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A Morphological and Nutritional Analysis of White Shrimp (*Litopenaeus setiferus*) Muscle

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Abstract

In response to the growing seafood demand and the environmental impact of traditional aquaculture and fishing methods, this study explored the potential of cellular agriculture for sustainable seafood production. As shrimp are the most consumed seafood in the United States, this project aimed to establish a comprehensive database of the morphological, physicochemical, and nutritional properties of shrimp muscle. This database is intended to support the development of cell-based seafood by providing a benchmark for texture, color, and nutritional content. To do so, thirty large white shrimp (*Litopenaeus setiferus*) were analyzed through texture analysis, colorimetry, Hematoxylin and Eosin (H&E) staining, and proximate analysis. Analysis was completed for both the surface and cross-section for the thirty shrimp. The findings indicated consistent texture profile analysis parameters across *L. setiferus's* surface and cross-section, while colorimetry values showed significant differences. H&E staining revealed similar cellular patterns, affirming the uniformity of shrimp texture. The proximate analysis offers a nutritional target for developing shrimp analogs. The study concludes that while shrimp texture is consistent, addressing color variation is crucial for the development of cell-based shrimp analogs. Future research should include characteristics of cooked shrimp and other seafood products to expand the database for cellular agriculture applications.

Introduction

The relentless surge in global population, expected to reach 9.7 billion by 2050 (United Nations, 2023), coupled with the remarkable doubling of seafood consumption per capita over the past 57 years (Issifu et al., 2022), poses unprecedented challenges in meeting the current and future demand for seafood. The aquaculture industry provided 57% of the world's seafood by volume in 2020 with traditional fishing accounting for 43% (Ritchie & Roser, 2021). Aquaculture plays an instrumental role in meeting the rising demand for seafood. However, it is not without adverse environmental consequences, including but not limited to habitat destruction, pollution, and disease transfer (Greenberg, 2019). Additionally, traditional fishing practices have strained 85% of the world's fisheries beyond their sustainable limits (Pew, 2022).

This shows the need for an alternative seafood production system to produce enough seafood for the growing demand in the coming decades.

Fish and shellfish are important sources of high-quality protein, omega-3 fatty acids, vitamins, and minerals (Mayo Clinic, 2023). Preserving these nutritional benefits in alternative production systems is crucial for consumer health and acceptance (Baker et al., 2022). Additionally, sensory characteristics such as taste, texture, and appearance play a vital role in consumer food choices and satisfaction (Kadim et al., 2015). Therefore, for alternative seafood products to succeed in the market, they must not only replicate the nutritional content of traditional seafood but also deliver a satisfactory sensory experience that aligns with consumer preferences.

Cellular agriculture has emerged in recent years as a potentially more environmentally friendly alternative to meeting seafood demand. This innovative technology involves the cultivation of agricultural products from cells in a controlled bioreactor environment (Samandari et al., 2023) Research suggests that cellular agriculture could significantly reduce global warming, air pollution, land use, and water consumption compared to conventional agricultural practices (GFI, 2021). The absence of antibiotic use in cellular agriculture further mitigates the risk of antibiotic resistance and disease spread (Nyika et al., 2021). Despite its potential, cultured seafood faces significant challenges, such as achieving a competitive price point and replicating sensory characteristics of traditionally sourced seafood to satisfy consumer preferences (Nyika et al., 2021). Notably, the absence of a database with morphological and physio-chemical properties of seafood products, including defined standards for nutritionally high-quality seafood, complicates comparison between cell-based and traditional seafood products during the prototyping of cell-based seafood products (Good Food Institute, 2021).

The central aim of this research was to investigate the morphology physicochemical, and nutritional properties of shrimp muscle, culminating in the establishment of a comprehensive database. A series of methods including texture profile analysis, colorimetry, Hematoxylin and Eosin staining, and proximate analysis were employed for the characterization. Shrimp were used in the study as the first seafood product to be characterized in the database as they are the most popular seafood in the USA (National Fisheries Institute, 2021) This database will serve as a foundational resource for future advancements in the field of cellular agriculture, paving the way for sustainable and palatable cell-based seafood products.

