

Characteristic of yellow tuna skin (*Thunnus albacares*) gelatin enriched with cinnamon (*Cinnamomum zeylanicum*) and roselle (*Hibiscus sabdariffa*) powder

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Article history:

Received: 13 May 2020

Received in revised form: 1
July 2020

Accepted: 16 July 2020

Available Online: 4
September 2020

Keywords:

Valorization,
Gelatin,
Roselle,
Cinnamon,
Antioxidant

DOI:

[https://doi.org/10.26656/fr.2017.4\(6\).225](https://doi.org/10.26656/fr.2017.4(6).225)

Abstract

The current practice condition of fish processing will generate some amounts of by-products such as skin waste. These fish processing by-products can be considered as an alternative raw material for the preparation of high protein ingredients such as gelatin. Gelatin extraction from the skin of yellowfin tuna (*Thunnus albacares*) was conducted by the acid process. Afterwards, the fish gelatin was mixed with cinnamon (GEC) and roselle powder (GER) to enhance the physicochemical and functional properties of gelatin. The effects of the addition of cinnamon (GEC) and roselle powder (GER) on the physicochemical and the functional properties such as tensile strength, hardness, viscosity, water holding capacity, oil holding capacity, total flavonoid, total phenolic, total antioxidant and flavor compounds of enriched gelatin (GEC and GER) from tuna skin were evaluated and compared with control (without the addition of cinnamon and roselle powder). The gelatin exhibited excellent tensile strength, WHC, OHC, total flavonoid, total phenolic, and antioxidant in the presence of cinnamon and roselle powder rather than control (without addition) ($p < 0.05$). The flavor analysis by using GC-MS showed more cinnamaldehyde as one important flavor compounds in GEC and where cyclohexanones can be identified as one important flavor compound in GER. Thus, this study confirmed the improvement of physicochemical properties of gelatin enriched with cinnamon and roselle powder.

1. Introduction

Gelatin is a denatured fibrous protein derived from collagen by partial thermal hydrolysis. It is an important functional biopolymer that has broad applications in the food, pharmacy, and photography industries (Shyni *et al.*, 2014; Sila *et al.*, 2017). Fish gelatin seems the preferred raw material in biodegradable packaging and also biodegradable film production as it has good film-forming ability and is abundant in nature (Hanani and Nor-khaizura, 2019). The development of biodegradable packaging materials from fish gelatin is an effective alternative to synthetic packaging material (Ahmad *et al.*, 2012).

Tuna is one of the worldwide favorite fish that was captured tuna is usually processed as canned food and sliced raw meat in a factory, and by-products of tuna are affluent and collected at once. Fish by-products from

freshwater are seldom used as a source of raw materials for gelatin extraction. They are mainly used for animal feed supplements due to their small size (Kasankala *et al.*, 2007). For this reason, if the physical properties of gelatin from tuna skin resemble mammalian gelatin, tuna skin can be a replacement resource of mammalian gelatin (Cho and Kim, 2005).

Several spices and herbs are enriched in gelatin to enhance its mechanical and barrier properties. Natural substances such as spices and herb powder are highly preferable as antioxidants compared to synthetic ones yet certain disadvantages limit their usage. It has been reported that cuttlefish skin gelatin films with different herb extracts had higher tensile strength, compared with the control film. The protein-polyphenol interactions took place via hydrogen bonds and hydrophobic interactions than can lead to film strengthening. The increased amount of cross-links via such interactions

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might form a film network with the decreased free volume of the polymeric matrix, resulting in better character (Karim and Bhat, 2009; Fakhreddin and Gómez-guillén, 2018). On the other hand, a term of active packaging contains an active substance such as antioxidants that can interact with the headspace and packaged product to prolong the shelf life and quality of the product. It possesses several benefits such as the gradual release of bioactive compounds. Therefore, the continued use-up of antioxidants during storage will be decreased and there is a lower chance that the natural extract will affect the taste of the food (Hanani *et al.*, 2019).

In Indonesia, cinnamon and roselle are mainly used for important spice and herbs that have been applied in many kinds of food. Cinnamon extract that contains many phenolic compounds, such as trans-cinnamaldehyde, limonene, eugenol, benzaldehyde, and cinnamyl acetate, have good antibacterial and antioxidant activities. Trans-cinnamaldehyde is a major phenolic compound, which is responsible for antibacterial and antioxidant activities (Kim and Song, 2018). Commonly roselle is added with hot water and drinks afterwards. The red anthocyanin pigments in the calyces are used as food coloring agents. The swollen calyces are the part of the plant of commercial interest as they are rich sources of Vitamin C, phytochemicals and are also used for several purposes (Ningrum *et al.*, 2019).

