LWT - Food Science and Technology 62 (2015) 955–961

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of ethanolic extract of coconut husk on gel properties of gelatin from swim bladder of yellowfin tuna

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ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form
19 January 2015
Accepted 5 February 2015
Available online 17 February 2015

Keywords:
Phenolic compounds
Gelatin
Coconut husk
Gel strength
Swim bladder

ABSTRACT

The impacts of ethanolic extract from coconut husk (EECH) rich in tannic acid at different levels (0.25, 0.5, 0.75, 1, 2, 3 and 5 mg g^{-1} dry gelatin) on gel properties of gelatin from yellowfin tuna swim bladder were investigated. Gel strength of gelatin increased when EECH concentrations increased up to 0.5 mg g^{-1} ($p < 0.05$). Nevertheless, the gradual decrease in gel strength was found with increasing EECH levels. When EECH at different levels was incorporated, no marked changes in protein patterns determined by SDS-PAGE were observed, suggesting that most of bondings were hydrogen bond or other weak bonds. Gel matrix with uniformity and larger strands were observed with gels added with 0.5 EECH mg g^{-1}. Gelling and melting temperatures were also increased when EECH at a level of 0.5 mg g^{-1} was incorporated. The color of all gelatin gels slightly increased with increasing concentrations of EECH. Therefore, ethanolic extract from coconut husk at an appropriate level could act as a natural gel enhancer of gelatin.

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1. Introduction

Gelatin is a fibrous protein produced by thermal denaturation, or partial degradation of collagen from animal skin and bone. It has been widely used in food, material, pharmacy and photography industries (Benjakul, Oungbho, Visessanguan, Thiansilakul, & Roytrakul, 2009). However, the outbreaks of bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD) as well as bird flu have resulted in anxiety among users of gelatin from land animal origin. Additionally, the gelatin obtained from pig skin or bone cannot be consumed for Muslim and Jewish, due to religious objections (Sadowska, Kolodziejska, & Niecikowska, 2003). The gelatin industry primarily uses mammalian skins and bones as raw materials. Byproducts 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 properties have been reported for the skins of many fish species, including African catfish (Alfaro, Biluca, Marquetti, Tonial, & Souza, 2014) and cobia (Silva, Bandeira, & Pinto, 2014). Recently, Kaewdang, Benjakul, Prodpran, Kaewmanee, and Kishimura (2014) successfully extracted gelatin from swim bladder of yellowfin tuna using an alkaline pretreatment. However, gelatin from swim bladder showed low gel strength. Generally, gelatins of fish origin have poorer gel strength, compared with mammalian counterpart, due to their lower imino acid content (Grossman & Bergman, 1992). However, chemical and physical treatments can be applied to modify the gelatin network through cross-linking of the gelatin chains to improve gel properties (Cao, Fu, & He, 2007). Cross-linking agents including glutaraldehyde, genipin, carbodiimides, calcium salts and transglutaminase have been used (Benjakul & Visessanguan, 2003; Chiou et al., 2006). Physical treatments, such as UV- and γ-irradiation (Chambi & Grosso, 2006) and high pressure technology (Montero, Fernández-Díaz, & Gómez-Guillén, 2002) have been also applied to enhance gelling property of gelatin.

Plant phenolics are defined as compounds possessing one or more aromatic ring bearing a hydroxyl substituent(s), and can be found in many foods and drinks from plant origin, e.g. fruits, vegetables, coffee (Parr & Bolwell, 2000). Phenolic compounds can interact with proteins through non-covalent and covalent interaction (Maqsood, Benjakul, & Shahidi, 2013). Covalent bonding seems to play an important role in protein-phenol interaction, which is used to improve functional properties of proteins (Sarker, Wilde, & Clark, 1995). Coconut is considered as an important crop in tropical countries. Coconut husk is one of the major agro-industrial waste generated in the developing countries each year. A portion of this

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http://dx.doi.org/10.1016/j.lwt.2015.02.006
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waste material is processed and used by the rope-making industry, but the majority remains unutilized (Dey, Sachan, Ghosh, & Mitra, 2003). Coconut husk is a major source of carbohydrate and phenolics (Sueli & Gustavo, 2007). Temdee and Benjakul (2014) used the extracts from kiam wood and cashew bark rich in tannic acid as the gel enhancer for gelatin. Phenolics in coconut husk could be used as the alternation protein cross-linker, which strengthens the gel network of gelatin, particularly from swim bladders. Therefore, the present study aimed to investigate the impact of the extract from coconut husk rich in phenolic on the gel properties of gelatin extracted from swim bladder of yellowfin tuna.

