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Utilization of Hydrolyzed Collagen from Milkfish (*Chanos chanos*) By products in Nutraceutical Gummies: A Scientific and Socioeconomic Perspective

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Abstract: The amount of fish waste in the Philippines is increasing, and there is a limited knowledge on managing fishery by-products in the country. Collagen is the most abundant protein found in animals and is commonly used in food, pharmaceutical, and biomedical industries. In present most of the collagen used in the market is porcine and bovine which gives rise to the risk of swine flu, mad cow disease or bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD). Moreover, religious constraints had alerted researchers to probe alternative sources of collagen. Fish Collagen has a lot of benefits on health and found to be a good source of antioxidants. Hydrolyzed Collagen (HS), also known as collagen peptide or collagen hydrolysate from fish bones and scales has good antioxidant and hypertensive properties. The development of hydrolyzed collagen extracted from milkfish wastes to produce chewable gummies presents a practical and effective approach in the production of bioactive nutrients and supplements in this modern world. The hydrolyzed collagen from milkfish wastes was extracted yield, antioxidant activity, and proximate composition. There was 12.3% hydrolyzed collagen extracted from the milkfish waste. The antioxidant activity of fish wastes was 0.26±0.01 mg TE/g, this indicates that fish waste could be a source of antioxidants. The average content of moisture is 7.3±0.17% while the ash content is 39.8±0.3% and is relatively high. It also has a consistent protein content of 43.5±0.25%; and the fat content is approximately 3.0±0.12%. It also has an average fiber content which is 18.2±0.25% while the carbohydrate content is 6.3±0.17%. Overall, this research study contributes to the growing body of knowledge on collagen extraction from fish waste and its potential use in developing functional food products like nutraceutical gummies. The findings can benefit the food, pharmaceutical, and biomedical industries, as well as provide a sustainable way of managing fish waste and promoting good health among the population.

Keywords: Fish Collagen, Hydrolyzed Collagen, Antioxidant Properties, Milkfish Waste Utilization

I. Introduction

Due to its remarkable qualities over mammalian-based collagen, including its high collagen content, low molecular weight, biocompatibility, ease of absorption by the human body, lack of risk for disease transmission, affordability, and minimal environmental impact, fish collagen has sparked interest in the field of research. According to Guowei Li et al. (2020), fish collagen is a good source of antioxidants and has several health benefits.

Collagen, which is widely used in the food, pharmaceutical, and biomedical industries, is predominantly sourced from porcine and bovine sources. However, these sources pose a risk of transmitting diseases such as swine flu, mad cow disease or bovine spongiform encephalopathy (BSE), and foot-and-mouth disease (FMD). Moreover, religious considerations must be taken into account. The utilization of fish waste as a collagen source can eliminate these risks and serve as a cost-effective solution. Collagen, being the most abundant protein in animal bodies, has limited applicability due to its high cost. Therefore, the utilization of fish processing waste as a collagen source provides a promising alternative that can also mitigate serious environmental pollution.

In the Philippine setting, there is limited knowledge on managing the fishery by-products. Most of the research conducted focused on the utilization and characterization of different fishery commodities (Copolla, 2021).

Furthermore, based on the regional trend in seafood production and consumption the total fish supply will increase from 154 million tons in 2011 to 186 million tons in 2030. In addition, the fastest aquaculture growth is expected for tilapia and shrimp, in India, Latin America, and Southeast Asian countries (Kobayashi et al., 2015). There is a great potential for turning these wastes into valuable products. Nurilmala (2020) had proven that fish wastes showed relatively high amounts of antioxidant activity. In recent years, the use of antioxidants as a functional ingredient in the diet of people increased significantly León-López (2019). According to nutrition research, antioxidants are nutritionally beneficial compounds that can be used to keep our immune system strong enough to fight COVID-19 and other diseases. Collagen, particularly in its hydrolyzed form, is a widely recognized functional ingredient in the food industry due to its antioxidant properties. Hydrolyzed collagen (HC), also known as collagen peptide or collagen hydrolysate, is a type of collagen that can be easily absorbed into the bloodstream when used as a supplement. In a study of León-López (2019), it was found that hydrolyzed collagen from fish bones and scales has good antioxidant and hypertensive properties. The antioxidant property is due to the repeating unit glycine-proline-alanine in their structure.

