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Research paper

Preparation of Fish Glue from Three Different Types of Freshwater Fishes (*Oreochromis niloticus*, *Labeo rohita*, *Labeo catla*)

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ABSTRACT

This study investigates the preparation of fish glue from three different species of freshwater fish: *Oreochromis niloticus* (Tilapia), *Labeo rohita* (Rohu), and *Labeo catla* (Catla). Fish glue, a natural adhesive derived from collagen-rich materials, has been historically significant in various applications, including woodworking, art conservation, and culinary uses. The project aims to utilize fish swim bladders, a byproduct of the fishing industry, to produce a sustainable adhesive, thereby promoting environmental sustainability and reducing waste. The methodology involves extracting collagen from the swim bladders through a multi-step process, including cleaning, soaking, and heat treatment. The quality of the produced glue is evaluated based on its adhesive properties and potential applications. The findings highlight the ecological and functional advantages of using fish glue over synthetic alternatives, emphasizing its role in enhancing the economic value of fishery resources.

1. Introduction

Fish glue, a historically significant adhesive, has been utilized since ancient times, with evidence dating back at least 3,500 years to Egypt, where it was produced by heating fish parts. The Roman scholar Pliny the Elder referred to it as ichtyokolla in the first century. In medieval Europe, it found applications in painting and gilding, as noted by Theophilus and Cennino Cennini. Isinglass, derived from sturgeon bladders, became particularly valued for its strong adhesive properties, remaining important in art conservation through the 20th century. Ancient civilizations likely discovered fish glue through trial and error, recognizing its adhesive properties when boiling fish parts, especially bladders and skins. The Greeks referred to it as ichtyokolla, with its use documented by figures like Dioscorides and Hippolytus from the 1st to 3rd centuries noting its applications in medicine and as a binding agent in art and manuscripts. To effectiveness for repairs in woodworking and gilding was acknowledged in ancient Egypt and throughout the Mediterranean, contributing to its continued use across various cultures.

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1.1 Ancient Egypt (1500-1000 BC)

In ancient Egypt, fish glue was utilized for wood furnishing and mural paintings, with artifacts found in tombs dating back to this period. Stone carvings depict the preparation and application of this adhesive, underscoring its role in preserving the legacies of pharaohs. Fish glue was particularly important for adhering decorative elements and ensuring the durability of artistic works in burial sites, reflecting the Egyptians' commitment to preserving their cultural heritage. Fish glue, a historically significant adhesive, was prepared through multi-step process in ancient Egypt. The preparation began by boiling fish parts, particularly skins and bones, in water over a fire to extract the collagen protein. The resulting gelatinous substance was then dried and ground into a fine powder. When needed, the powdered glue was mixed with water and reheated to the desired consistency for application. The molten fish glue was applied with brushes to adhere wood furnishings and bind pigments in mural paintings, as evidenced by stone carvings and artifacts found in pharaohs' tombs. The Egyptians valued fish glue for its strong adhesive properties and used it extensively in their craftsmanship and artistic works.

1.2 Classical Antiquity

During Classical Antiquity, the Greeks and Romans employed fish glue, known as ichtyokolla, for various purposes, including art and medicine. Notable figures such as Dioscoridess documented its applications, emphasizing its importance in daily life and artistic practices. Fish glue was used as a binding agent in manuscripts and artworks, contributing to the longevity of these cultural artifacts. Its versatility made it a preferred choice for artists and craftsmen throughout Mediterranean.

China (Warring States Period, 475-221 BC) In China, fish glue was utilized as early as the Warring States period for binding materials and preparing pigments. This indicates its critical role in artistic endeavours and craftsmanship. The use of fish glue in traditional Chinese painting and calligraphy demonstrates its importance in enhancing the quality and durability of artistic works. Additionally, it was employed in various applications, including the creation of inks and as a binding agent for pigments, showcasing its versatility and significance in Chinese art.

2. Material

2.1 Swim bladder

swim bladders are the primarily material for fish glue production.

2.2 Scissor

Scissor are crucial for cutting and shredding dried swim bladders into smaller pieces and for removing impurities, membranes, and connective tissues.

2.3 500ml beaker

Three 500 ml jars will be used to store the dried swim bladders, which will be soaked for 24 hours in a 3% hydrogen peroxide solution to clean and prepare them for further processing.

