± 0.88^{b}	7.00 ± 0.93^{b}	6.41 ± 1.30^{b}	6.19 ± 1.13^{b}	7.22 ± 1.03^{a}	6.69 ± 0.80^{b}
BG	7.63 ± 0.88^{a}	7.42 ± 1.02^{a}	6.83 ± 1.34^{a}	6.70 ± 1.18^{a}	6.97 ± 0.87^{b}	7.22 ± 0.95^{a}

Values are mean ± SD (n = 3). Different superscripts in the same column denote the significant differences ($p < 0.05$). SDGG, FDGG and BG represent spray-dried goat gelatin, freeze-dried goat gelatin and commercial bovine gelatin, respectively.

6.5 Conclusions

Drying of gelatin using spray drying method had an impact on characteristics and functional properties of resulting gelatin. SDGG had the better characteristics and properties including higher gel strength, shorter setting time as well as higher gelling and melting point than those from BG. SDGG had higher foaming property but lower emulsifying property than FDGG. Therefore, goat skin gelatin could be prepared by spray drying method, in which properties equivalent to commercial bovine gelatin could be obtained, except for sensory properties. Further improvement on color and odor/flavor is however still required for goat skin gelatin.

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CHAPTER 7

SUMMARY AND FUTURE WORKS

7.1 Summary

1. Appropriate pretreatment of goat skin was required prior to extraction. The optimal pretreatment condition included soaking the skin using 0.75 M NaOH, followed by treatment using 0.75M Na_2SO_4 and subsequent bleaching with 2 M H_2O_2.

2. Both extraction temperature and time affected the characteristics and properties of gelatin from goat skin. Degradation of gelatin from goat skin was enhanced with increasing extraction temperature and time. The highest gel strength was observed when gelatin was extracted at 50 °C for 2.5 h.

3. Spray drying conditions played a role in characteristics and gelling properties of gelatin from goat skin. Drying at high inlet temperature (200 °C) caused the pronounced degradation of gelatin. The highest gel strength was observed when gelatin was spray-dried at an inlet temperature of 160 °C. The obtained gelatin powder had higher gel strength, which was comparable to freeze-dried counterpart. Gelatin prepared by spray drying had higher gel strength, shorter setting time as well as higher gelling and melting point than commercial bovine gelatin

4. Spray drying also had an impact on functional properties of resulting gelatin. It had higher foaming property but lower emulsifying property than freeze-dried gelatin.

5. Goat skin gelatin could be prepared by spray drying with inlet temperature of 160 °C, in which properties equivalent to commercial bovine gelatin could be obtained, except for sensory properties.

7.2 Future works

1. Clarification of gelatin from goat skin using appropriate technology for quality improvement should be further studied.
2. Modification of gelatin from goat skin using several methods for property improvement should be investigated.

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List of Publication and Proceedings

Publication

1. Mad-Ali, S., Benjakul, S., Prodpran, T. and Maqsood, S. 2015. Characteristics and gel properties of gelatin from goat skin as affected by pretreatments using sodium sulfate and hydrogen peroxide. J. Sci. Food Agric. DOI: 10. 1002/jsfa.7336.

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