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The potency of spice extracts in fish skin collagen production

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Abstract. Spices can be used as an alternative washing agent in fish collagen production, replacing organic materials' commonly used washing agent. Many Asians, including Indonesian traditional culinary use spices to remove the fishy odor. Therefore this research was conducted to obtain a suitable spice used as a washing agent in fish collagen production. Ginger, galangal, lemongrass, bay leaves, lime leaves, and pandan leaves were used as the tested spices in the form of 5% liquid extract in water. The ANOVA and LSD test on fishy odor test data showed that most respondents preferred Pangasius skin soaked in galangal liquid extract and had no significant difference in odor compared to the control (ethanol). Pangasius skin soaked in galangal extract had the total protein and ash content lower than control, while the total fat and water content were higher. Soaking in galangal extract also made Pangasius skin have a higher degree in swelling and total protein content in its acid-soluble collagen (ASC) extract than in control. The higher value of the two latter parameters was also found in the descaling Tuna skin soaked in galangal extract.

1 Introduction

Collagen is a structural protein assembled by three helicoidal long chains amino acids formed by sequences of glycine and two amino acids, commonly proline and 4-hydroxyproline [1, 2]. Protein is the body connective tissue, i.e., cartilages, bones, tendons, ligaments, and skin is an extracellular matrix composed mainly of collagen [1].

Many studies found collagen consumption has benefits for health because collagen peptide content in the extract has many essential bioactivities, i.e., antioxidant [3], anti-inflammation [4], and anti-rheumatism [5]. It seems that the crucial medicinal properties of collagen are primarily based on its wound-healing activity. Collagen peptides have a role as a natural base material and as a framework in forming new tissues responsible for hemostasis, inflammation, proliferation, and remodeling [6].

Because of collagen importance in medical issues, attention to fisheries by-products as a new source of collagen is increasing. Some studies have shown that in addition to the ease of extraction with high yields, fishery-based collagen is free from the risk of animal diseases and pathogens. Besides, it has easy absorption due to its biocompatibility and low molecular weight, less significant religious and ethical constraints [7]. Those advantages can overcome the limitation of mammalian-based collagen applications.

The collagen peptide isolation principally includes preparation, extraction, and recovery. The preparation step, i.e., cleaning, cutting/mincing to reduce the sample size, and washing, are needed to facilitate the next sample pretreatment steps. The pretreatment using mild chemicals is subjected to remove non-collagenous substances and to increase the extraction yield. The following pretreatments can be performed in alkaline, acid, or enzymatic treatment according to the raw materials and the extraction method. The second pretreatment is used to break down the crosslinked collagen before the extraction process. The crosslink in collagen structure can inhibit the extraction process [8].

Organic solvents like ethanol and butanol are commonly used to eliminate odorous substances in fish skin in the preparation step [8, 9, 10]. Odorous substances can be classified as species-related fresh fish odor, microbial spoilage odor, oxidized odor, processing odor, and environmentally derived odor [11]. On the other side, some common spices are traditionally known for eliminating odorous substances from animal meat or fish. The U.S. Food and Drug Administration (FDA) classified spice as an aromatic vegetable substance in the whole, broken, or ground form. The significant function of which in food is seasoning rather than nutrition and from which no portion of any volatile oil or other flavoring principle has been removed. Many compounds isolated from spices have shown aromatic properties and antimicrobial activity against some of the most common microorganisms affecting food quality and shelf life [12]. Therefore, using spice to prepare raw material might benefit collagen production because the cost is lower than organic solvent and has no constraint in religious affairs. So this study aimed to reveal the potency of spice extract in the preparation of fish skin as raw material in collagen production.

2 Methodology

2.1 Spice extract preparation

Zingiber officinale (ginger) rhizome (A), *Alpina galanga* (galangal) rhizome (B), *Cymbopogon citrates* (lemongrass) stem (C), *Citrus hystrix* (kaffir lime) leaves (D), *Laurus nobilis* (bay) leaf (E), and *Pandanus amarylifolius* (pandan) leaves (F) were used in the study, with ethanol 70% as control soaking solution (G). When preparing the spice liquid extracts, all spices were obtained from a fresh market and used in new conditions. Spices were prepared as liquid extracts using water as solvent. As much as 50 g of clean minced spices were boiled in 1 L of water for ten minutes and then strained to separate the liquid extract solution.