One current research interest in drug delivery is the use of gummies as a delivery method. Gummies have become increasingly popular as a preferred form of vitamin and mineral supplements due to their appealing taste, texture, and ease of consumption. As a drug delivery method, gummies have several advantages over traditional tablet forms. The chewable nature of gummies allows for more efficient absorption of nutrients and drugs as they are more readily broken down in the mouth and stomach. This can result in faster onset of action and improved bioavailability. Additionally, gummies can be designed in various shapes, colors, and flavors, which can potentially improve patient compliance and adherence to medication regimens. Therefore, the use of gummies as a drug delivery method has the potential to improve patient outcomes and revolutionize drug delivery in the future.

This study generally aimed to extract hydrolyzed collagen from Milkfish (Chanos chanos) waste using an Acid Extraction Procedure and to develop nutraceutical gummies as a functional food. Specifically, the study aimed to: (1) determine the yield of collagen extraction; (2) evaluate the antioxidant properties of the extracted collagen; and (3) analyze the proximate composition of the collagen to achieve this goal. Additionally, (4) the study aimed to process the extracted collagen into gummies. By accomplishing these objectives, the research paper could have contributed to reducing food waste and developing a new functional food product with potential health benefits. Furthermore, the study could have shed light on the potential uses of fish waste as a source of hydrolyzed collagen and provided valuable information for the food and supplement industries.

The study was conducted at the Rice Chemistry and Food Science Division, Philippine Rice Research Institute, Maligaya, Science City of Munoz, Nueva Ecija from February-March 2023. This provided information in converting milkfish (Chanos chanos) waste into value added products like nutraceutical gummies as functional food. It may also help the government to ensure the strong immunity of the citizens regardless of the health problems facing the country, including COVID-19.

II. Methodology

Collection of Samples

Milkfish scales were collected from a milkfish processing plant at (BFAR - National Fisheries Development Center (NFDC), Bonuan Binloc, Dagupan City, Pangasinan, Dagupan, Philippines). The collected samples were transported in an iced condition. Samples were washed with distilled water and kept frozen at -20°C until processing and analysis.

Extraction of Fish collagen

The procedure for the extraction of collagen were based on the study of Jafari et al. (2020) with certain modifications. Instead of sodium hydroxide, sodium bicarbonate (baking soda) was used, and food-grade acetic acid replaced sulfuric acid. Throughout the process, distilled water was employed for both the washing of acid and alkali during pre-treatment and for the extraction itself. The fish samples, weighing 400 grams, were treated with a 0.2% solution (w/v) of food-grade sodium bicarbonate (Arm & Hammer) in a ratio of 1:7 (sample to solution) for 40 minutes. Following this, the samples were thoroughly rinsed with distilled water. Subsequently, the treated samples underwent a similar treatment with a 0.2% solution (w/v) of food-grade phosphoric acid (NECO Philippines, Inc.) in a ratio of 1:7 for 40 minutes.

This acid treatment process was repeated three times for each sample to ensure consistency and reliability of results. Following the acid treatment, the samples were treated with a 1% solution (w/v) of food-grade acetic acid (NECO Philippines, Inc.) for 12 hours. Thorough rinsing with distilled water was repeated three times. The use of acetic acid as a co-solvent enhances the extraction process by increasing the solubility of the target substance in the solvent. Acetic acid also serves as a pH modifier, which may have improved the extraction efficiency by adjusting the pH to an optimal range. The extraction process involved using distilled water in a ratio of 1:7 (sample to water) on a water bath at 60°C for 8 hours without stirring. After 8 hours, the samples were filtered to separate the extract, which was then heated in a water bath at 60°C for another 8 hours to reduce the volume to approximately one-third of the original. The resulting clear extract was filtered again using a strainer to remove any remaining solid particles, ensuring a final extract of high quality and free from impurities. Pulverizing the dried extract into a fine powder using a pulverizer is the final step in the process. This step ensures that the powder has a uniform particle size, making it easier to handle and incorporate into various products. The resulting powder can then be stored in an airtight container to preserve its quality and prevent any moisture or contaminants from entering.

The use of an airtight container is important to maintain the quality of the fish scale collagen powder. Moisture and oxygen can degrade the powder over time, reducing its efficacy and shelf life. By packaging the powder in an airtight container, the product can be preserved for longer periods, ensuring that it retains its effectiveness and quality. This enhanced methodology of fish collagen extraction, including the modifications and additional steps, aims to obtain a purified collagen extract suitable for various applications. The hydrolyzed collagen yield of the sample was calculated using the formula:

Yield of hydrolyzed collagen = (Weight of vacuum oven dried collagen/ Wet weight of fish waste) x 100

This formula is used to calculate the percentage of hydrolyzed collagen obtained from a given amount of wet fish waste. To calculate the yield, the weight of vacuum oven dried collagen was divided by the wet weight of fish waste and then multiplied by 100 to get the percentage. The resulting value represents the proportion of hydrolyzed collagen obtained from the initial amount of fish waste used in the process. A higher yield indicates a more efficient extraction process, while a lower yield may suggest that the extraction process was less efficient or that there was loss of material during processing.