2.4 Vessels

Four vessels will be utilized: one for soaking and cleaning the swim bladders after peeling away the collagen membrane and three for heat treatment.

2.5 Tray

Trays will be essential in the preparation of Isinglass from fish swim bladders, providing a flat surface for even soaking and drying.

2.6 Gas stove

A gas stove will be utilized as a consistent and controllable heat source for the heat treatment process in fish glue preparation, allowing for careful temperature monitoring.

2.7 Measuring cylinder

Measuring cylinder to accurately measure the hydrogen peroxide concentration for treating fish swim bladders.

2.8 Hydrogen peroxide

3% hydrogen peroxide solution to disinfect and clean the swim bladders, effectively removing impurities and pathogens while enhancing the transparency of the final product.

2.9 Wood

Preventing direct heat contact and allowing for gentle, even heating, crucial for maintaining the glue's adhesive qualities.

2.10 Polypropylene Wash Bottle

Polypropylene wash bottle will be used to add water to the barker for fish glue preparation from swim bladders will prove invaluable due to its excellent chemical resistance.

2.11 Silicon cover

A silicon cover will be used to prevent evaporation and maintain necessary moisture levels for optimal glue consistency.

3. Methodology

3.1 Site selection

The Siddipet Integrated Fish Market serves as an ideal location for collecting fish waste, particularly swim bladders of Tilapia, Rohu, and Catla catla, for the production of fish glue. This market generates approximately 50-80 kgs of fish per day, providing a substantial and reliable source of raw materials for your project. The high volume of waste produced at the Siddipet Integrated Fish Market ensures that you can obtain sufficient quantities of swim bladders to manufacture fish glue efficiently. By utilizing this readily available waste stream, you can effectively convert what would otherwise be discarded into a valuable by-product, contributing to the overall sustainability and economic viability of your project

3.1.1 Step 1: Collection of fish swim bladders

The first step in the fish glue production process involves the collection of swim bladders from three specific fish species: Tilapia, Labeo rohita, and Catla catla. These swim bladders serve as the primary raw material for the manufacture of fish glue. To ensure a consistent supply of swim bladders, a systematic collection process is established at the Siddipet Integrated Fish Market. Fish vendors and wholesalers are engaged to facilitate the collection of swim bladders from the daily catch. Proper storage facilities are provided to maintain the quality and freshness of the collected swim bladders until they are ready for processing.

Fig. 1 Tilapia [*Oreochromis niloticus*]

Fig. 2 *Labeo rohita*

Fig. 3 *Labeo catla*

Fig. 4 Collection of swim bladders

3.1.2 Step 2: Separation of swim bladders by fish species size

In the second step of the fish glue production process, the collected swim bladders are separated based on the size of the fish species. This classification is done on the basis of size of the fish directly correlates with the size of the swim bladders, which can impact the glue extraction process and the final product quality. The swim bladders are categorized into three groups based on the typical size ranges of the fish species:

1. **Small swim bladders from Tilapia**: Tilapia is a relatively small fish, typically ranging from 0.5kg to 1 kg in size. The swim bladders from this species are correspondingly small and are separated into their own category for processing.

2. **Medium-sized swim bladders from *Labeo rohita***: *Labeo rohita*, also known as the rohu, is a medium-sized fish, with an average weight of 1-2 kg. The swim bladders from this species are sorted into the medium-sized category.

3. **Large swim bladders from *Labeo catla***: *Labeo catla* is a larger fish species, with an average weight exceeding 2.5 kg. The swim bladders from this fish are the largest among the three species and This separation process is carried out meticulously to ensure that each size category is handled and processed separately. The size of the swim bladders is not directly related to their collagen content, as mentioned in the previous information. The size categorization is based solely on the typical size ranges of the fish species.

Fig. 5 Collected swim bladders of three fishes

3.1.3 Step 3: Peeling the collagen membrane carefully

The collagen membrane is removed from swim bladders during the preparation of fish glue to enhance the adhesive quality and ensure a smooth extraction process. The swim bladder primarily consists of collagen, which is the key protein responsible for the adhesive properties of fish glue. However, the collagen is often surrounded by membranes and other materials that can hinder the extraction and purification process.

