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EXTRACTION AND PARTIAL CHARACTERIZATION OF GLYCOSAMINOGLYCANS FROM *Litopenaeus vannamei* MUSCLE

EXTRAÇÃO E CARACTERIZAÇÃO PARCIAL DE GLICOSAMINOGLICANOS DO MÚSCULO DE *Litopenaeus vannamei*

Giancarlo Lavor Cordeiro[1], José Ariévilo Gurgel Rodrigues[1]*, José Gabriel de Sousa Cândido[1], Kelma Maria dos Santos Pires-Cavalcante[1], Johnny Peter Macedo Feitosa[2], Sandra de Aguiar Soares[2] & Ianna Wivianne Fernandes de Araújo[1]*

1 Departamento de Engenharia de Pesca, Universidade Federal do Ceará - UFC
2 Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará - UFC
*e-mail: arieviloengpesca@yahoo.com.br , iwfaraujo@gmail.com

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Resumo

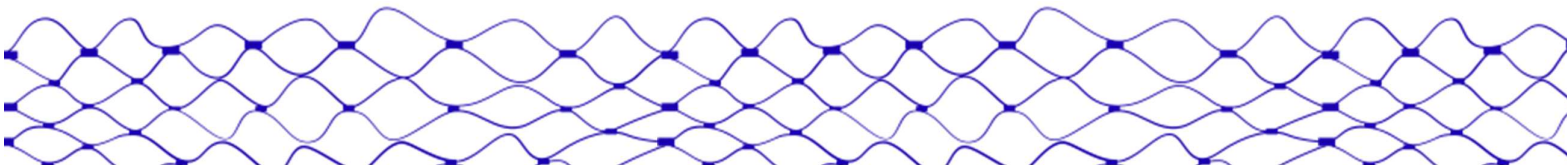
Exceto para camarões, descrita é somente a composição de glicosaminoglicanos (GAGs) para peixes teleósteos. Este estudo, do músculo de *Litopenaues vannamei* juvenis descartados durante o controle de qualidade, extraiu e examinou parcialmente GAGs (GAGsLv). Foi digerido com papaína o músculo triturado para obter GAGsLv metacromáticos, da matrix extracelular, os quais, em seguida, foram caracterizados com padrões de mamíferos corados com azul de toluidina combinado ou não ao corante 'stains-all' físico-quimicamente por eletroforese em gel de agarose; e, posteriormente, por técnica de infravermelho, seus grupos funcionais a partir da amostra analisados. Houve de camarões juvenis uma concentração de GAGsLv intramuscular de 0,07 ± 0,02% (m m^{-1}, 700 µg g $tecido^{-1}$) maior que a relatada para músculo esquelético de peixes teósteos. No entanto, não se revelou clara na amostra a composição metacromática para definir espécies de GAGsLv em razão ao nível elevado de contaminantes naturais refletindo, por ambas análises eletroforética e de infravermelho, a condição fisiológica corporal dos animais saudáveis cultivados em água mesohalina. Em suma, ferramentas bioquímicas combinadas provaram efetivas, para acessar e avaliar, GAGs sulfatados de matriz extracelular do tecido conectivo de *L. vannamei*.

Palavras-chaves: Matriz intramuscular, Digestão proteolítica, Polímeros sulfatados, Análises bioquímicas.

Abstract

Muscle glycosaminoglycans (GAGs) composition is only decribed for teleost fishes, but not for shrimps. This study extracted and partially examined for GAGs from juvenile *Litopenaeus vannamei* muscle (LvGAGs) discarded during quality control. Tritured muscle was digested with papain to obtain extracellular matrix metachromatic LvGAGs, which were then physically-chemically characterized by agarose gel electrophoresis with mammalian standards stained with toluidine blue combined or not to stains-all dye; and thereafter, their functional groups by infrared technique analyzed from the sample. Juvenile shrimps had intramuscular LvGAGs concentration of 0.07 ± 0.02% (w w^{-1}, 700 µg g $tissue^{-1}$) greater than that reported for teleost fish skeletal muscle. However, metachromatic composition revealed unclear to define LvGAGs' species due to high level of natural contaminants in sample by both electrophoretic and infrared analyses reflecting body physiological condition of healthy animals farmed in mesohaline water. In summary, combined biochemical tools proved effective for accessing and evaluating extracellular matrix sulfated GAGs from the muscle connective tissue of *L. vannamei*.

Keywords: Intramuscular matrix, Proteolytic digestion, Sulfated polymers, Biochemical analyses.



Introduction

North-eastern Brazil has been well-known worldwide for shrimp production as the second most significant commodity in Brazilian exportations over the years. In 2024, volume produced reached 146,831T and the State of Ceará accounted to the largest amount of cultured crustaceans in the country (IBGE, 2024). The technological advance allowed the better performance of the marine Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931), even in low-salinity aquaculture systems (Boyd, 2001; Rodrigues et al., 2009). It is the most commercialized shrimp species with prominence world compared to other crustaceans, such as *Penaeus monodon* and *Penaeus chinensis*, whose species have great value in the international market. Its adaptability to low-salinity water has been an alternative to native freshwater species (*Macrobrachium acanthurus* Wiegmann, 1836, *M. amazonicum* Heller, 1862 and *M. carcinus* Linnaeus, 1758) difficult to cultivate due to their aggressive behavior and high dietary demand (Valenti, 1996).