Methods

Sample Preparation

Thirty large white shrimp (*Litopenaeus setiferus*) were purchased from Northwest Seafood (Gainesville, FL). The shrimp were farm-raised and imported from Ecuador. The shrimp samples were stored at 2-4 °C for at least 2 hrs before preparation for analysis. To prepare the samples, each shrimp was cut into two 1 cm^2 pieces. Shrimp abdominal areas are divided into 6 sections as shown in Figure 1. The 2nd abdominal segment was used for surface textural analysis and the 3rd abdominal segment was used for cross-sectional textural analysis. Before texture analysis, samples were stored at 2-4 °C for 30 min to allow samples to cool down to 4 °C.

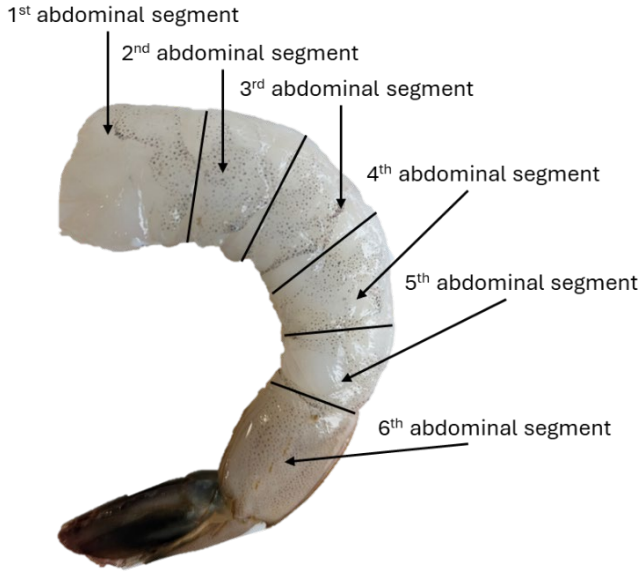

Figure 1. Anatomy of white shrimp *(Litopenaeus setiferus*)

Texture Profile Analysis

The texture analyzer (TA.XTplusC, UK) fitted with a 50 kg load cell was used to conduct texture profile analysis (TPA) of shrimp samples. Calibration was completed prior to use. The texture analyzer was set to a pre-test speed of 5 mm/sec, a test speed of 2 mm/sec, a post-test speed of 5 mm/sec, a distance of 5 mm, a time of 2 sec, a trigger force of 0.049 N. Each sample cube was individually compressed to a distance of 5 mm, representing a 50% reduction from its original height, using a 50 mm diameter cylindrical probe. The six parameters quantified from

the texture profile analyzer were hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness. These parameters were derived from the force/distance data obtained through a two-cycle compression test.

Colorimetry

Colorimetry was performed using MiniScan XE (HunterLab, Reston, VA, USA), and the lightness value (L*), the red/green value (a*), and the blue/yellow value (b*) were recorded. L* represents lightness from black to white on a scale of 0 to 100. Both a* and b* represent chromaticity, specifically, positive a* corresponds to red, negative a* to green, positive b* to yellow, and negative b* to blue (HunterLab, 2023).

Proximate Analysis

Proximate analysis including total moisture, crude protein, total fat, total carbohydrate, and ash was done in 6 shrimp samples using the service lab (Midwest Laboratories, Omaha, NE). Table 1 shows the methods that were used for proximate analysis.