If the collagen membrane is not removed from the swim bladders during the preparation of fish glue, it can negatively impact the quality and efficiency of the glue extraction process.

Fig. 6 Peeling of collagen membrane

Reasons to remove collagen membrane

The collagen membrane contains various impurities and contaminants that can interfere with the glue extraction process. These impurities can include fats, proteins, and other materials that are not part of the pure collagen needed for high-quality fish glue. Leaving the membrane intact can introduce these contaminants into the final glue product, affecting its purity and performance.

3.1.4 Step 4: Washing the swim bladders with water

After peeling the collagen membrane from swim bladders, common impurities that may remain include blood residues, connective tissue, and miscellaneous proteins. Blood and other fluids can stick to the swim bladder tissue, while connective tissues like blood vessels may also be present. To effectively remove these impurities, the swim bladder tissue is typically rinsed thoroughly with distilled water to remove blood and fluids After these cleaning steps, the swim bladder tissue can be used as a high-quality source of type l collagen for various applications.

Fig. 7 Washing of swim bladders

3.1.5 Step 5: - Removal of gas from swim bladders

When preparing fish glue from swim bladders, it is essential to remove the gas contained within them during the soaking process. Swim bladders are gas-filled organs that help fish maintain buoyancy in water. However, when these bladders are harvested for glue production, the presence of gas can pose several challenges.

Fig. 8 Removing of gas with knife

Firstly, gas-filled swim bladders can create air pockets that interfere with the glue's ability to dissolve and bond effectively when heated. This can lead to an inconsistent mixture that may not adhere properly, compromising the quality of the final product. By venting the gas, the swim bladder becomes more amenable to soaking in water allowing it to absorb moisture more uniformly. This absorption is crucial because it helps to break down the collagen and proteins within the swim bladder, which are the primary components that contribute to the adhesive properties of fish glue. Additionally, removing the gas ensures that the resulting glue has a smoother and more homogeneous consistency. This is particularly important for applications in crafting, woodworking, and conservation, where clarity and strength are paramount. A well-prepared fish glue will bond effectively and provide a durable adhesive solution, making the initial step of gas removal vital to the overall quality and performance of the glue. Thus, venting the swim bladders not only enhances the glue's effectiveness but also contributes to a more reliable and high-quality product and soaked the Swim bladders for 4-5 hours to hydrate and soften them, making it easier to extract the gelatine necessary for fish glue, also known as isinglass.

3.1.6 Step 6: Drying of swim bladders

After soaking the swim bladders for four hours, drying them for 48 hours is a critical step in the preparation of fish glue. This drying process removes excess moisture, which is essential for several reasons. Foremost moisture content affects the glue's adhesive properties. If the swim bladders retain too much water, the resulting glue may not achieve the desired viscosity and strength when rehydrated. Proper drying ensures that the glue can dissolve effectively in water, forming a strong bond when applied.

Furthermore, drying helps to preserve the swim bladders. Excess moisture can lead to spoilage or microbial growth, which could compromise the quality of the glue. By thoroughly drying the swim bladders, you enhance their shelf life and ensure that they remain suitable for glue production. Lastly, the drying process allows for a more uniform texture in the final glue product. Properly dried swim bladders break down more evenly during the cooking phase, resulting in a smoother, more consistent glue that is easier to work with and provides better adhesion.

Overall, this step is vital for producing high-quality fish glue that meets the necessary performance standards for various applications, including woodworking and conservation.

Fig. 9 Preparing the swim bladders for drying

Fig. 10 Drying swim bladders at room temperature

Reasons for drying of swim bladders

1. Preserves Collagen Structure: Maintains adhesive properties.
2. Enhances Shelf Life: Reduces risk of spoilage and microbial growth.
3. Facilitates Handling: Easier to transport and store.
4. Ensures Quality: Contributes to high-quality fish glue production.

3.1.7 Step 7: Preparation of fish glue

After the drying process of the swim bladders for 48 hours, the next crucial step in the preparation of fish glue involves collecting and cutting the dried swim bladders into smaller pieces. This step is essential for facilitating the extraction of collagen and ensuring a more efficient processing procedure.