The addition of herb and spice extract and also essential oils from various plants have been known to exhibit antioxidant properties, which can extend shelf-life by lowering lipid oxidation in foods. Therefore, the incorporation of gelatin with spice and herbs to gelatin could be an approach to enhance the physicochemical and functional properties of gelatin. Simultaneously, gelatin with functional properties such as antioxidant activity can be gained. However, rare information regarding the improvement of physicochemical property of fish gelatin enriched with cinnamon and roselle powder has not been reported. The purpose of this research was to characterize the physicochemical characteristic gelatin enriched with Cinnamon (GECF) and Roselle Powder (GERP).

2. Materials and methods

2.1 Materials

Yellowfin tuna (*Thunnus albacares*) skin was provided by Fresh Fish, Yogyakarta, Indonesia. The yellowfin tuna skin was parted into abdominal and dorsal skins, the dorsal skin of yellowfin tuna was used in this study. All chemicals were purchased from Sigma Chemical Co. All reagents used in this study were

analytical grade.

2.2 Preparation of gelatin

The yellowfin tuna skin was washed, chopped, and frozen until used. The cleaned skin was treated with an acid solution (acetic acid) for 1 day to remove the non-collagen protein and subcutaneous tissue after they were swollen. After the acid treatment, the skin was neutralized water and washed. For hot-water extraction, six volumes (v/w) of distilled water were added and heated at a temperature ranging 40–80°C for several hours. The extracted solution was heated until it concentrated and the filtered solution was dried at for 24 hrs in a cabinet-air dryer. Then the dried gelatin was crushed using a waring blender and then the obtained powder was obtained after 40 mesh. Obtained gelatin powder was mixed with cinnamon and roselle powder (1:1 w/w) then characterized further including the physical and chemical properties. Cinnamon and roselle powder obtained from the local market, Yogyakarta.

2.3 Physicochemical properties

2.3.1 Yield

Gelatin yield was calculated as the percentage of the weight gelatin powder divided by the skin fish.

2.3.2 Determination of gel strength and hardness

Gelatin solution with a concentration of 3.32% was stirred using a magnetic stirrer until homogeneous at a temperature of 60°C for 15 mins. The solution was incubated for 18 hours at 10°C. The gel strength and hardness were measured using the TAXT Plus-type texture analyzer.

2.3.3 Determination of viscosity

Gelatin solution was made 6.67% by weighing 6.67 g of gelatin then dissolved in 100 mL of distilled water. The viscosity was measured using the Brookfield viscometer.

2.3.4 Water holding capacity (WHC)

About 2.5 g gelatin was dissolved in 5 mL of distilled water and the suspension was stored at room temperature for 15 mins and shaken every 5 mins. The suspension is stirred using centrifugation at 3,000 rpm for 15 mins. The WHC value is calculated as the percentage of the weight residue gelatin divided by the weight of the dry sample.

2.3.5 Oil holding capacity (OHC)

About 2.5 g gelatin was dissolved in 5 mL of glycerol and the suspension was stored at room temperature for 15 mins and shaken every 5 mins. The

suspension is stirred using centrifugation at 3,000 rpm for 15 mins. The OHC value is calculated as the percentage of the weight residue gelatin divided by the weight of the suspension sample.

2.3.6 Total flavonoid

Total flavonoid was analyzed using aluminum chloride colorimetric method. Quercetin was used to make the calibration curve. 25 mg of quercetin was dissolved in 25 mL ethanol 96% and diluted to 2, 4, 6, 8 and 10 ppm. 1 mL of each concentration of standard solutions, as well as 1 mL of each sample solution, were mixed with 10 mL ethanol 96%, 1 mL of aluminum chloride 2% and 1 mL potassium acetate 120 mM. The mixture was incubated at room temperature for an hour. The absorbance was measured at 435 nm using a UV-Vis spectrophotometer. Total flavonoid was expressed as weight of quercetin equivalent (QE) per gram of fresh weight sample.