2.2 Fishy odor test

A fishy odor test was conducted using Pangasius skin obtained from *Pangasius* sp. reared in a biofloc fish farm in Cibinong, Indonesia. Pangasius fish were skinned and washed with tap water. The skin was then cut into 1x1 cm size and stored in the freezer before use. A total of 50 g Pangasius skin samples was soaked in 100 mL spice extract for 30 minutes and then washed with tap water three times. The test for the odor of Pangasius skin after spices treatment was conducted according to Suryaningrum et al. [13] with modifications. The test used 32 untrained respondents with four scales of odors that were very weak (score 1), weak (score 2), distinct (score 3), and strong odor (score 4). The average, total, and modus scores of each spice extract and control in the fishy odor test were counted and followed by statistical analysis using ANOVA and the least significant differences (LSD) test for further tests. For further study, the spice extract with the same or closest value and did not significantly differ from the control solution was selected as test extracts.

2.3 Proximate analysis

A total of 100 g cut Pangasius skin was soaked in each selected spice extract 5% and ethanol 70% for 30 minutes and then washed three times by demineralized water. The washed skin samples were then stored in the freezer before the analysis. The proximate analysis performed included analysis of moisture, ash, protein, and fat content in skin samples by referring to the SNI 01-2891-1992 method. The proximate analysis was conducted to study the effect of washing with selected spice extract and ethanol on Pangasius skin nutritional content. In addition, the appearance of spice-treated skin was also examined.

2.4 Acid soluble collagen extraction

The skin samples were washed with tap water to eliminate debris, cut the skins into 1x1 cm size, and then rinsed once with demineralized water. Acid soluble collagen extraction was performed according to [9, 10] with modification. As much as 100 g of fish skin were pretreated by soaking it in each 200 mL of 5% selected spice extract, control ethanol 7% for 30 minutes, and then washed with demineralized water. To eliminate non-collagenous protein, the skin samples were submerged in NaOH 0,05 M for 4 hours and washed until the neutral pH was reached. The next step was acid pretreatment which was carried out by submerging the NaOH pretreated skin for 4 hours in acetic acid 0,05 M with the solid to solution ratio of 1:10 (w/v). The treated skin was then washed until the pH of washing water reached neutral. The last step was hydro extraction using water as solvent. The mass of the skin was weighed before the hydro extraction process. The degree of skin swelling (Dsw) was counted by the equation (1) :

$$D_{sw} = \frac{(W_1 - W_0)}{W_0} \times 100\ \% \tag{1}$$

Dsw = Degree of swelling (%), W_0 = initial raw skin weight (g), W_1 = skin weight after acetic acid pretreatment (g)

The acid-pretreated skin was then submerged in demineralized water with a solid to solution ratio of 1:0,5 (w/v). The hydro extraction was held at a temperature of 40°C for 2 hours. The skin was then strained to separate it from the extract solution. The protein content in the extract solution was analyzed by Biuret Method [14] and counted as a percent of total protein using the following equation (2):

$$P = \frac{(C_B - V)}{W} \times 100\ \% \tag{2}$$

P = Total Protein (%), C_B = concentration total protein by Biuret analysis (mg/L), V= Volume of ASC extract (L), W = weight of rawskin

Acid soluble collagen (ASC) extraction was carried out on two types of fish skin, i.e., Pangasius and Tuna fish. Same as Pangasius skin, Tuna skin, also a side product in fish processing, could be used as raw material in collagen production. Tuna skin is different from Pangasius fish because Tuna has scales in their skin while Pangasius is not. Tuna skin in this study aimed to know whether the selected spice liquid extract had the same effect in different skin types. Tuna skin was obtained from the marine fish processing industry at Nizam Zachman Fishing Port, Jakarta. Tuna skin was descaling before use in this study. Tuna skin was described at low temperature (approximately15 – 20°C) to minimize skin decay.

3 Results

3.1 Selection of spice for washing solution

The fishy odor test result of Pangasius skin showed that the skin soaked in galangal extract was the lowest among the other samples (total score 54, mean 1.69) and closest to the score of ethanol (total score 44, mean 1.38) compared to the other five extracts (Figure 1). Even though lime leaf, bay leaf, and pandan leaf extract have the same modus score (Table 1), the respondents preferred most skins washed with galangal extract.

