

Squid Gladius: A Revolutionary Material for Advanced Technologies and Sustainable Innovation

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Abstract

Gladius, commonly known as the squid pen, is an important internal structure that is primarily composed of β -chitin, possessing superior mechanical strength, flexibility, and biocompatibility compared to crustacean α -chitin. Every year, squid processing produces a significant amount of waste, with gladius being a valuable yet underutilized byproduct that continues to be discarded despite possessing promising properties for value-added and sustainable utilization. Due to its novel properties, it is increasingly explored for applications in biomedical, environmental, and agricultural sectors, making it a key component for a circular economy. It demonstrates effectiveness in wound healing, drug delivery systems, antimicrobial agents, and water treatment, including the removal of heavy metals and pollutants.

Introduction

Squid is a member of the class Cephalopoda, which includes exclusively marine molluscs such as nautiluses, octopuses, and cuttlefish. In squids, the important muscle groups are attached to the pen, which is an internalized shell that provides structural support for the digestive organs (Gosline & DeMont, 1985; Thompson et al., 2014, 2016; Williams, 1909; Young et al., 1998). While dissecting a squid, it is easy to remove, and since they are transparent and flexible, it has an appearance resembling a long piece of plastic.

Gladius is found in numerous cephalopods belonging to the superorder Decapodiformes, particularly the squids, and as an exception in the living species of the Octopodiformes, the vampire squid (*Vampyroteuthis infernalis*). The Indian squid (*Uroteuthis duvauceli*) is the dominant species in Indian waters, which contributes about 97% of the total catch all over the country, whereas other species, such as *Sepioteuthis lessoniana* and *Doryteuthis sp.*, represent the remainder (Anusha & Fleming, 2014).

Fisheries Statistics and Waste Generation

According to the *Handbook on Fisheries Statistics 2023* (Department of Fisheries, 2023), the marine fish landings in India during FY 2022-2023 were 44.3 lakh tonnes, with squid

landings accounting for 0.57 lakh tonnes which is comparatively lower, yet of significant commercial importance and the states with the highest squid landings were Karnataka (0.24 lakh tonnes), followed by Kerala (0.23 lakh tonnes) and Tamil Nadu (0.04 lakh tonnes). From the total squid landings, almost 90% is contributed by the west coast of India. These figures highlight the regional concentration of squid fisheries and underscore their growing role in India's marine sector.

Gladius Availability

The processing of squid produces many byproducts, such as squid ink, pen, skin, milt, liver, and viscera, which account for roughly 52% of the bodyweight (USDA, 2016). According to studies, the gladius accounts for approximately 0.2% to 1% of the squid's total body weight, highlighting the amount of waste generated and the potential for its utilization. Following the substantial squid landings in India, there is an abundant amount of gladius waste (Fig.1).