Table 1. Proximate analysis methods

Component	Method
Protein (Crude) (%)	AOAC 930.03
Fat (Crude) (%)	AOAC 954.02
Moisture (%)	AOAC 930.15
Ash (%)	AOAC 2003.05

Tissue Fixation

A sample measuring 2 mm in thickness and 10 mm in width was carefully excised from the surface of the shrimp's 2nd segment and the cross-section from the 3rd segment. This step was repeated with triplicate samples. These samples were placed in cassettes and fixed in 10% formalin for 16 hr to preserve cellular structures. Following fixation, the samples were washed in phosphate-buffered saline (PBS), repeating this washing step twice to remove fixative residues. The samples were then stored at 4°C in preparation for paraffin embedding.

Paraffin Embedding

Paraffin processing was carried out using an automatic tissue processor (ASP300, Leica), where the tissue samples underwent dehydration through two successive 0.5-hr baths in 80% ethanol, followed by clearance in xylene for 1 hr at 65°C, and finally, infiltration with paraffin wax for 12 hrs. Once embedded, the paraffin blocks were sectioned at 4 µm thickness using a microtome (D11-33). The 4 µm sections were placed in a 42°C water bath to eliminate wrinkles and folds, then transferred onto microscope slides and left to dry overnight.

Hematoxylin and Eosin (H&E) Staining

For staining, the 4 µm thick sections were deparaffinized in xylene and progressively rehydrated through a graded ethanol series (100%, 95%, 70%, and 100% water). The rehydration process prepared the sections for staining, which involved applying hematoxylin to stain cell nuclei blue, followed by eosin to stain the cytoplasm and extracellular matrix pink. The final step involved imaging the stained sections using an Olympus slide scanner (VS200).

Statistical Analysis

ANOVA from the Fit Model platform of JMP Pro 15.2 (Statistical Analysis, SAS Institute, Cary, NC) was employed and the means were separated using the LSMeans procedure of JMP. The means were considered statistically significant at $p \leq 0.05$ and separated utilizing Tukey's HSD test.

Results and Discussion

Texture Profile Analysis

To better understand the textural characteristics of *Litopenaeus setiferus*, we conducted a comprehensive TPA. The results of this analysis, presented in Table 2, provide insights into the shrimp's hardness and other key parameters, both in surface and cross-sectional orientations. The hardness values, ranging from 5.48 to 6.00, suggested a moderate firmness. This indicates that white shrimp possesses a robust structure without being excessively tough. This observation aligns with reported hardness values in the literature, such as 6.5 for *Penaeus spp.* (Balfour et al., 2012) and 15.33 for *Litopenaeus vannamei* (Murthy et al., 2016).

The adhesiveness values, spanning from -23.57 to -21.47, reveal a slight tendency of raw shrimp to adhere, which could influence both the mouthfeel and the ease of handling. Although these values are much more negative than the -0.5 reported for *Penaeus spp.* (Balfour et al., 2012) , they still underscore a propensity for stickiness.

The observed springiness values were between 0.64 and 0.68 which indicates a moderate springiness which is important for mouthfeel. This finding is in line with previous research as *Metapenaeus ensis* shows a springiness value of 0.73 (Xu et al., 2016). The consistency of these springiness values across shrimp species suggests a narrow range, which is important to know when replicating textural properties in analog shrimp products.

Cohesiveness values were between 0.27 and 0.28, signifying tenderness and minimal chewiness of *L. setiferus*. Previous work had similar findings regarding cohesiveness, specifically, cohesiveness was measured as 0.56 for *Metapenaeus ensis* (Xu et al., 2016) and 0.23 for *Litopenaeus vannamei* (Murthy et al., 2016). While the values for *L. setiferus* were similar to other species, it was low comparatively to *Penaeus spp.* which had a cohesiveness value of 3.5 (Balfour et al., 2012). The high value of *Penaeus spp.* indicates a wide range of texture profiles across species.

Gumminess values, ranging from 1.42 to 1.70. These values denote a low degree of toughness, aligning with the desired texture attributes of tenderness and minimal chewiness. These results are slightly lower than those found in the literature, notably *Penaeus spp.* gumminess value was 3.0 (Balfour et al., 2012) and 3.25 for *L. vannamei* (Murthy et al., 2016). This suggests species and size variations may influence this texture characteristics.