L. vannamei-muscle proximate composition has shown as a farmed species with a wide nutritional atribute not only by its low content of lipid and cholesterol, but also by higher amount of protein (Araújo et al., 2012). Its nutritional value may also be variable according to the first-matter treatment, such as in cooked whole shrimp for consumption (Lima et al., 2022). As an adverse consequency, increased farmed *L. vannamei* production has generated uncontrolled processing waste discards (cephalothorax/exoskeleton) leading to several environmental impacts. Furthermore, increased seafood production has pressured the natural sources (*e.g.*, increasing demand, overfishing, landing of by-catch, climate change-related biodiversity changes, extinction of species and others) and biorefinary-associated concepts have emerged for the valorization of seafood discards and for the utilizing of molecules-rich wastes aiming at biotechnological perspectives within blue economy (Venugopal, 2022).

Of all the bioproducts, glycosaminoglycans (GAGs) are the main extracellular matrix-found components, as well as on the surface of animal cells (vertebrates and invertebrates), regulating different physiological events linked to proteins (growth factors, enzymes and cytokines) (Balbinot-Alfaro et al., 2021; Sahu et al., 2023). They have chemical quality depending on the animal origin (Pomin & Mourão, 2008; Rodrigues et al., 2025), type of tissue (Fernandes et al., 2025) and protocol used (Badri et al., 2018; Balbinot-Alfaro et al., 2021). On a structural basis, they are unbranches, made of repetitive disaccharide units of a hexosamine (D-glucosamine or D-galactosamine) and uronic acid (D-glucuronic or L-iduronic) or galactose units. GAGs are linear and highly charged varying in sulfation pattern, glycosidic linkage and specific sugar sequence. The more known classes are heparin (HEP, highly charged), chondroitin sulfate (CS), dermatan sulfate (DS), hyaluronic acid (HA, nonsulfated), keratan sulfate (KS) and heparan sulfate (HS) (Pomin and Mourão, 2008; Badri et al., 2018; Balbinot-Alfaro et al., 2021; Sahu et al., 2023). The versatile use of these compounds have been reported for potential therapeutic substitutes, since they are commercially produced from different origins (HEP, bovine/porcine lung and

intestine; HA, microbial fermentation; and CS, bovine/porcine trachea and shark cartilage); but, those derived from mammalian reveal potentially elevated viral risk (Volpi, 2011; Badri et al., 2018; Balbinot-Alfaro et al., 2021), therefore, motivating the search for aquatic sources, including marine-origin GAGs (Valcarcel et al., 2017).

Recovery of biomaterials has been a challenger field in the polymeric sciences, especially to those derived from crustaceans. From shrimp *Penaeus brasiliensis* Latreille, 1817 viscera, Dietrich et al. (1999) characterized a low molecular weight HEP-like GAG presenting anticoagulant effect. Brito et al. (2008) isolated a GAG analog to HEP with anti-inflammatory action from the shrimp (*L. vannamei*) cephalotorax. For *L. vannamei* head has also been reported as the major processing by-product for recovering various bioactive metabolites (protein hydrolysate, carotenoid, chitin, chitosan, and GAG) with feasibiltiy from a separation-integrated process (Cahú et al., 2012). Even the Norway lobster (*Nephrops norvegicus* Linnaeus, 1758) shell showed to be an alternative source for an anticoagulant DS devoid of cytotoxicity *in vitro* (Sayari et al., 2016). More recently, Amphan et al. (2025) discovered that the use of dextran sulfate and HEP as modulators against white spot syndrome and hepatopancreatic necrosis disease in shrimp *L. vannamei*. Other arthropod aspects as residual sources in GAGs were already mentioned in literature (Sahu et al., 2023). On the other hand, studies on the animal part-derived GAGs have been little debated regarding the compositional knowledge of the extracellular matrix (Page et al., 2005; Jridi et al., 2019), which biochemically composes the connective tissue with other structural elements, such as collagen (Tingbo et al., 2005), since structure and composition of the animal muscles could be determined by environmental differences (Hannesson et al., 2007). Somatic factors have also been pointed out for body GAGs composition among different stages (Hannesson et al., 2007; Zhang et al., 2009).

In invertebrate phylum, the muscle structure can vary consideravely, especially in predominance of fiber, depending on the animal species (Ogawa & Maia, 1999). Shrimp muscle is developed and covered by the chitin-segmented exoskeleton (Ogawa & Maia, 1999), whose growth is conditioned by ecdysis (Valenti, 1996; Rodrigues et al., 2009). Nevertheless, its GAGs composition in terms of tissue matrix is unknown, comparing to that of bony fish that contribute to the texture and muscle cohesion as important factors for processing (Tingbo et al., 2005; Hannesson et al., 2007).

Based on these considerations, the aim of this study was to obtain by an enzyme-assisted strategy *L. vannamei* GAGs (LvGAGs) from muscle samples discarded from a shrimp processing unit and partially characterize them by biochemical and structural procedures, as the first description on the occurrence of these compounds in the extracellular matrix of shrimp muscle tissue, considering the economical importance this species in the international scenario.

Material and Methods

Shrimp and muscle processing

Farmed samples of healthy juvenile shrimp (*L. vannamei*) discarded during the quality control from a local processing unit in the municipality of Jaguaruana, State of Ceará, Brazil, were used. According to the local information, animals were acquired from a mesohaline system (1‰, pH 8.3) with tank structure with a concave white mesh cover. A total of 34 specimens were obtained and the zootechnical indices corresponding to the juvenile phase were collected for the laboratorial study, as shown in table 1.