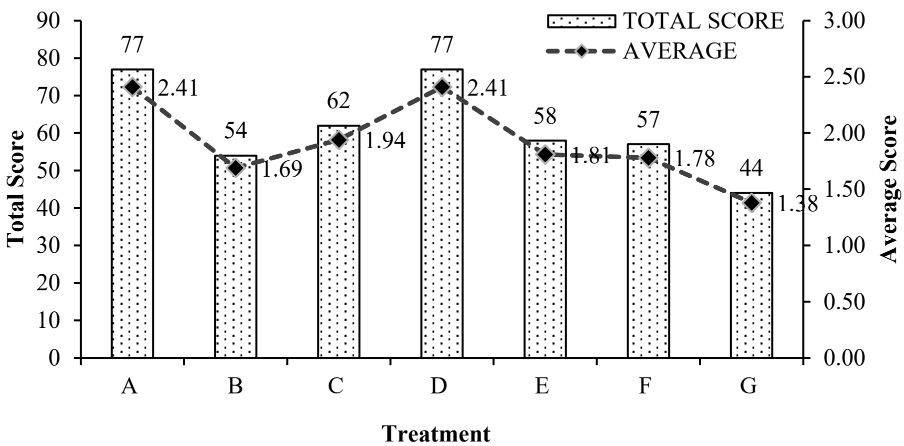

Fig.1. The total and average score of fishy odor tests on Pangasius skin soaked in extract solution of ginger rhizome (A), galangal rhizome (B), lemongrass stem (C), lime leaf (D), bay leaf (E), pandan leaf (F) and (G) ethanol

Tabel 1. Modus score of fishy odor test on Pangasius skin soaked in extract solution of ginger rhizome (A), galangal rhizome (B), lemongrass stem (C), lime leaf (D), bay leaf (E), pandan leaf (F), and (G) ethanol

Parameter	Spice Extract Treatment						Control
	A	B	C	D	E	F	G
Modus	2	1	2	1	1	1	1

The result of the ANOVA test on the survey data indicated that the Pangasius skin washed in several spice solutions had significantly different odors with a critical F value of 5,496. Further tests using the LSD analysis showed that the level of aromas of the skin washed using ginger, lemongrass, and lime leaves extract was significantly different from ethanol. Otherwise, the results from galangal, bay leaves, and pandan leaves extract produced a level of odors that were not substantially different from ethanol solution. Furthermore, the galangal solution's washing had the most considerable LSD value of 0.171 against the ethanol solution. This LSD value indicated that washing using galangal extract produced skin with an odor that was not significantly different from the results of washing using ethanol. Thus, based on the data above, 5% galangal plant extract was chosen as a washing solution for the raw material of fish skin in the collagen extraction process in further study.

3.2 Nutrition content of washed fish skin

The proximate analysis showed that washing with 5% galangal solution produced lower protein and ash content in fish skin than controls that washed with ethanol (Figure 2A and 2B). On the contrary, the water and fat content of the skin washed with 5% galangal were higher than those washed with ethanol (Figure 2C and 2D).

