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Characterization of Protein and Polysaccharides from Squid Ink *(Uroteuthis singhalensis)* Using SDS-PAGE and FTIR Methods

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ABSTRACT

Squid ink is a complex biological secretion containing various bioactive compounds, including proteins, polysaccharides, melanin, and other biomolecules. These components have attracted scientific interest because of their potential biological and biotechnological applications. This study aimed to characterize the protein profile and functional groups associated with proteins and polysaccharides in squid ink from *Uroteuthis singhalensis* using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Fourier Transform Infrared Spectroscopy (FTIR). Protein characterization using SDS-PAGE revealed two predominant protein bands with estimated molecular weights of approximately 50–51 kDa and 26 kDa. FTIR analysis identified several characteristic absorption bands, including O–H/N–H stretching at 3396.64 cm^{-1}, C–H stretching at 2927.94 cm^{-1}, amide I at 1624.06 cm^{-1}, and amide II at 1539.20 cm^{-1}. Additional absorption bands at 1384.89–1340.53 cm^{-1} and 1236.37–1068.56 cm^{-1} were associated with CH_2/COO^- bending, possible S=O vibrations, and C–O–C glycosidic structures. These findings indicate the presence of protein-related compounds and functional groups potentially associated with polysaccharides in *Uroteuthis singhalensis* squid ink. This study provides preliminary biochemical information regarding the molecular characteristics of squid ink and highlights the importance of further purification and structural identification to determine its specific protein and polysaccharide components. These findings provide a basis for further biochemical investigation of this species.

INTRODUCTION

Squid ink is a biological secretion produced by cephalopods as part of their natural defense mechanisms. It is a chemically complex material containing melanin as its major pigment, together with proteins, peptides, polysaccharides, amino acids, and other secondary metabolites (Derby, 2014). Marine-derived biopolymers are also recognized as valuable materials for biotechnological applications (Carvalho et al., 2024).

Among the various biochemical components of squid ink, proteins and polysaccharides are important biomolecules because of their structural diversity and potential biological functions. Proteins consist of amino acid chains with different molecular weights and structural characteristics, whereas polysaccharides are carbohydrate polymers composed of monosaccharide units linked through glycosidic bonds. Certain marine-derived

polysaccharides may also contain sulfate groups, contributing to their distinctive physicochemical properties (Gieroba et al., 2023; Pereira et al., 2013). A sulfated glycosaminoglycan-like polysaccharide has also been isolated and structurally characterized from Sepia recurvirostra ink, although this finding cannot be assumed to apply directly to *Uroteuthis singhalensis* (Cheng et al., 2025).

The biochemical characterization of these components is an important initial step in understanding the molecular composition of squid ink. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used to separate proteins according to their molecular weights under denaturing conditions. This method is based on the established discontinuous SDS-PAGE system described by Laemmli (1970).

Meanwhile, Fourier Transform Infrared Spectroscopy (FTIR) is a spectroscopic technique used to identify functional groups based on their characteristic infrared absorption bands. In protein analysis, characteristic absorption regions include amide I and amide II (Barth, 2007), whereas polysaccharide-related structures can be associated with hydroxyl, glycosidic, and sulfate-related vibrations (Gieroba et al., 2023; Pereira et al., 2013). Therefore, FTIR provides preliminary information regarding the chemical structures of biomolecules within complex biological materials.

Uroteuthis singhalensis is a squid species whose ink represents a potential source of marine-derived biomolecules. However, detailed characterization of its protein profile and polysaccharide-associated functional groups remains important to improve the understanding of its biochemical composition.

Therefore, this study aimed to characterize the protein profile of *Uroteuthis singhalensis* squid ink using SDS-PAGE and identify its protein- and polysaccharide-associated functional groups using FTIR. The results are expected to provide preliminary information regarding the biochemical characteristics of squid ink and support further investigations into the identification and purification of its constituent biomolecules.

METHOD

Study Period and Sample Collection

The study was conducted from October to November 2025. Fresh squid *Uroteuthis singhalensis* was obtained from fish traders at Pabean Market, Surabaya, East Java, Indonesia. The extraction process was conducted at the Semi-Outdoor Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga. FTIR analysis was performed at the Pharmaceutical Analysis and Instrumental Chemistry Laboratory, Faculty of Pharmacy, Universitas Airlangga.

Extraction of Squid Ink

Fresh squid were dissected carefully through the ventral mantle, and the ink sacs were separated to minimize contamination from surrounding tissues. Approximately 7 g of fresh squid ink was collected and immediately stored at approximately 4°C.