Table 1. Zootecnical cultivation parameters of *L. vannamei* obtained by industrial unit.

parameters*	specimens (n = 34)
total biomass (g)	~ 125
length (cm)	8.81 ± 0.79
weight (g)	3.66 ± 0.99
salinity (‰)	1
pH	8.3
cultivation system	tank structure with a concave white mesh cover

Shrimp were sacrificed by thermal shock and then tranported in ice (1:1 k biomass: ice ratio) at sialed isothermal boxes and received by the Marine Biochemical (MarBio) laboratory located at Aquaculture Biotechnology Center, Department of Fisheries Engineering, Federal University of Ceará (FUC) for the experimental analyses. Animals were authorized through our registration with SISGEN (National System for the Management of Genetic Heritage and Associated Traditional Knowledge). In MarBio laboratory, shrimp were manually washed with distillated water for removing of solid particules and salt before the biometry, as adopted for bony fish (Fernandes et al., 2025). For this, the biometric sampling of the *L. vannamei* individuals (Table 1) was performed using a rudimentary ichthyometer and a commercial balance on a 1 g precision scale, respectively, before the removal of the exoskeleton, as illustrated in experimental design (Figure 1).

Experimental design

After biometric determination, previously washed *L. vannamei* juvenile samples were then subjected to deheading (cephalotorax) (Brito et al., 2008; Cahú et al., 2012) followed by removal of the exoskeleton to separate the muscle portion from the whole shrimp body, being all the pretreated samples stored in freezer (-20 ± 1°C) until experimental analyses (Figure 1).

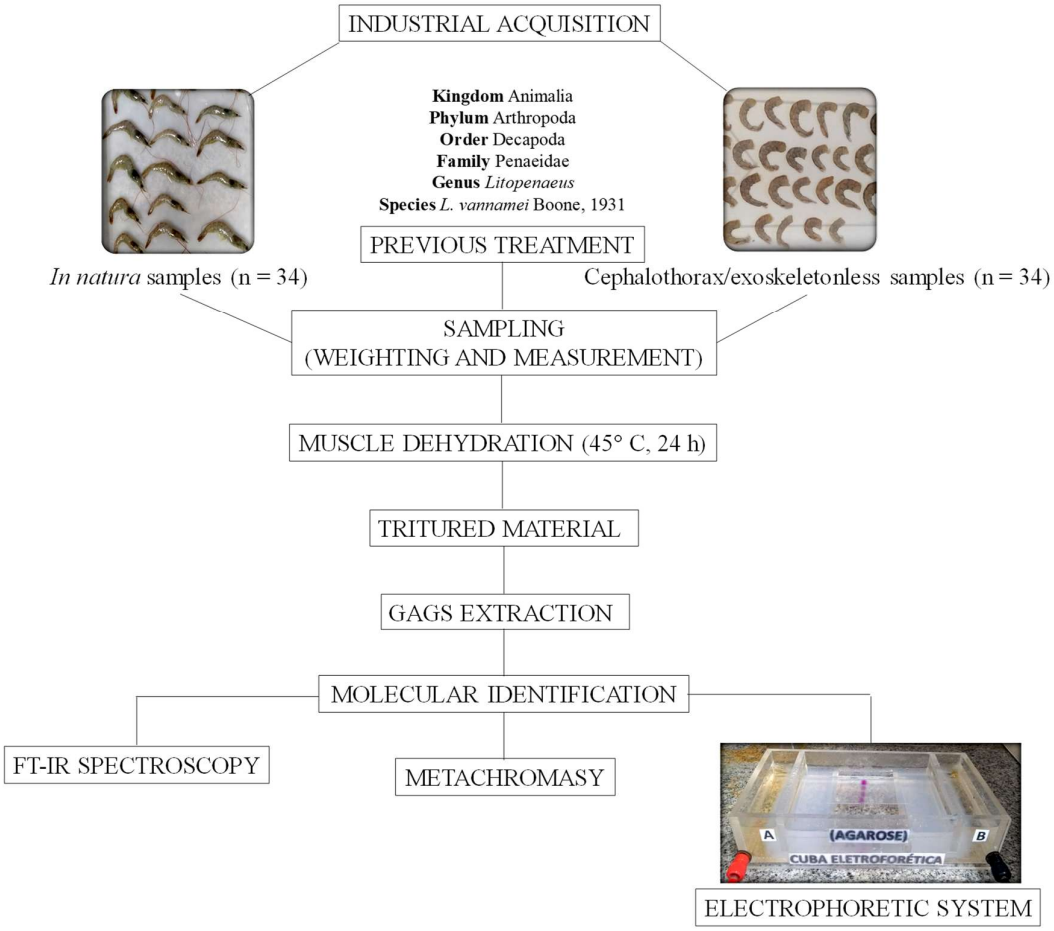


Figure 1. *L. vannamei* preparation scheme for extraction and partial charactization procedures of industrially-discarded individuals during the quality control.

Before LvGAGs extraction, the muscle portion was initially dehydrated (45 °C, 24 h) by gentle drying in an over under with air circulation based on fish' wastes (Fernandes et al., 2025), whose dehydrated material was then tritured and maintained in closed recipient until GAGs isolation according to the also adapted protocol for fish' biomass discards (Fernandes et al., 2025; Rodrigues et al., 2025).

Further, extracted GAGs mass was biochemically / structurally analyzed by anionic character according to the metachromatic property followed by both the eletrophoretic and Fourier Transform Infrared (FT IR) spectroscopy analytical procedures, respectivelly (Figure 1), to infer molecular features for sulfated materials from the analyzed polymer sample (Jridi et al., 2019; Fernandes et al., 2025; Rodrigues et al., 2025), and such experimental protocols are described below.

