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# Effect of oxidized kiam wood and cashew bark extracts on gel properties of gelatin from cuttlefish skins

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## ABSTRACT

The effects of ethanolic kiam wood extract (EKWE) and ethanolic cashew bark extract (ECBE) oxygenated at different pHs (8, 9 and 10) on gel properties of gelatin from cuttlefish skins were investigated. With the same extract, gel strength of gelatin increased as the pH for oxygenation of the extract increased ( $p < 0.05$ ). The increases in  $a^*$  and  $b^*$ -values of gelatin gels were noticeable with the addition of either extracts oxygenated at increasing pHs ( $p < 0.05$ ). Cuttlefish skin gelatin gels with EKWE or ECBE oxygenated at pH 9 had the fewest free amino groups, suggesting the cross-linking of gelatin via amino groups. The greater number of non-disulfide covalent bonds was noticeable in the gels with extracts oxygenated at higher pH. An increase in gel strength was observed when the concentration of extracts (1–8%, w/w) increased ( $p < 0.05$ ). At the same concentration of extract, gelatin with EKWE had a higher gel strength than those with added ECBE ( $p < 0.05$ ). The  $\alpha^*$ -values of the gels increased as the concentration of oxygenated EKWE or ECBE increased ( $p < 0.05$ ). The larger strands and larger voids in the gel matrix were observed with gels with added oxygenated EKWE or ECBE, compared with the control gel. Therefore, either oxidized extracts could act as a potential gel enhancer of gelatin.

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## 1. Introduction

Gelatin is commercially derived from collagen and the source, age and type of collagen influence the properties of the gelatin (Johnston-Banks, 1990). Gelatin has broad applications in the food, pharmaceutical, cosmetic, and photographic industries. However, limitations still exist among some consumers of gelatin from land animals (Asher, 1999) due to religious constraints. Both Judaism and Islam forbid the consumption of any pork-related products or non-religiously slaughter cattle products, while Hindus do not consume cow-related products. Furthermore, the occurrence of bovine spongiform encephalopathy

(BSE), and foot and mouth disease have led to human health concerns with the use of animal products (Badii & Howell, 2006). The gelatin industry primarily uses mammalian skins and bones as raw materials. By-products from fish processing, such as fish skins have been recognized as a promising alternative material for gelatin extraction (Yang et al., 2007). The effects of the extraction conditions on gelatin yield and the corresponding physical properties have been reported for the skins of many fish species, including channel catfish (Yang, Wang, Zhou, & Regenstein, 2008), megrim (Montero & Gómez-Guillén, 2000), tilapia (Choi & Regenstein, 2000), yellowfin tuna (Cho, Gu, & Kim, 2005), Alaska pollock (Zhou, Mulvaney, & Regenstein, 2006),

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horse mackerel (Badii & Howell, 2006) and skate (Cho, Jahncke, Chin, & Eun, 2006).

Cuttlefish have become an important fishery product in Thailand and are mainly exported around the world. During processing, skins are generated as a byproduct with low market value and are mainly used as animal feed (Aewsiri, Benjakul, & Visessanguan, 2009). The extraction of gelatin from cuttlefish skins could increase its profitability. Aewsiri et al. (2009) used H<sub>2</sub>O<sub>2</sub> as a bleaching agent for pretreatment of cuttlefish skins prior to gelatin extraction and found that such a pretreatment influenced the yield and properties of the resulting gelatin.

Generally, gelatins of fish origin have poorer bloom strength, compared with mammalian gelatins, due to their lower imino acid content (Grossman & Bergman, 1992). Bloom strength of fish gelatin has been improved by chemical modification (glutaraldehyde, genipin, carbodiimides and phenolic compounds) (Chiou et al., 2006; Strauss & Gibson, 2004) or enzyme modification (transglutaminase) (Gómez-Guillén, Sarabia, Solas & Montero, 2001; Kołodziejaska, Kaczorowski, Piotrowska, & Sadowska, 2004; Strauss & Gibson, 2004). Phenolic compounds can interact with proteins through non-covalent and covalent interactions (De Freitas & Mateus, 2001; Rawel, Kroll, and Riese, 2000). Covalent bonding seems to play an important role in protein–phenol interactions, which influences the functional properties of proteins.