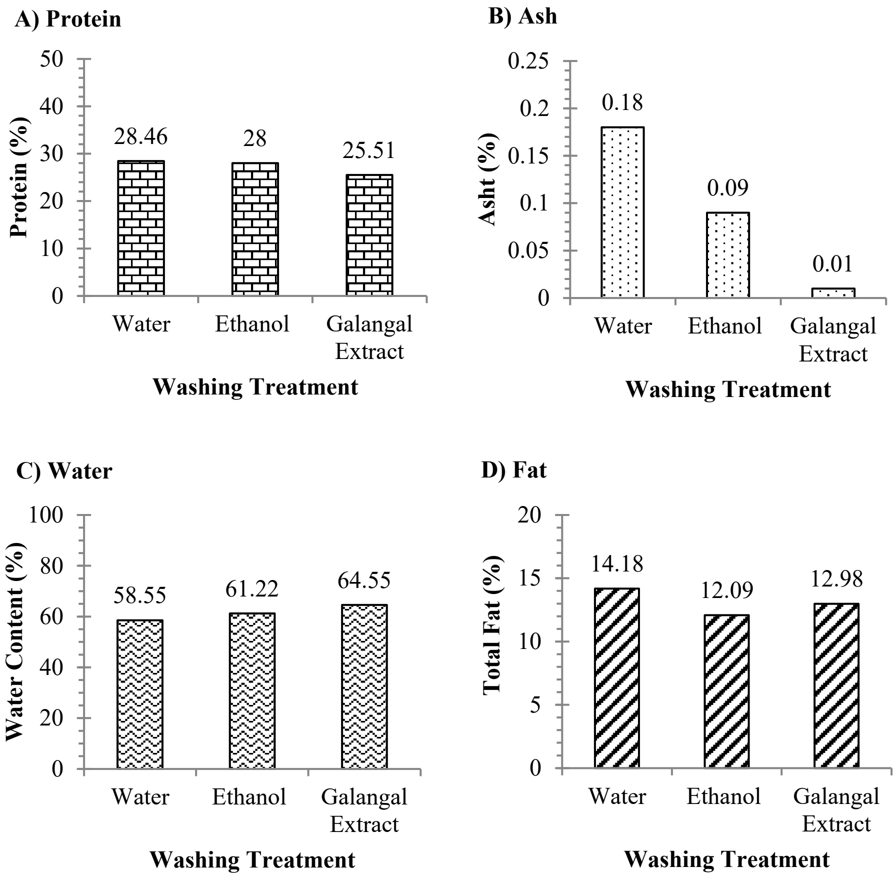

Fig.2. The nutritional composition of washed Pangasius skin using water, ethanol, and galangal extract

The higher water and fat content and strengthened by the lower content of ash in fish skin washed with galangal extract might cause a soft and fluffy Pangasius skin appearance compared to the skin washed with ethanol (Figure 3). In addition, the higher water content in the skin washed with galangal extract was an indication that the skin experienced the earlier stage of collagen protein breakdown than control.

A) B)


Fig.3. The appearance of Pangasius skin washed using galangan extract (A) and ethanol (B)

3.3 Effect of galangal extract washing in ASC extraction

In the ASC extraction process, washing the skin with galangal extract gave a higher degree of skin swelling after soaking with 0.05M acetic acid than skin washed with ethanol in both Pangasius and Tuna skin (Figure 4). The pH of the galangal extract was 6.7, while the ethanol solution was 7.1.

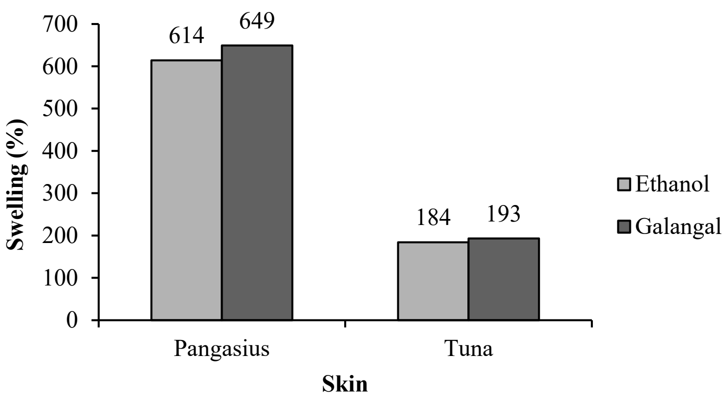

Fig.4. Degree of swelling of Pangasius and Tuna skin in ASC extraction with different washing solution

The higher value in the degree of skin swelling from the skin soaked in galangal was followed by the collagen extract's increase in total protein content. Pangasius and Tuna skin washed using galangal extract produced ASC extract with total protein as much as 19.25% and 2.47%, respectively, while in control made lower, i.e., 18.44% and 2.11 %.

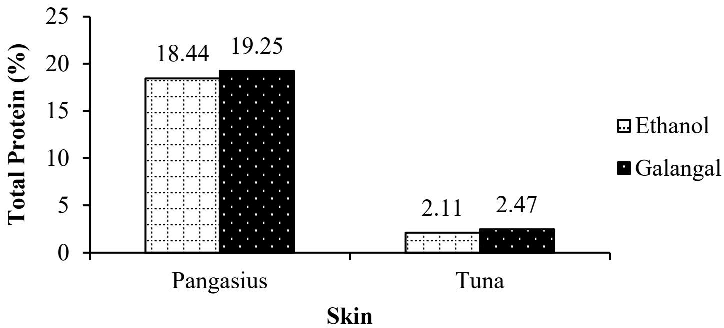

Fig.5. Total protein of Pangasius and Tuna skin in ASC extraction with different washing solution

Moreover, there is an indication that the lower ash content of the skin samples washed with galangal extract had the relationship to the higher total protein content (Figure 2B) in the ASC extract.