Product Development

The collagen that was extracted was utilized to create gummies. To begin with, 15 grams of collagen were bloomed in 3 tablespoons of water and kept aside for 10 minutes. In a small saucepan, ¼ cup of water was combined with 20 grams of Stevia, which is a natural sweetener, and then 2 tablespoons of lemon juice were added. To this mixture, ¼ cup of corn syrup is added before heating the pan. The saucepan is placed over medium heat, and the mixture is cooked until it reaches a boiling point, a candy thermometer was clipped on the side of the pan. The solution was then cooked for 8 minutes until it reached 240°F, following with the addition of ¼ cup of honey. The solution was immediately removed from the heat and carefully poured into the collagen mixture while the mixer was turned on at low speed. Once all the syrup was added, the speed was increased to high. The solution was allowed to cool for 5 minutes, after which it was filled into a gummy mold using a dropper to prevent bubbles from forming in the gummies. The gummies were cured for 4 hours and then dusted with confectioner's powdered sugar (Peotraco). Finally, the gummies were packed in clear resealable pouches.

Diagram of the Experimental Procedure

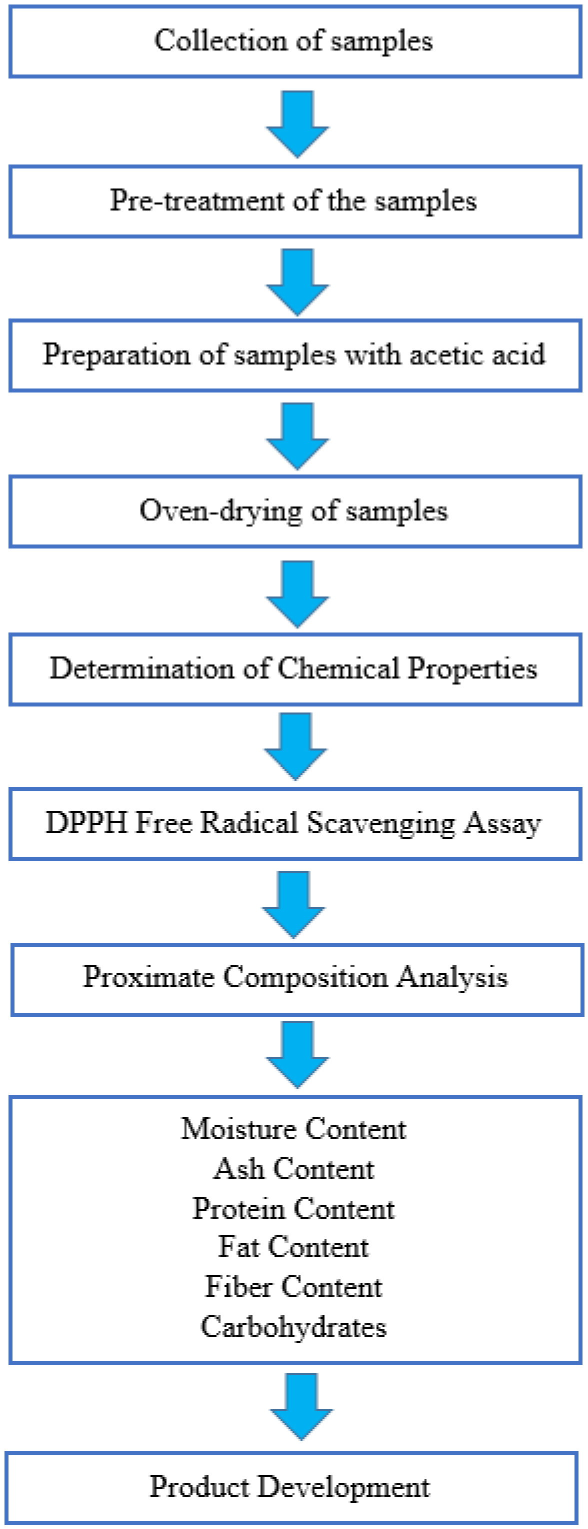

Figure 1: Flow of the Experimental Procedure

III. Result and Discussion

This study generally aimed of utilizing an acid extraction procedure to extract hydrolyzed collagen from the waste of Milkfish (*Chanos chanos*) and to create functional food in the form of nutraceutical gummies. The study focused on determining the yield of collagen extraction, assessing the antioxidant properties of the extracted collagen, and analyzing the proximate composition of the collagen.