The addition of phenolic compounds into gelatin solutions could increase the bloom strength of gelatin (Struss & Gibson, 2004). Recently, Balange and Benjakul (2009) found that the incorporation of tannic acid into mackerel surimi increased its gel strength. Phenolic compounds, especially tannin, are abundant in plants, especially in barks or woods. The use of these extracts from bark or wood containing potential phenolic compounds could be a “natural” means to improve the bloom strength of gelatins from fish or cuttlefish. The information obtained would be of benefit in using the natural extract containing phenolic compounds for strengthening gelatin gels. Due to the presence of large amounts of kiam and cashew products in southern Thailand and tropical countries, they can be used as the potential source of phenolic compounds. Additionally, saw dust, a byproduct of wood cutting, from those trees or others can be used as an alternative source of phenolic compounds. Pieces of wood from the kiam tree and bark from the cashew tree have traditionally been placed in sugar palm sap containers in Thailand to prevent or retard microbial fermentation (Chanthachum & Beuchat, 1997). The incorporation of phenolic compounds from kiam wood and cashew bark extracts may improve the functional properties of gelatins from cuttlefish, especially gel formation. The objective of this study was to investigate the impact of kiam wood and cashew bark extracts oxidized at different pHs on the properties of gelatins from cuttlefish skins.

## 2. Materials and methods

### 2.1. Collection and preparation of cuttlefish skins

Dorsal skin of cuttlefish (*Sepia pharaonis*) was obtained from a dock in Songkhla province, Thailand. Cuttlefish skins were

stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. Upon arrival, cuttlefish skins were washed with tap water and cut into small pieces (1 × 1 cm), placed in polyethylene bags and stored at –20 °C until use. Storage time was no longer than 2 months. Prior to extraction frozen skins were thawed using running water until the core temperature reached 10 °C

### 2.2. Extraction of gelatin

Prior to gelatin extractions, the pretreatment was done to remove the non-collagenous proteins in the skins. The thawed cuttlefish skins were treated with 0.4 M NaOH containing 0.75% H<sub>2</sub>O<sub>2</sub> at a skin/solution ratio of 1:10 (w/v). The mixture was stirred for 3 h at 4 °C using a stirrer equipped with a propeller (IKA<sup>®</sup> Laboratory Equipment, Staufen, Germany). Thereafter, the solutions were drained and the same volume of freshly prepared solution was added. The pretreatment was done 4 times in total. For bleaching, the pretreated skins were soaked in 10% H<sub>2</sub>O<sub>2</sub> for 48 h using a pretreated skin/solution ratio of 1:10 (w/v). The mixtures were stirred continuously at 4 °C. The pretreated and bleached skins were then washed using tap water until a neutral pH of the wash waters was obtained using a Model Docu pH Meter (Sartorius AG, Elk Grove, IL, USA).

To extract gelatin, the prepared skins were transferred to a beaker and 5 volumes of warm (50 °C) water were added. The mixtures were stirred continuously using a stirrer equipped with a propeller for 18 h, followed by centrifugation at 8000g for 30 min (Avanti J-E, Beckman Coulter Inc., Newton, CT, USA) at room temperature (28–30 °C) to remove insoluble material. The supernatant was collected and freeze-dried (CoolSafe 55, ScanLafA/S, Lyngø, Denmark). The dry matter referred to as ‘gelatin’ was placed in polyethylene bags and kept at 4 °C until used.

### 2.3. Preparation of kiam wood and cashew bark extracts

#### 2.3.1. Collection and preparation of kiam wood and cashew bark

Kiam (*Cotylelobium lanceotatum craih*) wood was obtained from a forest in Phattalung province, Thailand. The tree was about 15–20 years old. The tree was cut using a saw and the trunk was sun-dried for three months. Cashew (*Anacardium occidentale*) bark was obtained from a forest in Songkhla province, Thailand. The tree was approximately 15–20 years old. The tree was peeled using a knife and the bark was collected. The pieces of wood and bark with an average thickness of 1–1.5 cm were dried in a hot air oven at 70 °C for 8 h. Prepared wood and bark were ground using a colloid mill (Model GY-JMS, Guanyu, Guangdong, China) to a sieve size of 6 mm. This coarse material was blended using a Moulinex AY46 blender (Group SEB, Lyon, France) and finally sieved using a stainless steel sieve of 80 mesh size. The powder obtained was used for the preparation of ethanol extracts.

#### 2.3.2. Preparation of ethanol extracts

Ethanol extracts from kiam wood and cashew bark powder were prepared as per the method of Santos, Yoshie-Stark,

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