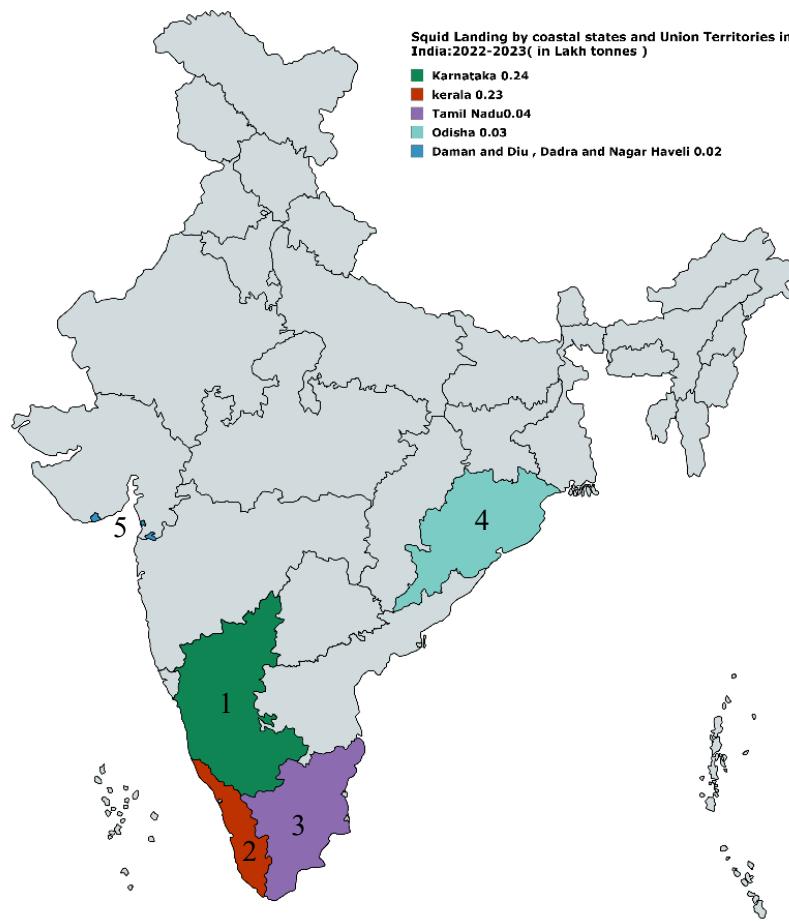


Fig.1 Squid Landing in India 2022-2023(DOF, 2023)

Chemical Composition and Properties

The Gladius, more commonly known as the squid pen, is an essential internal structure that is mostly made up of β -chitin, thus possessing superior mechanical strength, flexibility, and biocompatibility to α -chitin found in the crustacean shells. The main components

of squid pens are proteins and chitin, while moisture and minerals are present only in trace amounts. Even though the pen is protein-rich, its mineral content is somewhat lower and is mainly in the form of Calcium Carbonate (CaCO₃) and Magnesium. These properties make the squid gladius(pen) both flexible and strong, while the minerals give it some rigidity and a certain level of mechanical support for the squid's body.

Table 1: Proximate composition of *Loligo duvoceli*

Parameter	Value (%)
Protein	15.02 ± 0.455
Moisture	83.08 ± 0.504
Fat	0.74 ± 0.05
Ash	1.13 ± 0.046

Source: (Gajendra et al., 2020)

Morphometric Analysis

The morphometric parameters for Needle Squid (*Doryteuthis sibogae*) were recorded following standard cephalopod taxonomic descriptions (Roper & Voss, 1983).



Fig.2: The chitinous gladius (internal shell) of a Needle Squid (*Doryteuthis sibogae*) collected from the Veraval coast. (Image credit: Anjali Chaudhary)

Sr. No.	Parameter (Abbreviation)	Average Value ± SD
1.	Mantle Length (ML)	6.17 ± 0.62 cm
2.	Total Length (TL)	16.32 ± 1.33 cm
3.	Average Pen Weight (APW)	0.034 g ± 0.012
4.	Average Body Weight (ABW, dried)	12.8 ± 2.15 g

5.	% Gladius Contribution (dried weight)	0.27 % \pm 0.11
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Applications of Chitin and Chitosan Extracted from Squid Gladius

1. Antibacterial Properties

Squid pen chitosan exhibits significant antibacterial properties, as noted by Abdelmalek et al. (2017); chitosan derived from European squid (*Loligo vulgaris*) effectively inhibits foodborne pathogens such as *Escherichia coli* and *Staphylococcus aureus*, producing significant inhibition zones. Similarly, phosphorylated chitosan from Bigfin reef squid (*Sepioteuthis lessoniana*) gladius exhibited strong antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus mutans*, highlighting its potential use in biomedical applications (Sharma et al., 2024). In addition, Mooduto et al. (2019) observed strong inhibitory effects against *Porphyromonas gingivalis*, a key pathogen in periodontal disease. All of these results highlight that squid pen chitosan presents its potential as an environmentally friendly, natural antibacterial agent with a variety of uses, from coatings, sutures, wound healing, and food safety.

2. Antifungal activity

Chitosan from *L. vulgaris* gladius inhibited fungi like *Fusarium solani*, *Botrytis cinerea*, and *Alternaria solani*, with the highest effect against *B. cinerea* (Abdelmalek et al., 2017). The antifungal effectiveness of squid chitosan nanoparticles was observed against *Lasiodiplodia pseudotheobromae*, *Alternaria alternata*, and *Penicillium digitatum* (Cuong et al., 2022). These findings emphasize the potential of natural preservatives to control fungal spoilage.