Lastly, chewiness values, between 1.00 and 1.15, further emphasize the tenderness and minimal chewiness of the shrimp. The results were lower than the 4.0 and 5.0 values reported for *Penaeus spp.* (Balfour et al., 2012) and *L.vannamei* (Murthy et al., 2016), respectively. This variance could be attributed to differences in size and species supporting the findings for gumminess. However, even with the slight deviations seen for the gumminess and chewiness values, the overall TPA findings are corroborated by the literature.

There were no statistically significant differences between the surface and cross-section orientations across all the TPA parameters examined (Table 2). This lack of statistically significant difference suggested a uniformity in the textural properties of shrimp, regardless of the orientation in which they were measured. Without the need to account for variations due to

orientation, recreating the texture of shrimp in analog products may be more straightforward than previously anticipated.

Table 2. Comparison between shrimp's muscle surface and cross-section within each TPA parameter

TPA Parameters	Muscle Orientation	Mean	SD	SEM	p-value
Hardness	Surface	5.48	2.110	0.384	0.258
	Cross-section	6.00	1.405	0.257	
Adhesiveness	Surface	-23.57	5.110	0.933	0.221
	Cross-section	-21.47	7.720	1.409	
Springiness	Surface	0.68	0.056	0.010	0.062
	Cross-section	0.64	0.085	0.016	
Cohesiveness	Surface	0.27	0.046	0.008	0.156
	Cross-section	0.28	0.038	0.007	
Gumminess	Surface	1.47	0.585	0.107	0.119
	Cross-section	1.70	0.568	0.104	
Chewiness	Surface	1.00	0.373	0.068	0.187
	Cross-section	1.15	0.490	0.089	

Colorimetry

Table 3 presents the colorimetry parameters for *L. setiferus*, analyzed in both surface and cross-sectional orientations. The L* values range from 50.93 to 52.12, indicating a moderate level of brightness without a statistically significant difference between the two orientations. This consistency, regardless of orientation, simplifies product development by eliminating the need to account for variations in perceived lightness. The L* values for white shrimp fall between the lower lightness value reported of 37.88 for Metapenaeus ensis (Xu et al., 2016) and the higher value of 85.16 for unspecified Chinese shrimp (Qi & Li, 2024).

The a* values for *L.setiferus* range from -1.58 to -0.8. These a* values indicate *L.setiferus* has a slight greenish tint. These findings were compared to *Metapenaeus ensis* with an a* value of 0.69 (Xu et al., 2016) and 3.26 for unspecified Chinese shrimp (Qi & Li, 2024) which is less red compared to *L.setiferus.* There is a statistically significant difference

in the a* value between the surface and cross-section orientations of the white shrimp ($p < 0.001$). The a* values are more negative in the cross-section orientation, suggesting a stronger green component.

The b* values for *L.setiferus* ranging from -4.68 to -2.81, indicate a slight bluish tint, positioning them between Metapenaeus ensis at -2.13 (Xu et al., 2016) and the unspecified Chinese shrimp at -8.8 (Qi & Li, 2024). The observed significant differences in a* and b* values between surface and cross-section orientations highlight the inherent color variation in shrimp. Addressing these variations is essential for the development of cell-based shrimp analogs, where achieving uniform color may necessitate the careful manipulation of color-imparting ingredients or processing techniques.