The fresh ink was diluted with phosphate-buffered saline (PBS) at a ratio of 1:10 and homogenized using a refrigerated shaker at approximately 170–180 rpm and 4°C overnight. The homogenized suspension was subsequently centrifuged at approximately 10,000 rpm for 15 minutes at 4°C to obtain the supernatant.

The supernatant was precipitated by adding ethanol at a ratio of 1:3 and incubated overnight at low temperature. The precipitated fraction was separated by centrifugation and rehydrated with distilled water at a ratio of 1:3. The resulting solution was freeze-dried at approximately −40 to −80°C, followed by sublimation for approximately 30 hours to obtain dried squid ink extract.

Protein Characterization Using SDS-PAGE

Protein characterization was performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), following the general principles of the Laemmli system (Laemmli, 1970). Approximately 0.1 g of squid ink sample was finely ground under liquid-nitrogen cooling and transferred into a 1.5 mL microtube.

One milliliter of extraction buffer containing 100 mM Tris-HCl (pH 8.0), 20% glycerol, 4% SDS, and 2% β-mercaptoethanol was added to the sample. The mixture was homogenized and centrifuged at 12,000 rpm for 5 minutes. The supernatant was collected as the protein fraction. Loading buffer was subsequently added, and the sample was heated for 2 minutes before electrophoresis. Protein separation was performed using a 12.5% resolving gel and a 5% stacking gel.

Following electrophoresis, the gel was stained and destained using a solution containing 10% glacial acetic acid, 50% methanol, and 40% distilled water. Protein bands were compared with a molecular-weight marker to estimate the molecular weights of the detected protein fractions. Band intensity and thickness were evaluated using Gel Analyzer 19.1.

Functional Group Characterization Using FTIR

FTIR analysis was performed to identify the functional groups present in the dried squid ink extract, particularly those associated with proteins and polysaccharides. Approximately 1 mg of dried extract was mixed with approximately 200 mg of potassium bromide (KBr), finely ground until homogeneous, and compressed into a KBr pellet. The pellet was subsequently placed in the FTIR instrument for spectral acquisition.

The resulting spectrum was evaluated to identify characteristic absorption bands associated with hydroxyl, amino, amide, glycosidic, and sulfate-related functional groups. The interpretation of absorption bands was used to assess the presence of protein- and polysaccharide-associated structures in the squid ink extract.

Data Analysis

SDS-PAGE results were analyzed qualitatively by comparing the migration distances of protein bands with the molecular-weight marker. Molecular weights were estimated using the electrophoretic migration profile, while band intensity and thickness were evaluated using Gel Analyzer 19.1. FTIR spectra were interpreted based on characteristic absorption frequencies and their corresponding functional group assignments. The results were analyzed descriptively to determine the biochemical characteristics of protein- and polysaccharide-associated compounds in the squid ink extract.

RESULT AND DISCUSSION

Protein Profile of Squid Ink Based on SDS-PAGE

SDS-PAGE analysis was performed to characterize the protein profile of *Uroteuthis singhalensis* squid ink extract and estimate the molecular weights of the detected protein fractions. The electrophoretic profile revealed several protein bands with different migration distances, indicating the presence of protein fractions with different molecular weights. Based on densitometric analysis, two predominant protein bands were consistently detected at approximately 50–51 kDa and 26 kDa.

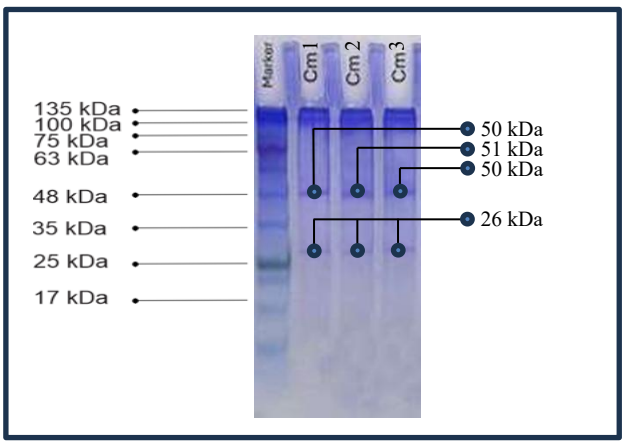


Figure 1. SDS-PAGE profile of red squid ink extract Cm1 = Sampel Cumi Merah 1, Cm2 = Sampel Cumi Merah 2, Cm3 = Sampel Cumi Merah 3.

The SDS-PAGE electrophoretic profile of Uroteuthis singhalensis squid ink extract revealed two prominent protein bands in the sample lane (Cm), with estimated molecular weights of approximately 51 kDa and 26 kDa, based on their migration distances relative to the protein molecular weight marker (Figure 1).