2. Materials and methods

2.1. 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 purchased from GE Healthcare UK Limited (Buckinghamshire, UK). Tannic acid was obtained from Sigma Chemical, Ltd, St. Louis, MO, USA with a purity of 98 g 100 g^{-1}.

2.2. Collection and preparation of swim bladder

Swim bladders of frozen yellowfin tuna (*Thunnus albacares*) were obtained from Tropical Canning Public Co., Ltd., Songkhla, Thailand. After tuna were thawed using running water, fish were eviscerated and swim bladders were collected. Swim bladders with the length of 8–12 cm were placed in polyethylene bags, inserted in ice using a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Songkhla. Upon arrival, swim bladders were washed with tap water and cut into pieces with the length of approximately 2 cm. The prepared samples were then placed in polyethylene bag and frozen at −20 °C. The samples were stored at −20 °C until used. The storage time was not longer than 3 months. Prior to extraction, frozen swim bladders were thawed using a running water until the temperature was 0–2 °C.

2.3. Extraction of gelatin from swim bladder

Firstly, swim bladders were pretreated with alkaline solution as per the method of Kaewdang et al. (2014). Prepared swim bladders were added with the mixed alkaline solution (Na_2CO_3:NaOH; 7:3) having the concentration of 4 g 100 mL^{-1} at a ratio of 1:10 (w/v). The mixture was stirred for 12 h at room temperature (28–30 °C) using an overhead stirrer model W20.n (IKA®-Werke GmbH & CO. KG, Staufen, Germany). The alkaline solution was changed every 6 h. The pretreated samples were washed with tap water until a neutral or faintly basic pH was obtained.

To extract gelatin, alkali-pretreated samples were immersed in distilled water (60 °C) using a swim bladder/water ratio of 1:5 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany). The extraction was performed for 24 h with a continuous stirring at a speed of 150 rpm. The mixture was then filtered using a Buchner funnel with a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England). The filtrate was freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The dried gelatin was placed in polyethylene bag and kept at 4 °C.

2.4. Preparation of coconut husk

2.4.1. Collection and preparation of coconut husk

Coconut husk was obtained from a local market in Hat Yai, Songkhla, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk was prepared following the method of Vazquez-Torres, Canche-Escamilla, and Cruz-Ramos (1992). The obtained powder was placed in a polythene bag, sealed and kept at room temperature until use.

2.4.2. Preparation of ethanolic extract from coconut husk

Coconut husk powder was subjected to extraction according to the method of Santoso, Yoshie-Stark, and Suzuki (2004). Dried extract was powdered using a mortar and pestle and was kept in an amber bottle and stored in a desiccator at room temperature (28–30 °C) until use. The obtained powder was referred to as ‘ethanolic extract from coconut husk, EECH’. The yield of EECH was 48.5 g 100 g^{-1} husk powder.

2.4.3. Determination of total phenolic content

Total phenolic content was determined using a colorimetric method using the Folin–Ciocalteau reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). Total phenolic content in EECH was calculated from a standard curve of tannic acid (0.01–0.1 mg L^{-1}) and expressed as g tannic acid equivalent kg^{-1} dry matter (Singleton et al., 1999).

2.4.4. Determination of tannic acid content

Tannic acid in EECH was determined using an HPLC equipped with a variable wavelength detector (VWD) following the method of Tian et al. (2009) with slight modifications. The HPLC system consisted of an Agilent 1100 series HPLC (Alginet, Wilmington, DE, USA), quaternary pump with the seal wash option, degasser, solvent, cabinet and preparative auto-sampler with thermostat equipped with a diode array detector. The separations were done using a Hypersil ODS C18 4.0 × 250 mm, 5 μm column (Cole–Parmer, London, UK). HPLC conditions were as follows: mobile phase: 0.4 g 100 mL^{-1} formic acid: acetonitrile (85:15), flow rate: 0.8 ml min^{-1}, temperature: 25 °C. Detection was carried out at 280 mm. The concentration of extracts was 25 mg ml^{-1} and each injection volume was 20 μl. Standard tannic acid was used for peak identification.

2.5. Preparation of gelatin gels containing EECH

The gelatin with the concentration of 6.67 g 100 mL^{-1} was dissolved in 40 °C water bath with the aid of mechanical stirring until it was completely dissolved. To gelatin solution, EECH was added to obtain the final concentrations of 0.25, 0.5, 0.75, 1, 2, 3 and 5 mg g^{-1} dry gelatin. The mixtures were stirred using a magnetic stirrer (IKA Labortechnik stirrer, Selangor, Malaysia) for 1 h at room temperature (28–30 °C), followed by setting at 10 °C for 16–18 h (BSI, 1975). The resulting gels were subjected to analyses.

2.5.1. Determination of gel strength

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

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