Fig. 11, 12, 13 Dried swim bladders of Tilapia, Rohu, Labeo catla

3.1.7.1 Sub Step 1: Collecting the dried swim bladders

Once the swim bladders have been thoroughly dried, collection is the first step. It is important to handle the dried swim bladders carefully to avoid any damage or breakage. At this stage, you should ensure that the swim bladders are free from any contaminants or impurities that may have accumulated during the drying process. Proper collection sets the foundation for the next steps in the glue preparation process.

Fig. 14 Collection of dried swim bladders fishes

3.1.7.2 Sub Step 2: Cutting the dried swim bladders into small pieces

After collecting the dried swim bladders, the next sub-step is to cut them into smaller pieces. this is a critical action for several reasons:

Increased Surface Area: Cutting the swim bladders into smaller pieces increases the Surface area, which facilitates the extraction of collagen during the subsequent processing stages. A larger surface area allows for more efficient interaction with water and heat leading to better glue quality.

Easier Handling: Smaller pieces are easier to handle and process, making it more convenient during the cooking phase. This can improve workflow and efficiency in the production process.

Fig. 15 Cutting of dried swim bladders separately

Uniform Processing: Cutting the swim bladders into uniform sizes ensures that they cook evenly, leading to a consistent glue product. This uniformity is crucial for achieving the desired viscosity and adhesive properties in the final fish glue.

In summary, Step 7 involves the careful collection and cutting of dried swim bladders into smaller pieces. These actions are essential for maximizing the effectiveness of the collagen extraction process and ensuring the production of high-quality fish glue.

3.1.8 Step 8: Soaking the cutting swim bladder pieces in hydrogen peroxide solution

After cutting the dried swim bladders into small pieces, the next step in the preparation of fish glue involves soaking the cut pieces in a hydrogen peroxide solution. This step is crucial for enhancing the transparency of the final product, particularly when preparing isinglass.

3.1.8.1 Sub-Step I: Collecting the cutting swim bladder pieces

Begin by carefully collecting the cut pieces of dried swim bladders in a clean, 500 ml beaker. Ensuring that the beaker is free from any contaminants or impurities that may interfere with the soaking process.

Fig. 16 Collecting the small pieces of swim bladders in beaker

3.1.8.2 Sub-Step II: Soaking the small pieces in the hydrogen peroxide solution

Carefully pouring the hydrogen peroxide solution into the beaker containing the cut swim bladder pieces. Ensure that all the pieces are fully submerged in the solution. Cover the beaker and allow it to soak for 24 hours.

Fig. 17 Pouring Of Distilled Water in Beaker for Concentration

The addition of hydrogen peroxide serves two main purposes:

1. Enhancing Transparency: Hydrogen peroxide acts as a bleaching agent, helping to remove any impurities or discoloration from the swim bladder pieces. This results in a clear and more transparent final product, which is particularly desirable for isinglass.

Fig. 18 Adding of hydrogen peroxide in concentration

2. Facilitating Collagen Extraction: The hydrogen peroxide solution helps to break down the collagen fibres within the swim bladder pieces, making them more accessible for extraction during the subsequent processing stages. This can improve the efficiency of the glue-making process and contribute to a higher-quality final product. It is important to note that the concentration of hydrogen peroxide used in this step is relatively low (3%) and is not harmful to the collagen structure. However, it is crucial to ensure proper ventilation and handle the solution with care, as hydrogen peroxide can be irritating to the eyes and skin.

Fig. 19 Concentrating the solution for 24 hours

3.1.9 Step 9: Concentrating and heating the swim bladder mixture

After soaking the cut swim bladder pieces for 24 hours, the next step involves concentrating the samples and preparing them for heating treatment. This step is crucial for dissolving the isinglass solution completely, ensuring a high-quality adhesive.

Fig. 20 The concentration of swim bladers soaking for 24   ours

3.1.9.1 Sub-Step I: Collecting the concentrated samples

Begin by carefully collecting the concentrated swim bladder pieces from the soaking solution. Use a sieve to remove the hydrogen peroxide solution, ensuring that only the soaked pieces remain. This process helps to eliminate any residual liquid that could affect the consistency of the glue.