The presence of these bands indicates that the squid ink extract contains protein fractions with different molecular weights. The protein bands were further analyzed using densitometric measurements to determine their relative migration distances (Rf), peak values, raw volumes, and estimated molecular weights. The results of this analysis are presented in Table 1.

Table 1. Molecular weight of protein bands detected by SDS-PAGE

Sample	Lane	Band	Rf	Peak Value	Raw Volume	Molecular Weight (kDa)
CM-1	5	1	0.280	120	903	50
CM-1	5	2	0.479	143	1043	26
CM-2	6	1	0.276	121	1131	51
CM-2	6	2	0.475	146	1208	26
CM-3	7	1	0.279	113	828	50
CM-3	7	2	0.269	136	844	26

Table 1. indicates that squid ink contains proteins with distinct molecular weight characteristics. The consistency of these bands across the analyzed samples suggests a relatively similar protein profile among the samples examined.

Cephalopod ink is known to contain various biochemical constituents, including proteins, peptides, and melanin, which contribute to its complex chemical composition (Derby, 2014). Polysaccharide fractions have also been structurally characterized in ink from other cephalopod species, including a sulfated glycosaminoglycan-like polysaccharide from Sepia recurvirostra (Cheng et al., 2025). The identification of protein fractions through SDS-PAGE provides preliminary molecular information that can be used as a basis for further protein characterization.

However, SDS-PAGE only provides an estimation of molecular weight and does not directly identify the amino acid sequence or specific protein identity. Therefore, additional techniques, such as protein purification and mass spectrometry, are required to identify the specific proteins represented by the detected bands.

Functional Group Characteristics of Squid Ink Based on FTIR

FTIR analysis was performed to identify the functional groups present in the red squid ink extract, particularly those associated with proteins and polysaccharides.

The FTIR spectrum showed several characteristic absorption bands corresponding to different chemical bonds within the extract.

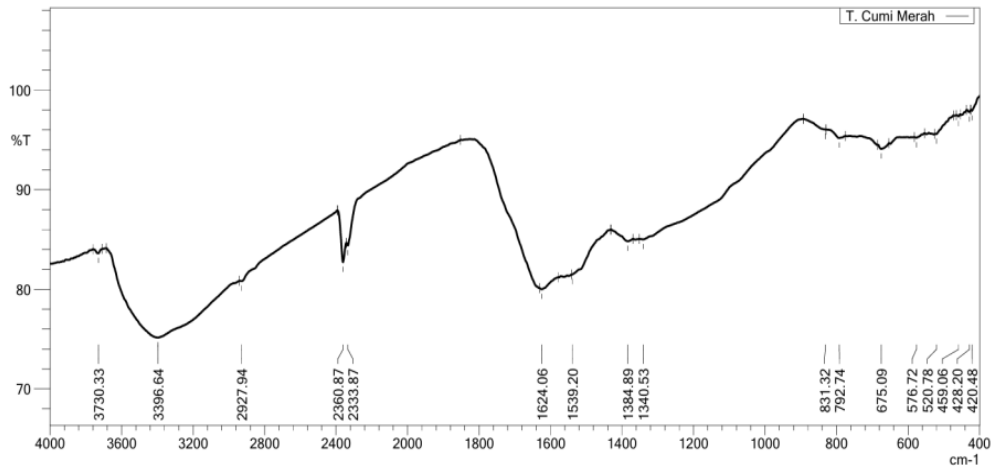


Figure 2. FT-IR spectrum of red squid ink extract

The FTIR spectrum of red squid ink extract from Uroteuthis singhalensis showed several characteristic absorption bands across the wavenumber range of 4000–400 cm^{-1} (Figure 2). The spectrum revealed absorption signals at several wavenumbers, including 3730.33, 3396.64, 2927.94, 2360.87, 2333.87, 1624.06, 1539.20, 1384.89, 1340.53, 831.32, 792.74, 675.09, 576.72, 552.78, 476.76, 428.20, and 420.48 cm^{-1}. These absorption bands indicate the presence of various chemical bonds and functional groups within the squid ink extract. In particular, the absorption regions associated with amide groups suggest the presence of protein-related compounds, while the fingerprint region may contain signals associated with carbohydrate and polysaccharide structures. The identification and interpretation of these absorption bands are presented in Table 2.