GAGs extraction and metachromatic property

From *L. vannamei* dehydrated muscle, it was cut into small peaces and tissue samples of about 13 g were further incubated with crude protease (10 % papain, w w^{-1}) in a thermostatic bath adjusted to 60° C, in the presence of 100 mM sodium acetate buffer (pH 5.0) added of both 5 mM EDTA and 5 mM cysteine. The extraction process was overnight developed at 60°C based on Fernandes et al. (2025) and Rodrigues et al. (2025), as shown in figure 2*a*.

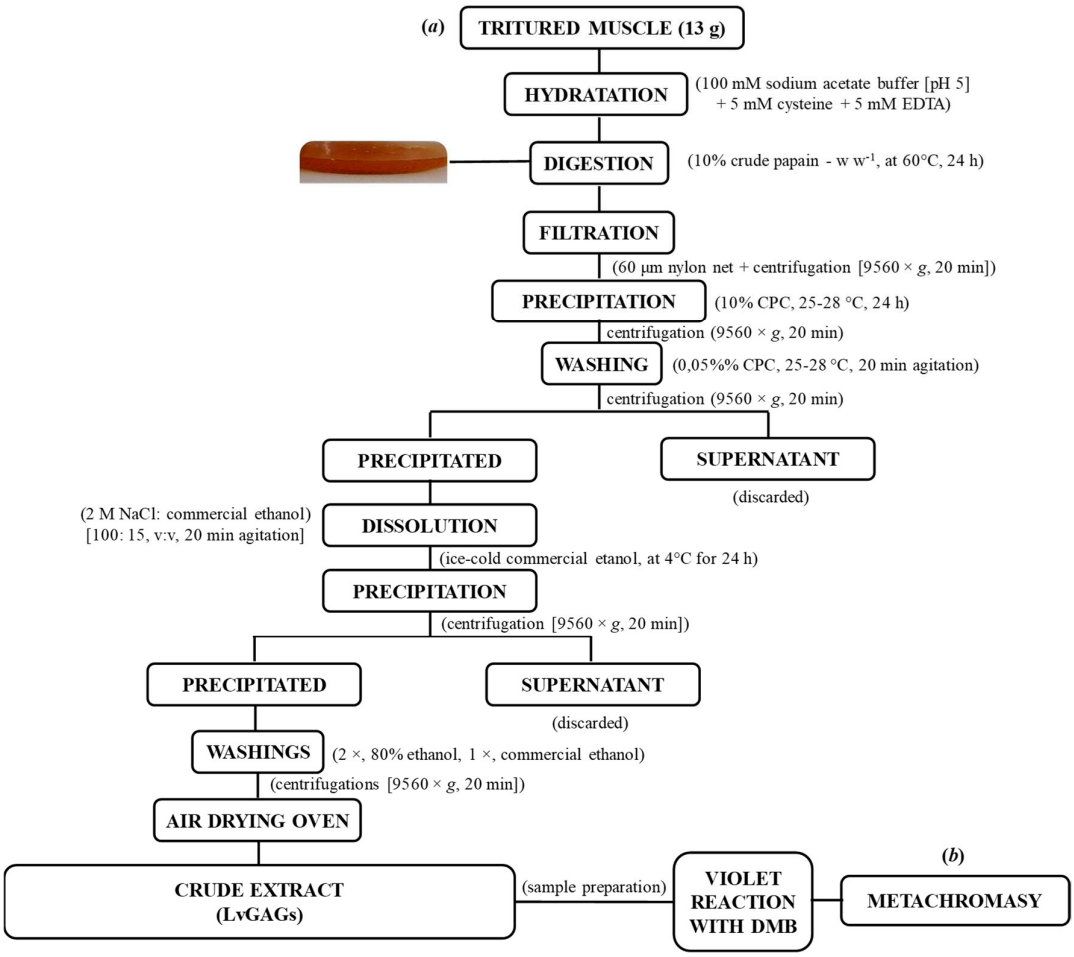


Figure 2. Tritured muscle of juvenile *L. vannamei* shrimp subjected to proteolytic digestion (*a*) and metachromasy checking (*b*) of extracted LvGAGs samples.

After 24 h incubation period, the mixture was continually filtered using a 60 µm nylon net and the supernatant was saved and then centrifugated (9.560 × g for 20 min). GAGs that were present in medium were precipitated with 10 mL of 10% cetylpyridinium chloride (CPC) solution at room temperature (25-28°C) for 24 h. The mixture was then centrifuged at 9.560 × g for 20 min and the *pellets* containing the GAGs were washed with 100 mL of 0.05% CPC solution, dissolved (under mechanical stirring for 20 min) in 100 mL of a 2 M NaCl: 92.8% ethanol (100:15 ratio, v:v) solution, followed by precipitation (24 h, 4°C) with addition of 100 mL of 92.8% ice-cold commercial ethanol. The recovered precipitate was centrifugated (9.560 × g for 20 min) and washed with 100 mL of 92.8% commercial ethanol. After each centrifugation (9.560 × g for 20 min) among the mentionated steps, the partially purified material was dried using an oven with air circulation (60°C, 6 h) to obtain the crude LvGAGs extract. The extraction yield was calculated as the percentage (w w^{-1} %, n = 3) based on dehydrated first-matter (g), according to the Equation 1 below:

LvGAGs mass yield (%, w w^{-1}): shrimp muscle material $weight^{-1}$ × 100 (1)

Metachromasy analysis from the extracted sample was checked from a solution of crude LvGAGs extract previously prepared and an aliquot containing 27 µg (w v^{-1}) was used in the presence of 1,9 dimethylmetilene (DMB) blue dye that is an indirect indicator of colorimetric reaction based on complex formed (Farndale et al., 1976). The *in vitro* assay was performed following Fernandes et al. (2025) and Rodrigues et al. (2025) for fish waste-derived GAGs, in triplicate, using glass tubes (24-28°C) and the violet property visualized to be specific for sulfated GAGs (Figure 2*b*). The results were recorded by photographic images from a portable device of 50 megapiexels resolution.