Fig. 21 Collection of concentrated swim bladders

3.1.9.2 Sub-Step II: Sieving the swim bladder pieces

Once the solution is removed, sieve the small swim bladder pieces together to ensure uniformity. This step helps to separate any undissolved particles and ensures that only the well-soaked, concentrated pieces are used for the next stage. Uniformity in size is important for even processing during heating.

Fig. 22 Sieving of soaked swim bladders

3.1.9.3 Sub-Step III: Adding distilled water

After sieving, measure out 20 grams of the sieved swim bladder pieces and transfer them back into a clean container. Add distilled water to the mixture, ensuring that the pieces are adequately submerged. The addition of distilled water is essential for facilitating the dissolution of the collagen during the heating process.

Fig. 23 Adding of water of heat treatment

3.1.9.4 Sub-Step IV: Covering the container

Once the distilled water is added, cover the container tightly with a silicone cover. This step is important to prevent evaporation of the solution during heating, which could lead to inconsistencies in the glue's concentration and quality.

Fig. 24 Covering the beakers with silicon cover

3.1.9.5 Sub-Step V: Heating in a double boiler

Place the covered container in a double boiler setup for heating. This method involves placing the container over a pot of simmering water, allowing for gentle and even heating. Heat the mixture for approximately 5 hours. The double boiler method is crucial as it prevents direct contact with high heat, which could damage the collagen and affect the glue's properties.

Fig. 25 Preparing beaker for the heat treatment

Fig. 26 Heating of concentrated solutions in the double boiler

During this heating period, the collagen from the swim bladders will dissolve into the water, creating a viscous isinglass solution. Stir the mixture periodically to ensure uniform heating and dissolution.

In summary, Step 9 involves collecting, sieving, and preparing the swim bladder pieces for heating. This meticulous process is essential for achieving a high- quality isinglass solution, which is characterized by its clarity and adhesive properties.

4. Trail No 1

4.1 Step I: Adding distilled water

After sieving, measure out 20 grams of the sieved swim bladder pieces and transfer them back into a clean container. Add 100 mL of distilled water to the mixture, ensuring that the pieces are adequately submerged. The addition of distilled water is essential for facilitating the dissolution of the collagen during the heating process.

4.2 Step II: Covering the container

Once the distilled water is added, cover the container tightly with a silicone cover. This step is important to prevent evaporation of the solution during heating, which could lead to inconsistencies in the glue's concentration and quality.

4.3 Step III: Heating in a double boiler

Place the covered container in a double boiler setup for heating. This method involves placing the container over a pot of simmering water, allowing for gentle and even heating. Heat the mixture for approximately 5 hours. The double boiler method is crucial as it prevents direct contact with high heat, which could damage the collagen and affect the glue's properties.

Fig. 27 Heating of swim bladder concentration with 100 ml of water

5. Result & Discussion

The failure in preparing fish glue from the swim bladders of Tilapia (*Oreochromis niloticus*) Rohu (*Labeo rohita*) and Catla (*Labeo catla* may stem from low collagen content and inadequate adhesive properties of key amino acids. Swim bladders generally contain lower collagen levels compared to other fish parts, which affects the glue's effectiveness. Essential amino acids like glycine, proline, and hydroxyproline are crucial for adhesive properties. However, their concentrations in swim bladders may not suffice for optimal performance.

High-quality collagen typically exhibits a significant presence of these amino acids, which enhances its adhesive capabilities. In contrast, the failure to achieve stickiness in glues derived from freshwater fish like Tilapia (*Oreochromis niloticus*) Rohu (*Labeo rohita*) and Catla (*Labeo catla*) may stem from several factors. The type and quality of collagen in these species may differ significantly from that in sturgeon. Sturgeon collagen has a unique structure that contributes to its superior adhesive properties.

Gorin, I. & Cherkasova, Z. (1977): The success of Sevruga sturgeon (*Acipenser stellatus*) and Russian sturgeon (*Acipenser gueldenstaedtii*) swim bladder glue, particularly isinglass, can be attributed to its unique composition and preparation methods. Sturgeon swim bladders are rich in collagen, which forms a flexible and strong adhesive when processed correctly. The glue is produced by soaking the dried bladders to hydrate them, followed by gentle heating to extract the collagen without damaging its structure, resulting in a high-quality adhesive with excellent bonding properties.

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