Table 3. Colorimetry Results

Parameter	Orientation	Mean	SD	SEM	P-Value
L*	Surface	50.93	3.286	0.6	0.245
	Cross	52.12	4.478	0.818	
a*	Surface	-0.8	0.696	0.127	<0.001
	Cross	-1.58	0.393	0.072	
b*	Surface	-2.81	1.698	0.31	<0.001
	Cross	-4.68	1.439	0.263	

Proximate Analysis

The proximal analysis of shrimp, as detailed in Tables 4 and 5, provides a comprehensive overview of the nutritional composition of shrimp on both a wet and dry basis. Table 4 indicates that white shrimp primarily consists of moisture, with an average content of 84.0%, and the dry matter predominantly composed of protein (14.2%), followed by ash (1.40%) and fat (0.48%). This composition highlights shrimp's value as a lean protein source, which is a critical attribute to replicate in shrimp analogues for maintaining its nutritional appeal.

Table 4. Proximate Analysis Wet Basis (%)

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Average	84.0	14.2	0.48	1.40

SD	0.56	0.65	0.10	0.14
SEM	0.23	0.27	0.04	0.06

When comparing these findings to literature data, which provides a range of values for moisture (72.97% to 77.57%), protein (12.33% to 15.09%), fat (0.48% to 0.80%), and ash (1.33% to 2.07%) across five different species of shrimp (Liu et al., 2021), it is evident that our analysis aligns well with these reported ranges. The slight variations can be attributed to species-specific differences and environmental factors affecting the shrimp's nutritional composition. However, the consistency in the low-fat content and high protein levels across different studies and species reinforces the importance of these nutritional attributes in shrimp.

When analyzed on a dry basis in Table 5, the protein content significantly dominates the nutritional profile, averaging at 88.8%. This highlights shrimp as an excellent source of protein. The fat content, at an average of 2.99%, remains low, reinforcing shrimp's status as a lean protein source. The ash content, averaging at 8.79%, represents the total mineral content of the shrimp.

Table 5. Proximate Analysis Dry Basis

Sample	Protein (%)	Fat (%)	Ash (%)
Average	88.8	2.99	8.79
SD	1.64	0.66	1.14
SEM	0.67	0.27	0.47

In developing shrimp analogues, it is crucial to replicate not only the sensory attributes of real shrimp but also its nutritional profile. The previous data serves as a benchmark for the nutritional standards that shrimp analogues should aim to meet or exceed. Material consideration in the development of shrimp analogues should prioritize sources that provide similar or improved nutritional content, particularly focusing on maintaining high protein and low-fat levels. This approach will ensure that the analogues can serve as a suitable dietary substitute for real shrimp.

Furthermore, if there are differences in the nutritional content between the shrimp analogues and conventional shrimp, it is imperative to employ clear and transparent labeling. This

transparency is crucial to avoid misleading consumers about the nutritional benefits of the analogues. Accurate labeling that reflects the nutritional composition of shrimp analogues will help consumers make informed dietary choices.

Hematoxylin and Eosin Staining

The Hematoxylin and Eosin (H&E) staining technique, as depicted in Figure 2, provides a microscopic view of the cellular architecture of shrimp in both surface and cross-section orientations.