Agarose gel electrophoresis (AGE)

LvGAGs from muscle samples were physical-chemically characterized by charge density and homogeneity pattern using an AGE system (Dietrich & Dietrich, 1976). For this, the test sample (~ 12 µg) was applied to a 0.5% agarose gel prepared in 0.05 M 1,3-acetate diaminopropane buffer at pH 9.0 and the run developed at constant voltage (100 V, 1 h). After that, the gel was treated with 0.1% *N*-cetyl-*N*, *N*, *N*-trimethylammonium bromide solution for ~ 24 h to fix the LvGAGs and then it was dehydrated using an oven with air circulation (55°C, ~ 6 h).

Finally, LvGAGs present were stained with 0.1% toluidine blue, combined or not with stains-all for 30 min or 24 h, respectively, depending on the cationic dye. Then, the gels were destained with a solution containing absolute ethanol, distilled water and acetic acid or using distilled water only. As known markers of molecular mass, chondroitin-6-sulfate (C-6-S,~60 kDa), chondroitin-4-sulfate (C-4-S,~40 kDa), sulfated dextran (DexS,~8 kDa), dermatan sulfate (DS,~40 kDa) and/or HEP (~15 kDa) were applied as standards to partially identify the analyzed polymer sample (Fernandes et al., 2025).

Fourier Transform Infrared (FT-IR) spectroscopy

The structural features of LvGAGs from muscle samples were recorded by FT-IR using a spectrometer (IRPrestige-21 Shimadzu, Japan). For this, test sample was weighed (~10 mg) and then pressed in potassium bromide (KBr) (Fernandes et al. 2025; Rodrigues et al., 2025). The measurements were generated from *pellets* at a resolution of 4 cm^{-1}, with 64 scans min^{-1} at wavenumber 500-4000 cm^{-1}. The spectral value and the graphicals were assigned and represented using the Origin software version 8.0 as the Statistical Analysis Software (USA).

Results and discussion

As yet no literature reports on the GAGs concentration from the *L. vannamei* muscle structure, our study intented to examine its biochemical profile in muscle sample this marine shrimp when acquired by industrial processing discards during its quality control, whose animals were farmed under mesohaline conditions (Table 1). On a mass-to-mass basis, tritured *L. vannamei* muscle was susceptible to proteolytic action by crude papain during the 24 h incubation period (Figure 2) as for fish wastes (Fernandes et al., 2025; Rodrigues et al., 2025), since the GAGs mass was to be an average yield of 0.07 ± 0.02% from the dehydrated tissue (w w^{-1}) considered greater

than other muscle sources (Table 2). Increased global demands for GAGs have encouraged utilizing various origins and types of body-related part (Cahú et al., 2012; Sayari et al., 2016; Sahu et al., 2023; Amphan et al., 2025; Fernandes et al., 2025; Rodrigues et al., 2025), although with limited availability in animal tissues (Tingbo et al., 2005; Badri et al., 2018; Balbinot-Alfaro et al., 2021).

Table 2. Yield of *L. vannamei* muscle GAGs in comparison with other aquatic sources.

Organism	Tissue/origin	Yield (w w^{-1})	Gags g $tissue^{-1}$	Reference
L. vannamei	muscle	0.07 ± 0.02%	700 µg	this study
G. morhua	muscle	-	325 ± 58 µg	Hannesson et al.
Norwegian red	muscle	-	510 ± 42 µg	(2007)
L. vannamei	head	-	79 ± 2 mg	Cahú et al. (2012)
			-	Jridi et al. (2019)
Sepia offcinalis	muscle	8.6%		
Oreochromis	skin	0.18 ± 0.02%	-	Fernandes et al.
niloticus	eyeball	0.16 ± 0.03%	-	(2025)
	intestine	0.36 ± 0.10%	-	
	gonad	0.40 ± 0.08%	-	
O. niloticus	skin (freshwater)	0.06 ± 0.00%	1.53 ± 0.03 mg	Rodrigues et al. (2025)
	skin (mesohaline)	0.19 ± 0.01%	1.91 ± 0.08 mg	

*Yield calculaded on the basis of dehydrated fish part (%, w w^{-1}) or expressed as µg or mg g $tissue^{-1}$.

Studies on the connective tissue involving the animal muscular fiber have revealed to a GAGs content varying with the somatic factor. Considering animal body (Table 2), Hannesson et al. (2007) analyzed the general architecture and the concentration of GAGs in the skeletal muscle of fish cod (*G. morhua*) wild caughted and then in cage-type captivity and young bulls (*Norwegian red*). The authors found a distinct profile of GAGs present between both animals, with a comparatively higher level in bovine muscle (510 ± 42 µg GAGs g wet $weight^{-1}$) than that of fish one (325 ± 58 µg GAGs g wet $weight^{-1}$). Studying the cuttlefish (*Sepia offcinalis*), Jridi et al. (2019) found an average extraction yield 8.6% (w w^{-1}) from the animal muscle. The amount of GAGs recoverd from the *L. vannamei* juveniles muscle in this study was to be 0.7 ± 0.03 mg GAGs g dehydrated $weight^{-1}$ i.e., being on the same unit of mass, at least, about 1.37-fold higher than those obtained by Hannesson et al. (2007) for bovine and fish cod adult muscles. Higher recovery rate for LvGAGs compared to other related studies could be the result of the dehydrated tissue used than in *in natura* (wet) preparation during papain incubation (Rodrigues et al., 2025), besides the content of carotenoid present in animal (Cahú et al., 2012) participating as a metabolic stimulus on the production of body GAGs as previously described for fish rainbow trout (*O. mykiss*) supplemented with dietary carotenoid (Page et al., 2005).