2.3.7 Total phenolic

Phenol compound analysis can be approached by using UV-VIS Spectrophotometer method with the Folin Ciocalteu reagent. Samples 200 mL will be introduced to test cuvettes and then 1 mL of Folin Ciocalteu reagent and 0.8 mL of Na₂CO₃ will be added. The absorbance of all samples will be measured at 765 nm by using a UV-VIS spectrophotometer after incubation at 30°C for 1.5 h. Results will be expressed as milligram of gallic acid equivalent per gram of fresh weight sample

2.3.8 Total antioxidant

The radical scavenging activity against DPPH of the methanolic extract will be analyzed for measuring total antioxidant will be measured (Vadivel *et al.*, 2012; Kumar *et al.*, 2018). The sample (0.1 mL) was added to 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (6×10^{-5} mol/L) in methanol and incubated for 30 min at room temperature (30±1°C). The absorbance was measured at 515 nm. DPPH solution was used as a control and methanol was used as a blank.

2.3.9 Volatile compounds

One gram of gelatin was a mixture with pentane: dichloromethane (2:1 v/v). The mixture was stored in the freezer for 24 hrs. After that, the solution was filtered through a 0.45 µm membrane filter. The mixture was added sodium anhydrous and evaporated. The free volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS) using the following temperature program: initial temperature 80°C ramped to 250°C at 10°C/min, and held for 10 mins. The mass scan range was set to m/z 50–300. Helium was

used as carrier gas at a constant flow rate of 1.7 mL/min. The identification of volatile compounds was based on the comparison of the mass spectra of each component with mass spectral library WILEY.

2.4 Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model in Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA) and Tukey's multiple comparison tests for the physicochemical parameters (significance level $p < 0.05$).

3. Results and discussion

3.1 Yield

Gelatin yield is key parameters because of their economic importance for the gelatin industry and its application. In this study, the protein yield of fish gelatin was 6.29%. The protein yield of gelatin samples varies depending on the raw material and extraction methods used. In the case of gelatin obtained from the skin; proximate composition, amino acid composition, acid, and alkali used in pretreatments and extraction conditions applied may show great influence on protein yield achieve. The appearance of obtained gelatin control, enriched with roselle powder and cinnamon powder can be seen in Figure 1.

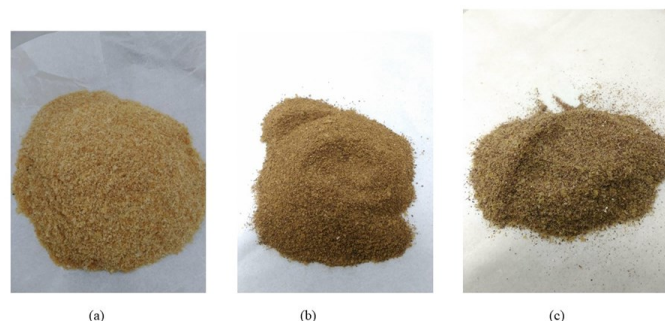


Figure 1. Appearance of (a) gelatin control extract, (b) gelatin enriched with cinnamon powder and (c) gelatin enriched with roselle powder.

3.2. Gel strength, hardness, and viscosity

Physical or mechanical properties of gelatin containing roselle and cinnamon powder are shown in Table 1. GERP and GECP showed the higher TS, but lower hardness and higher viscosity, compared with the control (without the addition of spice and herb powder).

Amongst all gelatin added with cinnamon powder showed the highest TS ($p < 0.05$). It indicated that the cinnamon powder components might increase the bonds in gelatin so that the tensile strength is increased. The incorporation of cinnamon powder (GECP) to gelatin could enhance the development of heterogeneous matrix,

Table 1. Physicochemical and functional properties of control, GERP and GECP

Sample	Tensile Strength (MPa)	Hardness (N)	Viscosity (cP)	WHC (%)	OHC (%)	Total Flavonoid (ppm)	Total Phenolic (%)	Total Antioxidant (%)
Control	16.46 ^a	0.43 ^b	4.2 ^a	1.47 ^a	0.51 ^a	0.02 ^a	190.10 ^a	9.41 ^a
GERP	17.12 ^b	0.03 ^a	5.4 ^b	1.61 ^b	0.72 ^b	0.03 ^b	1243.13 ^b	36.95 ^b
GECP	17.78 ^b	0.04 ^a	5.6 ^b	1.57 ^b	0.92 ^c	0.04 ^b	4244.38 ^c	69.57 ^c

Means with alphabet superscripts within columns are significantly different ($p < 0.05$)

leading to an increase of tensile and hardness (Ejaz *et al.*, 2018).