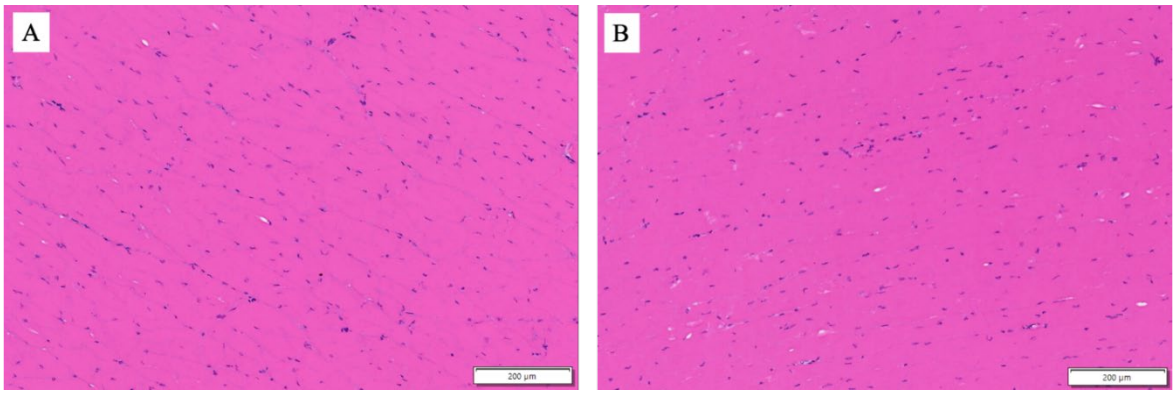

Figure 2. (A) H&E Stain of Shrimp Surface and (B) H&E Stain of Shrimp Cross Section.

The H&E staining of the shrimp surface and cross-section revealed similar cellular patterns across both orientations. The uniform cellular patterns observed in the H&E-stained sections affirm the textural uniformity of shrimp, as previously suggested by the TPA parameters. This histological evidence supports the notion that shrimp possess a consistent texture, which is an important quality attribute for both fresh shrimp and their analogs. The textural uniformity observed at the microscopic level could explain the lack of significant differences in textural properties between the surface and cross-section orientations, as reported in the TPA results.

Conclusion

This study contributes to the field of cellular agriculture, particularly in the sustainable production of shrimp. The research focused on the morphological, physicochemical, and nutritional properties of white shrimp muscle, aiming to establish a comprehensive database to support the development of cell-based seafood analogs. The findings revealed consistent texture profile analysis parameters across the shrimp's surface and cross-section. The uniformity in the

texture of white shrimp simplifies the process of prototyping analog products. This consistency removes a layer of complexity, allowing developers to focus on other aspects of product development without worrying about variations in texture.

Colorimetry revealed significant color variation by orientation in white shrimp. This poses a challenge that future research must address to ensure the visual appeal of cell-based shrimp products. The visual appeal of analog products is a crucial aspect of consumer acceptance.

Proximate analysis highlighted shrimp's potential as a high-protein, low-fat food source, emphasizing the importance of replicating these nutritional attributes in shrimp analogs. The study suggested the need for clear and transparent labeling to inform consumers about any nutritional differences between analogs and conventional shrimp.

H&E staining confirmed the uniformity of shrimp texture at the cellular level, supporting the textural findings and suggesting that the cellular structure of shrimp muscle is consistent across different orientations. This histological evidence further reinforces the potential for creating texturally accurate shrimp analogs.

This study provides valuable insights into the properties of white shrimp muscle, offering a solid foundation for the development of cell-based shrimp analogs. The findings on texture uniformity, color variation, nutritional composition, and cellular structure present both opportunities and challenges for researchers and developers in the field of cellular agriculture. As the cellular agriculture industry continues to evolve, studies like this one will play a pivotal role in advancing sustainable food production methods and addressing the growing global demand for seafood in an environmentally responsible manner.

Future Research

Expanding the species range is essential for gaining a comprehensive understanding of the textural, sensory, and nutritional properties of various marine life forms. Characterizing a broad spectrum of species allows researchers to discover unique textural profiles that could shape consumer preferences and inform processing techniques. The influence of cooking methods on the texture, color, and nutritional quality of seafood is profound. Investigating the effects of these methods on seafood is vital for the creation of seafood analogs that accurately replicate the desired qualities of cooked seafood, not just in their raw state. This research is crucial for

diversifying the range of alternative seafood products and developing a range of alternative seafood products that meet consumer expectations in terms of texture, color, and nutrition.

Additionally, future research should focus on refining techniques to replicate the consistent texture of white shrimp, address color variations, and match the nutritional profile of conventional shrimp. Exploring consumer perceptions and preferences for cell-based seafood will be crucial for successful market integration. As the cellular agriculture industry continues to evolve, interdisciplinary collaboration and open knowledge sharing will be essential to address technical challenges, ensure food safety, and develop sustainable and economically viable cell-based seafood products that can contribute positively to global food systems.

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