Specific literature reveals that the vertebrate' extracellular matrix could be an important biological basis to examine the differences amoung the aquatic species aiming at better industrial utilization against post mortem (Tingbo et al., 2005; Hannesson et al., 2007). It has been reported that the connective tissue in animals operates during muscle contraction, as well as participating like a supports to muscle

architecture and in its growth and differentiation of the muscle fibers involved in the biomechanical role (Ogawa & Maia, 1999). In this study, the revelant GAGs level found in *L. vannamei* muscle tissue using papain method (Figure 3), as an invertebrate organism-analyzed edible product, reflected the animal's rearing conditions (Table 1), since the salinity could also influence the osmotic balance in aquatic animal (vertebrate and invertebrate) extracellular matrices-GAGs concentration, providing useful data on the physiological status of the species regarding the relationship between structure and adaptative-function (Pomin & Mourão, 2008; Rodrigues et al., 2025). Dietary carotenoid (astaxanthin and canthxanthin) supplementation significantly impacted on the increased mucopolysaccharide levels in experimentally cultured rainbow trout (*O. mykiss*) (Page et al., 2005). Zhang et al. (2009) suggested that the developmental stage (0.5 days to adult) determined the GAGs levels in zebrafish (*Danio rerio*).

As the *L. vannamei* revealed to be a valuable source of intramuscular connective tissue GAGs (Table 2), LvGAGs composition in its juvenile phase was also thereafter examined by the apparent charge density from the extracted material sample. Using the DMB dye, the colorimetric method of Farndale et al. (1976) strongly evidenced the presence of sulfated components in test sample, since it had a specific DMB dye-binding ability, as demonstrated by the violet reaction typical for sulfated polymeric structures (Figure 3) similar to other animal tissue' sources (Fernandes et al., 2025; Rodrigues et al., 2025).

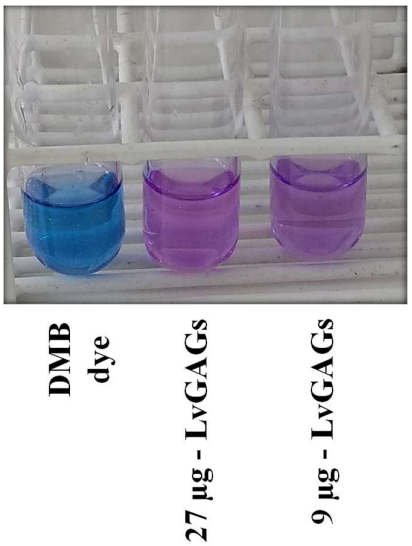


Figure 3. Metachromasy assay using 9 or 27 µg of LvGAGs from muscle samples (LvGAGs) in the presence of DMB cationic dye.

The metachromatic property occured with a minimum preparation (9 µg) that suggested LvGAGs chains with a stronger degree to protein binding (Fernandes et al.., 2025; Rodrigues et al., 2025) because the extracellular matrix also is constituted of collagen together acting as modulators in the overall organization of the muscle tissue avoiding, for example, the gaping phenomenon post morten (Ogawa & Maia, 1999), as the loss of fiber-to-myocommata attachment during prolongated storage,

typical in fish (Tingbo et al., 2005). Collectively, observations revealed to an important charge density of *L. vannamei* muscle-derived GAGs as a viable anionic scenario when cultured in mesohaline water (Table 1) because shrimp physiological data submitted to these growing conditions are yet limited (Boyd, 2001; Rodrigues et al., 2009). Considering aquatic animals, antioxidant-based GAGs have been found in skeletal muscle (Jridi et al., 2019), whose property is implied in animal immunity (Hannesson et al., 2007; Amphan et al., 2025; Rodrigues et al., 2025) and food preservation (Ogawa & Maia, 1999). GAGs are involved in various bioactivities (Valcarcel et al., 2017; Badri et al., 2018; Balbinot-Alfaro et al., 2021; Sahu et al., 2023) even since microbial community to speciation (Pomin & Mourão, 2008).

The anionic character noted in polysaccharidic sample from *L. vannamei* muscle led us to partially identify, thereafter, by the AGE technique the chemical class (es) of GAGs present in the extracellular matrix of the connective tissue of shrimp edible part.

Partial identification by AGE

AGE procedure analyzed the *L. vannamei* muscle-extracted LvGAGs sample by determinating of charge density by means of two well-known cationic dyes (toluidin blue and stains-all), combined or not, due to their high sensivity to interact with sulfated (Dietrich & Dietrich, 1976) and uncharged sugar residues even in small sample amounts, respectively (Figure 4) (Fernandes et al., 2025).

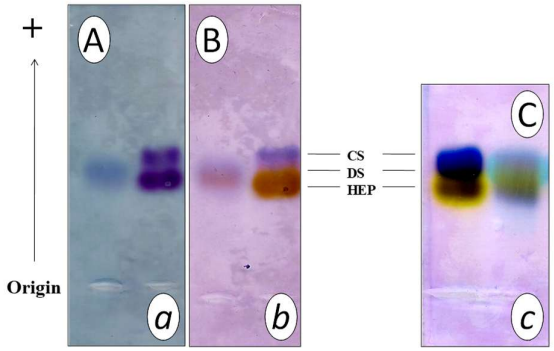


Figure 4. AGE (A-C) of LvGAGs from muscle samples comparing with mammalian standards chondroitin-6-sulfate (CS, ~60 kDa), dermatan sulfate (DS, ~40 kDa) and heparin (HEP,~15 kDa) present on gels stained with 0.1% toluidine blue (*a*) combined (*b*) or not to stains-all (*c*).