3. Antioxidant activity

The squid gladius was treated as waste for a very long time, but it is now considered a valuable source of bioactive chitosan. Abdelmalek et al. (2017) observed that the chitosan from *Loligo vulgaris* gladius showed strong antioxidant activity in vitro. It has also been tested as a clarifying agent in apple juice, improving clarity without reducing nutritional attributes. Altogether, squid gladius chitosan represents a sustainable alternative for applications in food, health, and pharmaceuticals.

4. Biodegradable Films, Nanofibers, and Food Preservation

Pinpru et al. (2024) reported that D-chitosan extracted from squid gladius has a strong ability to form biofilms. Huang and Wang (2023) found that gelatin-based packaging films incorporated with squid pen chitosan obtained by High Hydrostatic Pressure Processing had higher tensile strength, lower water vapor permeability, improved thermal stability, and delayed lipid oxidation. These features help to extend the shelf life of perishable foods and preserve their nutritional value, making the films a sustainable way to reduce food waste. Likewise,

Cabrera-Barjas et al. (2021) demonstrated that chitin nanofibers from *Dosidicus gigas* squid pens can form flexible films with good mechanical strength, moderate swelling capacity, and high lightness, suggesting possible applications in food packaging, agriculture, and biomedical materials.

5. Bone tissue engineering and wound healing

Shavandi et al. (2015) fabricated biocomposite scaffolds through the combination of squid pen chitosan with hydroxyapatite and tricalcium phosphate. These scaffolds exhibited cell growth support, controlled biodegradation, and showed potential for bone regeneration. In a different study, Shavandi et al. (2016) formulated injectable gels made of squid pen chitosan, glycerol phosphate, and calcium phosphate, which upon warming to body temperature, formed stable gels that absorbed water efficiently and exhibited good cytocompatibility. Recently, Ramachandran et al. (2024) found that the addition of zinc oxide nanoparticles to, chitosan improved wound healing in zebrafish by decreasing inflammation, facilitating tissue repair, and speeding up wound closure. Collectively, these studies demonstrate the potential of squid pen chitosan in bone tissue engineering and wound healing.

6. Drug delivery

Chitosan from squid pens has also been utilized in drug delivery systems. Garcinuo et al. (2022) demonstrated that chitosan from squid pens is an excellent material for preparing thermoresponsive hydrogels for risperidone release. The system of the hydrogel made it possible for the drug to be released slowly; thus, it could be useful for long-acting treatments. Moreover, Magnabosco et al. (2020) in their experiment illustrated that chitin extracted from squid pens might be an ideal natural carrier to deliver doxorubicin. The material was very compatible with HeLa cells, and after the drug was loaded, it exhibited a potent cytotoxic effect on cancer cells. Overall, the evidence points to chitosan from squid pens as new biomaterial sources for the creation of controlled and targeted drug delivery systems.

7. Larvicidal activity

Squid pen-derived chitosan has demonstrated larvicidal activity against *Culex* and *Aedes* mosquito larvae (Sree Vidhya et al., 2023), and the bacterium *Serratia marcescens* TKU011 can produce prodigiosin, a pigment with insecticidal properties, using squid pen powder (Wang et al., 2011). All of these studies point to squid pen products as potential larvicidal and insecticidal substitutes for chemical pesticides.

8. Wastewater treatment and dye removal

Unlike conventional chemical treatments, squid pen-based materials provide an eco-friendly option for wastewater purification. Fermented squid pen powder from *Bacillus cereus* showed strong dye removal ability (Liang et al., 2015), while β -chitosan squid pen protein

hydrogel beads captured heavy metals such as copper and zinc (Moralez et al., 2023). Together, they show the potential of squid pens as sustainable and biodegradable resources for wastewater treatment.

Conclusion

The Gladius no longer remains a mere remnant in ruins; it is slowly but surely emerging as a boon, finding use in advanced materials and sustainable innovations. The presence of β -chitin makes it superior to other conventionally used sources, and it is used in biomedical advancements and environmental solutions. This enormous amount of gladius generated as waste by the squid processing industry highlights the untapped potential that, when efficiently harnessed, will contribute to creating a circular economy. Now the gladius is not just a worthless piece of discard but a material of the future and fortune with vast possibilities yet to be explored.

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