4 Discussions

Z. officinale, *A. galanga*, *C. citrates*, *C. hystrix*, *L. nobilis*, and *P. amarylifolius* were chosen in this research because they were common ingredients in culinary recipes as aromatic to eliminate unpleasant odors that emerge from fish. Those spice plants also contained bioactive compounds such as gingerol, galanga-isoflavonoid, citral, citronellal, camphor, and 2-acetyl-1-pyrroline, which have antimicrobial activity [12]. The presence of aromatic compounds and antimicrobial activity in the spice are the advantages of using spices as a washing agent for fish skin. They both provide benefits through skin deodorization and spoilage prevention.

In this research, galangal (Alpinia galanga), which was considered more potential as a washing agent, is a native perennial herb from Indonesia, spreading to India, China, Arabic gulf areas, Malaysia, Egypt, and Sri Lanka [15]. Much research has been conducted to explore its bioactive properties because galangal is a traditional medicine used in Asia besides its culinary function as an aromatic herb. *A. galanga* has antioxidant, anticancer, antidiabetic, anti-inflammatory, antimicrobial, anti-fungal, and anti-ulcer properties [15 16]. Organic substances from phenylpropanoid, diterpene, curcuminoid, alkaloid, and flavonoid were reported as the primary bioactive compounds in *A. galanga* [15 17]. Those aromatic and organic compounds in galangal extract might be acting as a deodorizer for the fish skin. The combination of acids, phenol, p-cresol, indole, skatole, sulfur compounds, and amines such as trimethylamines contributes to the overall unpleasant odors of spoiled fish [18].

Although washing with galangal resulted in lower skin protein levels, these levels were still in the expected range for Pangasius, according to Suptijah et al. [10]. They found that the average protein, ash, water, and fat content in Pangasius skin was 30.3%, 0.2%, 65,6%, and 10.6%, respectively. Our finding was almost identical to the Prommajak and Raviyan [19] study except in crude lipid content, which was 2.19%. This different level of crude lipid content may be due to the other Pangasius species used by Prommajal and Raviyan. They used Thai panga fish *Pangasius bocourti* Sauvage as research material.

The lower pH value of galangal extract was thought to cause crosslinking in the collagen protein structure to break down earlier than skin washed with ethanol. As a result, in the pretreatment using 0.05 M acetic acid, the breakdown of protein crosslinks becomes more optimal, and the protein can be split into shorter fragments. Moreover, Liangan et al. [20] found that galangal has properties that can increase the process of cell apoptosis in the presence of the compound Acetoxy Chavicol Acetate or ACA. ACA can increase apoptosis through caspase-3 activation [15]. Caspases are enzymes that break down proteins by one class of proteases that can cause cellular apoptosis. The cellular apoptosis might lead to more water molecules entering the collagen protein fibers to dissolve more protein fragments. Compared to the control, the higher total protein content in the collagen extract solution from Pangasius and Tuna skins washed with galangal strengthened the assumption stated before (Figure 5).

Sreeyani et al. were found that galangal extract could inhibit the formulation of undissolved calcium oxalate [17]. This property was underlying the presence of alkaloids, glycosides, flavonoids in the galangal extract. Many organic substances include those three substances known as organic ligands that can form complexes with metals [21]. The formation of metal complexes by organic compounds present in galangal seems to cause the lower ash content in the skin soaked in galangal extract compared to the control. The relationship might be caused by the dissolution of minerals in the skin such as calcium, magnesium, and zinc [22], causing the deformities of skin integrity, leading to the attenuation of hydrolyzed collagen protein content in the ASC extract.

5 Conclusions

This research found that soaking the fish skin with the galangal solution gave less odor, almost as much as the fish soaked in ethanol 70%. Moreover, the skin had a higher degree of swelling and total protein content in the ASC extract solution than the fish skin soaked in ethanol 70%. Based on the data above, we conclude that galangal extract 5% has the benefit of being used as a washing solution for fish skin in the pretreatment process of the acid-soluble collagen production process.

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