Table 2. FT-IR absorption bands and functional group interpretation

Wave Number (cm^{-1})	Interpretation
3396,64	O–H / N–H stretching
2927,94	C–H stretching
1624,06	Amide I
1539,20	Amide II
1384,89–1340,53	CH_2/COO^- bending atau S=O vibration
1236,37; 1163,08; 1068,56	C–O–C glycosidic / S=O
871,82–738,74–675,09	C–H bending
459,06–420,48	Fingerprint Area (C–O, C–S, C–N)

The absorption band at 3396.64 cm^{-1} was associated with O–H and/or N–H stretching vibrations, indicating the presence of hydroxyl- and amino-containing compounds. The absorption band at 2927.94 cm^{-1} corresponded to C–H stretching vibrations, which are commonly associated with organic compounds. The absorption bands at 1624.06 and 1539.20 cm^{-1} were assigned to amide I and amide II, respectively. These characteristic bands provide supporting evidence for the presence of proteinaceous compounds in the squid ink extract. Amide I is primarily associated with C=O stretching vibrations, whereas amide II is associated with N–H bending and C–N stretching vibrations (Barth, 2007).

In addition to protein-related functional groups, the FTIR spectrum showed absorption bands at 1384.89–1340.53 cm^{-1}, which may be associated with CH_2/COO^- bending and/or other vibrations. These signals should not be considered definitive evidence of sulfate groups because the more commonly reported sulfate-ester S=O absorption region in marine sulfated polysaccharides is approximately 1220–1270 cm^{-1} (Je et al., 2021; Pereira et al., 2013; Vandanjon et al., 2023). The fingerprint region is particularly relevant to polysaccharide characterization. Absorption bands in the 1000–1200 cm^{-1} region are commonly associated with carbohydrate C–O and C–O–C vibrations, while sulfate-ester S=O stretching is commonly reported around 1220–1270 cm^{-1} in sulfated marine polysaccharides (Gieroba et al., 2023; Je et al., 2021; Pereira et al., 2013; Vandanjon et al., 2023). These assignments provide comparative references for interpreting polysaccharide-related FTIR signals.

The identification of these absorption bands suggests that the squid ink extract contains functional groups potentially associated with carbohydrate and polysaccharide structures. However, FTIR analysis alone cannot establish the specific polysaccharide type, monosaccharide composition, or degree of sulfation. Marine-derived biomaterials are recognized as important sources of diverse biopolymers, including proteins and polysaccharides, with potential applications in biotechnology and biomedical research (Carvalho et al., 2024). Cephalopod ink also represents a chemically complex biological material containing melanin and other biochemical constituents, including protein- and polysaccharide-related components (Derby, 2014; Cheng et al., 2025).

Therefore, the FTIR results provide preliminary biochemical evidence regarding the presence of protein- and polysaccharide-associated functional groups in U. singhalensis squid ink. Further analyses involving carbohydrate-specific assays, monosaccharide composition,

and sulfate quantification are required to confirm and characterize the polysaccharide fractions.

Integrated Characterization of Protein and Polysaccharide Components

The combination of SDS-PAGE and FTIR provides complementary information regarding the biochemical composition of *Uroteuthis singhalensis* squid ink. SDS-PAGE revealed two predominant protein fractions with estimated molecular weights of approximately 50–51 kDa and 26 kDa. These findings demonstrate the presence of distinct protein fractions in the extract.

Meanwhile, FTIR analysis identified characteristic amide I and amide II absorption bands, supporting the presence of protein-related compounds (Barth, 2007). The interpretation of polysaccharide-associated structures should be based on the actual absorption bands observed in the spectrum and compared with established marine-polysaccharide FTIR assignments (Gieroba et al., 2023; Pereira et al., 2013; Vandanjon et al., 2023). The combination of these analytical techniques provides preliminary evidence that squid ink contains protein components and functional groups potentially associated with polysaccharides. Nevertheless, the molecular identity of the individual proteins and polysaccharides cannot be conclusively determined using these techniques alone. Further investigations involving protein sequencing, carbohydrate composition analysis, chromatographic separation, and sulfate determination are necessary to establish the specific molecular composition and structural characteristics of the identified biomolecules.

CONCLUSION

The characterization of *Uroteuthis singhalensis* squid ink using SDS-PAGE and FTIR demonstrated the presence of protein fractions and functional groups potentially associated with polysaccharides. SDS-PAGE consistently identified two predominant protein bands with estimated molecular weights of approximately 50–51 kDa and 26 kDa. FTIR analysis revealed characteristic absorption bands associated with O–H/N–H, C–H, amide I, and amide II functional groups, as well as absorption regions potentially associated with glycosidic and sulfate-related structures. These findings provide preliminary biochemical evidence of protein- and polysaccharide-associated components in *Uroteuthis singhalensis* squid ink. However, further purification and molecular identification are required to determine the specific protein and polysaccharide structures present in the extract.

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