Hydrolyzed Collagen Yield of Milkfish Wastes

Hydrolyzed collagen extracted from milkfish (Chanos chanos) wastes was found to be 12.3%. This value was higher than the collagen reported from other fish species, including sole (8.3%), megrim (7.4%), cod (7.2%), hake (6.5%), bigeye snapper skins (6.5%), Nile perch bone (2.4%), and tuna fins (1.25%) (Aewsiri et al., 2010). However, the yield obtained in this study is lower than that reported in some other fish species, such as farmed Amur sturgeon (24.11%), Brownbanded bamboo shark (19.06-22.81%), freshwater carp (13.5%), black tilapia (11.5-18.3%), and Atlantic salmon skin (11.3%) (Nikoo et al., 2014). There was no mentioning of the part of the fish species analyzed.

The use of acetic acid as solvents for the extraction of the hydrolyzed collagen was found to be effective method, as shown by the 12.3% yield obtained. The results of this study suggest that this method could be a promising approach for the extraction of other target substances in the future. Further studies can be conducted to optimize the conditions of the extraction process and to characterize the extracted substance.

The process of filtering the clear extract obtained from fish scales using a strainer, followed by oven drying, and pulverizing it into a fine powder with a pulverizer, is an essential step in producing high-quality fish scale collagen powder.

The use of a strainer helps to remove any impurities or solid particles that may have been present in the initial extract, ensuring that the resulting powder is pure and free from contaminants. After straining, the extract is then subjected to oven drying, which helps to remove any remaining water or moisture, further increasing the purity and stability of the product.

The use of an airtight container is important to maintain the quality of the fish scale collagen powder. Moisture and oxygen can degrade the powder over time, reducing its efficacy and shelf life. By packaging the powder in an airtight container, the product can be preserved for longer periods, ensuring that it retains its effectiveness and quality.

The process of filtering, drying, and pulverizing the extract obtained from fish scales is an essential step in producing high-quality fish scale collagen powder. By following these steps and packaging the powder in an airtight container, the resulting product can be preserved for longer periods, ensuring that it remains effective and of high quality.

Determination of Chemical Properties of Hydrolyzed Collagen from Fish Wastes

Table 1: Antioxidant Activity of Fish Waste Hydrolyzed Collagen

Sample	**DPPH Radical Scavenging Activity (mg TE/g)**
R1	0.25
R2	0.25
R3	0.27
Average	0.26±0.01

The evaluation of the DPPH radical scavenging activity of collagen extracted from fish scales of (Chanos chanos) indicates its possession of antioxidant properties. The recorded value of 0.26±0.01 mg TE/g suggests a moderate level of antioxidant activity in the collagen derived from fish scales. These findings align with previous research that has also reported the antioxidant attributes of fish scale collagen.

The observed antioxidant activity of collagen can be ascribed to its amino acid composition, particularly the presence of hydrophobic amino acids like proline, glycine, and hydroxyproline. These specific amino acids have demonstrated antioxidant properties and the ability to scavenge free radicals within the human body.

The moderate antioxidant activity exhibited by the fish scale collagen from (Chanos chanos) implies its potential utilization as a natural antioxidant within the food and pharmaceutical industries. Antioxidants play a vital role in the prevention and mitigation of chronic diseases such as cancer, diabetes, and cardiovascular ailments. Hence, the development of natural antioxidants from sustainable sources such as fish waste can contribute to both human health and environmental sustainability.

Based on the results, the average DPPH radical scavenging activity of fish scale collagen from (Chanos chanos) was found to be 0.26±0.01 mg TE/g, indicating its potential as a natural antioxidant. Further studies can explore the potential of fish scale collagen as a functional food ingredient and investigate its effect on human health.

The present study aimed to develop collagen gummies that incorporate fish collagen as a novel ingredient and natural sweeteners to enhance their nutritional value. The successful creation of collagen gummies is deemed a significant achievement in meeting this objective. Furthermore, the incorporation of fish collagen in the product formulation offers promising health benefits, specifically in promoting skin and joint health.

The use of Stevia as a natural sweetener, as opposed to conventional sugar, is a judicious choice that enables a reduction in the overall calorie content of the gummies while still providing a satisfying sweetness. Moreover, the inclusion of honey in the recipe may provide a subtle flavor and aroma to the gummies, further enhancing their palatability.