As can be seen, the molecular scenario verified by the AGE analysis was very complex (Figure 4), since the electrophoretic profile of *L. vannamei* sample stained with stains-all only indicated distinct components on gel, as mainly observed in the color mixture (Figure 4C). On this basis (Figure 4A), a single band species with discrete resolution was observed in sample similar to *O. niloticus* intestine/gonad-derived GAGs, when stained with toluidine blue only (Fernandes et al., 2025). This approach permited suggest the initial presence of DS and/or HEP GAGs structures in the analyzed shrimp muscle, accordingly to intermediate mobility from the diamine' buffer between respective standards that exhibited intense metachromatic

composition as mammalian sulfated GAGs, since the cationic dye interacted with the extracted polymer (Dietrich & Dietrich, 1976).

Obviously, vertebrate and invertebrate are different animal phylum, revealing higher degree of molecular heterogeneity and body distribution, although GAGs conserved evolutionarily (Hannesson et al., 2007; Pomin & Mourão, 2008; Valcarcel et al., 2017; Badri et al., 2018; Balbinot-Alfaro et al., 2021; Sahu et al., 2023). In crustaceans, significant amount of processing rejects is routineraly negligenciated, being head waste one the main byproducts of shrimp industry, together with carapace and tail (Venugopal et al., 2022) produced by ecdyses (Rodrigues et al., 2009). Because of their consequences, natural products have been more explored from the shrimp (*L. vannamei*) head (protein, chitin, carotenoids and GAGs) than edible part, in which CS and HS were metachromatically identified by the toluidine blue staining (Brito et al., 2008; Cahú et al., 2012); from the Norway lobster (*N. norvegicus*) shell revealing a DS-type GAG (Sayari et al., 2017); and, for other arthropods, as diverse sources for various GAG classes (Sahu et al., 2023).

On the other hand, unique use of toluidine blue as metachromatic indicator for detects other natural components was not reasonable, since its combination with stains-all dye maintained the single band (Figure 4B) (Fernandes et al., 2025), although more visible on gel and sample in orange/yellow color as the standard HEP similar to HS structure (Badri et al., 2018; Balbinot-Alfaro et al., 2021). These combined observations suggested the preliminary presence of HS/HEP in the *L. vannamei* muscle tissue; by contrast, fish muscle have shown a dominance of GAGs depending on the species (Tingbo et al., 2005; Hannesson et al., 2007) with bioactivities (Jridi et al., 2019).

The electrophoretic migration of GAGs-containing sample stained with stains-all alone showed to be challenger (Figure 4C). Although in figures 4A and B exhibiting a well-defined electrostatic environment, sample of muscle GAGs indicated for a colorimetric diversity based on AGE-separated component in mixture obtained, elevating the production cost for its purity as industrial option (Badri et al., 2018), since dark blue+bright-blue (suggestive for HA (Fernandes et al., 2025), only nonsulfated GAG (Valcarcel et al., 2017; Badri et al., 2018; Balbinot-Alfaro et al., 2021) to yellow+bright-red (reinforcing HS/HEP - Fernandes et al., 2025) colors were observed, respectively. This molecular scenario allowed us to speculate for an elevated protein contamination because the stains-all dye reacted with the proteins (Tingbo et al., 2005; Hannesson et al., 2007) origined from the muscular structure constituted by various fibers related to tissue contraction (Ogawa & Maia, 1999), which resulted in protein degraded-peptides and DNA residues generated by proteolytic (papain) action to obtain *L. vannamei* muscle GAGs (Figure 1), as aforementioned for fish-extracted products, difficulting biochemical identification (Fernandes et al., 2025; Rodrigues et al., 2025). To data, the muscular portion of *L. vannamei* has been reported with a high level of proteins, not only *in nature* (21.9 g 100 g^{-1}) (Araújo et al., 2012), but also in cooked whole shrimp (17+4.8%) (Lima et al., 2022), therefore, representing a food of high nutritional value.

On this molecular perspective, further step was to characterize the LvGAGs from muscle sample by FT-IR spectroscopy to better elucidate its structural composition comparing with other animal sources.

Structural features of LvGAGs from muscle samples by FT-IR

Structural profile of GAGs derived from the *L. vannamei* muscle was analyzed by FT-IR spectroscopy that revealed features for sulfated materials as shown in figure 5. According to the spectrum (4000-500 cm^{-1} region), the absorption values revealed a low resolution profile, since it did not indicate the GAGs-related class (es) from the investigated polymer sample, similar to extracted samples of cuttlefish (*S. officinalis*) muscle by Jridi et al. (2019). In this study, the previous physical-chemical characterization by the AGE analysis suggested a high contamination level in the examined polymer sample when stained with stains-all (Figure 4C), and a masking of GAGs by protein contaminants occurred (Tingbo et al., 2005), since the extracellular matrix is rich of proteoglycan-structures variably formed by GAGs chains linked to protein core (Badri et al., 2018; Balbinot-Alfaro et al., 2021).

On the other hand, the spectrum of LvGAGs from muscle tissue samples showed typical bands related to the three types de amides (3431, 1674 and 1591 cm^{-1} for amides I, II and III, respectively) featuring a polysaccharidic compound extracted by papain method and related steps to obtain final mass similar to other studies (Jridi et al., 2019; Fernandes et al., 2025; Rodrigues et al., 2025).