Viscosity is also a key commercial characteristic of gelatin and it also varies according to not only average molecular weight and gel strength but also conditions such as pH and temperature. As similar to tensile strength and hardness, GECP had a higher viscosity than GERP and control ($p < 0.05$) (Table 1). The addition of other components, especially cinnamon powder, in the gelatin can increase the viscosity. The viscosity of commercial gelatins generally ranges from 2.0 to 7.0 cP and for some applications, this value is desired to be almost 13.0 cP. Viscosity is greatly influenced by differences in measurement procedures and conditions, raw material characteristics, and gelatin extraction methods (Tuğçe *et al.*, 2019).

3.3 WHC and OHC

Water holding and fat binding capacities are techno-functional properties that are closely related to texture by the interaction between components such as water, oil, and other components (Shyni *et al.*, 2014). The influence of using different sources in gelatin extraction on the techno-functional properties of the resultant gelatins was given in Table 1. GECP and GERP had a higher WHC than control ($p < 0.05$). WHC was related to the number of hydrophilic amino acids and whether there were high amounts of pores and spaces within the gel network. The higher amount of hydrophilic amino acids and Hyp content caused to higher water holding capacity (Tuğçe *et al.*, 2019).

Besides that factor, the amount of water bound by protein depends on several factors, such as amino acids composition of the protein, several polar groups within the particle, availability of hydrophilic spots, pH of the environment, ionic strength, temperature and protein concentration (Tkaczewska *et al.*, 2018). The water-binding capacity of the solubilized gelatin makes it a suitable material for reducing drip loss and impairs the juiciness in frozen fish or meat products when thawed or cooked, and where the denatured protein has suffered a partial loss of its WHC (Tkaczewska *et al.*, 2018).

In terms of oil holding capacity (OHC), statically significant differences were detected among the samples ($p < 0.05$) as shown in Table 1, GECP had a highest

OHC. Significant differences in gelatins might be caused by various levels of hydrophobic residues and tyrosine that may be affected by extraction methods (Tuğçe *et al.*, 2019). The presence of selected essential oil in roselle and cinnamon powder also can enhance the oil holding of gelatin.

The higher oil-binding capacity of GERP and GECP suggests the presence of a large proportion of hydrophobic as compared to hydrophilic groups on the surface of the protein molecules. The mechanism of fat-binding by proteins is not fully understood, but it appears to be affected by protein and lipid-protein complexes content. It is suggested that the oil absorption capacity is due to the non-polar side chains of the protein, as well as due to the different conformational features of the proteins. Our results suggest that GERP and GECP had better fat-holding capacity (Tkaczewska *et al.*, 2018).

3.4 Total flavonoid

Flavonoids are a class of secondary plant phenolics with important and powerful antioxidant activities. Therefore, the amounts of total flavonoids in the extracts were determined. The total phenolics in crude extracts of gelatin were determined by the Folin–Ciocalteu method. As seen in Table 1, the highest level of total flavonoid was found in the GERP and GECP rather than control.

In GERP there are several possible flavonoids, where apparently, most of the flavonoids can be conjugated with major pigment anthocyanins in Roselle (Tsai and Huang, 2004). Flavonoids such as quercetin, luteolin or gossipetin, and their respective glycosides are also present in roselle and can be conjugated with major anthocyanin in roselle (Borrás-linares *et al.*, 2015). The most frequent anthocyanins of roselle are cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-sambubioside, and delphinidin-3-sambubioside (Borrás-linares *et al.*, 2015). Major flavonoids in cinnamon such as a flavonol (-)-(2R,3R)-5,7-dimethoxy-3',4'-methylenedioxy-flavan-3-ol (MFO) were able to have high antioxidant activity (Li *et al.*, 2019). There are several evidence in vitro and in vivo certified that consumption of cinnamon is the benefit to human health because of its pharmacological functions on anti-diabetes, inhibition of oxidative stress, anti-inflammation, anti-hypertension, anti-microbial, and inhibition of cardiovascular disease (Borzoei *et al.*, 2018;

Li et al., 2019).