The method used in the preparation of the gummies, as described in the methodology section, is critical in achieving the desired texture and consistency of the product. In particular, the cooking process, which involves heating the sweetener mixture to a specific temperature and subsequently adding it to the bloomed collagen mixture, is an essential step. The use of a candy thermometer is essential to ensure that the sweetener mixture attains the correct temperature, which is crucial for proper gummy formation. The use of a gummy mold and dropper to pour the mixture carefully is also an important detail that contributes to the overall quality of the final product.

The dusting of the gummies with confectioner's powdered sugar and their packaging in clear resealable pouches add to the product's appeal. These final touches provide a polished appearance and make the product more marketable. Overall, the findings suggest that the methodology utilized in producing collagen gummies using fish collagen, Stevia, and honey was successful in creating a high-quality product,

The gummies made with fish collagen had a smooth texture and were firm enough to hold their shape. However, the color of the gummies was not very appealing and had a slightly off-white hue, likely due to the natural color of the fish collagen. Additionally, there was a slight fishy odor present in the gummies.

Socio-economics importance

Significant economical significance is attached to the development of new products from fish byproducts, such as hydrolyzed collagen in nutraceutical gummies. Through the utilization of fish leftovers, industry can lower waste and improve the sustainability of fishing methods. This strategy not only minimizes waste and helps manage environmental concerns, but it also encourages a circular economy in the seafood sector. This may result in new business ventures and the provision of jobs for fishing-dependent communities. Fish byproducts can be used to make high-value goods like nutraceuticals, which can boost livelihoods, stimulate economic growth, and help fishing communities maintain their financial stability.

Additionally, the public's health may benefit from the development of novel fish-based goods. Hydrolyzed collagen included in nutraceutical gummies may have positive effects on joint health and skin suppleness. There is a chance to improve general wellbeing and treat nutritional inadequacies by making these items more widely available. From a wider economic angle, the expansion of the nutraceutical market may encourage funding for R&D, resulting in additional innovations and diversification within the seafood sector. The interplay of public health, economic growth, and environmental sustainability highlights the many advantages of creating novel fish-based goods.

IV. Summary and Conclusion

This study aimed to extract hydrolyzed collagen from Milkfish (Chanos chanos) wastes using the acid extraction method and investigate its potential as a source of natural antioxidants for developing effective and safe food supplements. The Acetic Extraction method was employed, ensuring the production of clean and high-quality collagen extract from Milkfish wastes. Approximately 12.3% of collagen was successfully extracted from 400g of

Milkfish scales using this method. The use of acetic acid as a co-solvent proved advantageous by enhancing the solubility of the target substance during extraction. Additionally, the process of filtering, drying, and pulverizing the extract was crucial in obtaining high-quality fish scale collagen powder. The proximate composition analysis of the extracted collagen revealed its antioxidant properties, indicated by a DPPH Radical Scavenging Activity of 0.26±0.01 mg TE/g. These properties have the potential to protect cells and tissues from oxidative damage caused by free radicals, slow down the aging process, and extend the shelf life of food products.

Furthermore, the proximate composition analysis showed that Fish Waste Collagen has an average moisture content of 7.3±0.17%, which contributes to its stability and prevents microbial growth. The collagen also exhibited an average crude ash content of 39.8±0.3%, indicating its richness in calcium, phosphorus, and magnesium. With an average crude protein content of 43.5±0.25%, the collagen demonstrated good nutritional value due to its high protein content. The average crude fat content of 3.0±0.12% suggested advantageous health effects, such as providing energy, insulation, and organ cushioning. The collagen also contained an average crude fiber content of 18.2±0.25%, making it a potential source of dietary fiber, especially for individuals who struggle to consume vegetables or meet their daily fiber requirements. Lastly, with an average carbohydrate content of 6.3±0.17%, the collagen served as a low-carbohydrate protein source suitable for individuals managing their blood sugar levels or those with diabetes.

In terms of drug delivery formats, gummies were found to be the most favorable option due to their ease of use, portability, and availability of raw materials. Collagen gummies offer additional health benefits, including improved skin, hair, and nail health, as well as support for joint and bone health when used as a gelling agent. The use of natural sweeteners like stevia and honey in collagen gummy production makes them a healthier alternative to conventional gummy candies. Collagen gummies have the potential to enhance patient compliance and satisfaction with medication regimens, particularly for individuals who struggle to swallow pills or other forms of medication.

Overall, Fish waste collagen has demonstrated potential for use in functional foods and nutraceuticals, meeting the demand for natural and healthy food products. Fish scale collagen emerges as a promising ingredient with various applications across industries, offering a reliable source of hydrolyzed collagen with significant benefits.

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