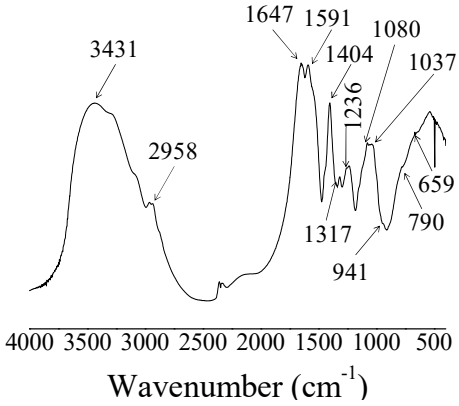


Figure 5. FT-IR spectra of the LvGAGs from muscle samples obtained from kBr *pellet* at 500-4000 cm^{-1}.

Additionally, at 2958 cm^{-1} indicated to the stretching of C-H for alkyl functionality common in polysaccharides and, in parallel, the presence in the sample of uronic acids (1317-1404 cm^{-1}) (Jridi et al., 2019; Fernandes et al., 2025; Rodrigues et al., 2025) like the cyan character found in it by AGE profile that revealed in the presence of stains-all dye (Figure 4C), since GAGs have structurally important compositions in nonsulfated sugars making part of the polymer (Badri et al., 2018; Balbinot-Alfaro et al., 2021). By these interpretations, was not suggested the presence of HA in shrimp sample, a high molecular size GAG (formed by uronic acid and hexosamine) of gelatinous consistency primarily found in skin and cartilage possessing clinical importance (*e.g.*, anti-ageing and skin repair, wound healing and

tissue repair properties) (Badri et al., 2018; Balbinot-Alfaro et al., 2021) since it did not previously appear from the toluidine blue+stains-all-treated gel as a cyan band (Figure 4B), as commonly coextracted from the fish eyeball when analyzed by FT-IR (Fernandes et al., 2025). Teleost fishes are considered as crucial biological models to examine GAGs regarding their evolutive roles at level of structural composition along the developmental stages (Zhang et al., 2009).

More relevant was to the confirmation of sulfated groups in the material extracted from the *L. vannamei* intramuscular tissue located at 1236 cm^{-1} (S=O) of the spectrum (Figure 5) as was also observed in cuttlefish (*S. officinalis*) muscle polysaccharide spectrum by Jridi et al. (2019), demonstrating the biomechanical and physiological importance of these polymers to the animal connective tissue considering other sources (Ogawa & Maia, 1999; Page et al., 2005; Tingbo et al., 2005; Hannesson et al., 2007). Shrimp heads are preponderantly-rich sources in HEP-like GAGs exhibiting anti-inflammatory (Brito et al., 2008) and anticoagulant (Brito et al., 2008; Cahú et al., 2012) actions. This GAG has been implicated in cell–cell recognition and growth control (Badri et al., 2018; Balbinot-Alfaro et al., 2021; Sahu et al., 2023), as well as modulators against white spot syndrome and hepatopancreatic necrosis disease in shrimp *L. vannamei* (Amphan et al., 2025).

Other signals found in the spectrum related to *L. vannamei* muscle isolated GAG was at 941 cm^{-1} (at low intensity), suggesting the occorrence of 3,6-anhydrogalactose in the analyzed polymer sample (Jridi et al., 2019). Based on this structural component, it could be attributed the presence of KS chains of similar mobility as CS based on combined AGE analysis (Figure 4), since KS contains galactose sugar and is devoid of uronic acid, and its degree of charge density varies according to the animal tissue (Balbinot-Alfaro et al., 2021), as found for some fish muscles (Tingbo et al., 2005; Hannesson et al., 2007). Degradation of proteoglycan-structures that support the matrix organization by proteases (like papain) releases GAGs-peptide components as a result of papain-assisted extraction (Figure 2), leading to a wide chemical diversity as visualized in figure 4, especially due to the high content of natural products (protein, cholesterol, lipids and carotenoids) (Araújo et al., 2008; Cahú et al., 2012; Lima et al., 2022), challenging more consistent structural biological elucidation (Sahu et al., 2023). Therefore, the presence of KS was unclear from the FT-IR analysis that revealed regions that remain inconclusive (Figure 5), and additional structural studies, as well as the previous treatment of the first-matter before the shrimp GAGs extraction followed by its chromatographic separation (Brito et al., 2008) are strongly recommended.

Given the commercial importance of *L. vannamei*, this study contributes to the partial knowledge related to the molecular biochemical of intramuscular conective tissue-derived LvGAGs when extracted of specimens discarded from a shrimp processing unit and farmed in mesohaline conditions. The use of biochemical tools implies as an additional strategy to evaluate the metabolic status of the animal for its meat quality by means of GAGs composition (Tingbo et al., 2005; Hannesson et al., 2007), which appear as polymeric arrangements supporting the functionality of the

extracellular matrix aiming at industrial improvement in terms of texture and muscle cohesion during food utilization post mortem (Ogawa & Maia, 1999). Herein, the methodology proved effective for accessing and biochemically evaluating extracellular matrix GAGs present in the muscle connective tissue of the shrimp *L. vannamei* farmed in mesohaline water.

Conclusion

Juvenile *Litopenaeus vannamei* sampled from an industrial unit and farmed in mesohaline water revealed a complex profile of intramuscular glycosaminoglycans extracted using papain method, with high protein contamination level. By classical biochemical (electrophoresis and infrared analyses) techniques allowed partially characterize some molecular aspects that could be implicated to matrix structure when in healthy animals. Using such tools could govern thereafter studies as matrix components that might be related in food stuffs connected to animal body (edible part) condition during sampling periods.

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