3.5 Total phenolic

The results for the total phenolic is presented in Table 1. GERP and GECP have higher phenolic compounds than control. Similar result with a little difference was reported by previous research, where the addition of herbs and spices extracts will enhance the total phenolic content in gelatin enriched with spice and herb extract (Tongnuanchan, et al., 2014; Figueroa-lopez et al., 2018; Kim et al., 2018). Several phenolic compounds have been identified in cinnamon extract e.g. gallic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, protocatechuic acid, salicylic acid, syringic acid, vanillic acid, vanillin, caffeic acid, quercetin, tannic acid, chlorogenic acid, ferulic acid, p-coumaric acid, cinnamic acid, sinapic acid and eugenol (Hu et al., 2018). Roselle as a herbal commodity also rich in several phenolic compounds. It has strong antioxidant properties mainly due to the abundant content of polyphenolic compounds, including anthocyanin, phenolic acids, flavonoids, and many others (Wu et al., 2018).

3.6 Total antioxidant

Cinnamon and roselle have been confirmed to exert strong antioxidant activity (Mardiah et al., 2015; Rahadian et al., 2017; Borzoei et al., 2018; Zhang et al., 2019). It can perform as antioxidants by interruption of chain oxidation reactions, by donating a hydrogen atom, as an acceptor of free radicals, or by chelating metals. Antioxidant tests represent measuring of DPPH radical scavenging activity is attributed to hydrogen donating ability of test compounds (Dou et al., 2018). And the antioxidant capacity was positively correlated with total phenolics and total flavonoids content in the film. The adding of cinnamon and roselle would also enhance the antioxidant capacity of gelatin (Table 1).

3.7 Volatile compounds

The volatile determination was also performed by GC MS analysis. Several aroma compounds can be identified in Control, GERP, and GECP (Table 2). The dichloromethane peak can be identified on three samples since the extraction for those samples used dichloromethane as a solvent

Cyclohexanone as hydrocarbon derived compounds can be identified in GERP and GECP (Table 2). On the other hand, cinnamaldehyde as an important flavor compound in cinnamon can be identified in GECP. Cinnamaldehyde is one important flavor compound in cinnamon that displayed the excellent antimicrobial activity against a variety of bacteria. Gelatin enriched with essential oil from cinnamon has a long-lasting antibacterial activity on *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* due to lease the antimicrobial active substances in the sample (Hu et al., 2018).

Another research also investigated the broad spectrum of a microorganism to see the antimicrobial activity of the cinnamon extract in gelatin. Gelatin films enriched with cinnamon oil were more effective against *Aspergillus niger*, *Rhizopus oryzae* and *Paecilomyces varioti* than *Escherichia coli* and *Staphylococcus aureus* (Wu et al., 2017). It is suggested that the investigated antimicrobial films can be used for prolonging the shelf-life of packed foods due to their bacteriostasis and barrier property of ultraviolet light (Wu et al., 2017). Another report also has shown that hagfish and chicken bone gelatin films containing cinnamon extract had good antibacterial and antioxidant activities (Kim et al., 2018).

4. Conclusion

Tuna skins were used in gelatin extraction as a valorization approach to reduce food waste. GERP and GECP may present better quality in several

Table 2. Aroma compounds in control, GERP and GECP

Control			
No	Retention Time (min)	Compound	%Relative Abundance
1	1.775	Dichloromethane	97.48
2	6.202	Torreyol,1-naphtalenol	0.91
3	6.899	Vallencene,1-naphtalene	0.99
4	14.869	Tetracosahexaene	0.62
GERP			
No	Retention Time (min)	Compound	%Relative Abundance
1	1.775	Dichloromethane	99.94
2	12.74	Cyclohexanone	0.06
GECP			
No	Retention Time (min)	Compound	%Relative Abundance
1	1.775	Dichloromethane	89.61
2	7.23	Cinnamaldehyde	9.93
3	12.74	Cyclohexanone	0.46

physicochemical and functional properties compared to control (without the addition of roselle and cinnamon powder). Physicochemical properties including tensile strength, viscosity revealed, WHC, OHC Total flavonoid that GERP and GECP are more desirable than control. Differences were observed also in flavor properties in GERP and GECP. Physicochemical and functional properties of gelatins from tuna skin and enriched with roselle and cinnamon powder showed that seafood skins, which are mostly underutilized, as obtained processing waste by-products can be used as alternative raw materials in gelatin production. This study provided data suggesting that tuna skin enriched with roselle and cinnamon powder gelatins can be used further in food, pharmaceutical, nutraceuticals application industries.

Conflict of interest

The authors declare no conflict of interest in the manuscript.

Acknowledgment

Authors thankful to Toray Foundation, Japan and RTA, UGM for supporting this research.

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