Research article

Comprehensive Comparison of Nutritional Components of Red Swamp Crayfish (*Procambarus clarkii*) and Signal Crayfish (*Pacifastacus leniusculus*)

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Abstract Crayfish are consumed worldwide as a food resource. Red swamp crayfish (*Procambarus clarkii*) are not widely recognized as a food source, but signal crayfish (*Pacifastacus leniusculus)* are consumed in Japan. Because nutritional studies and information on red swamp crayfish are scarce, comprehensive comparisons of red swamp crayfish and signal crayfish are lacking. This study aimed to determine the nutritional value of red swamp crayfish as a food source by comparing pigments in the exoskeletons of red swamp and signal crayfish and by making a comprehensive comparison of their nutrients. The exoskeleton of red swamp crayfish is typically reddish-black, whereas that of signal crayfish is brown. A comparison of the exoskeleton pigments between the two crayfish colors using thin-layer chromatography showed bands corresponding to the ester and free forms of astaxanthin in all samples. The pigments in the exoskeletons of both species were the same regardless of body color. A comparison of the three major nutrients in the tail muscles showed differences in carbohydrates and fats between the two species, but the protein content was the same. It is assumed that this is due to differences in food composition and activity levels due to the habitat in which they grow. The fatty acid composition differed between the two crayfish. A comprehensive analysis of hydrophilic compounds in the muscles was conducted using triple quadrupole gas chromatography-mass spectrometry, which identified 107 common components in each sample, including various sugars, nucleic acid-related substances, vitamins, and functional compounds. Principal component analysis by GC/MS showed that the clusters of both crayfish species were largely divided between samples, reflecting the differences in their nutritional components. The presence of higher nutritional components in red swamp crayfish than in signal crayfish may lead to new applications regarding the potential use of red swamp crayfish as a food resource. This study demonstrates the significant nutritional value of red swamp crayfish and can be used to promote the potential use of red swamp crayfish as a valuable food resource and to expand the culinary and nutritional options in Japan.

Keywords *Procambarus clarkii*, *Pacifastacus leniusculus,* pigment, food resource

INTRODUCTION

Crayfish are distributed as a food source in China, the USA, and other parts of the world (FAO, 2018, 2020). Red swamp crayfish (*Procambarus clarkii*) are not well recognized as a food source, but signal crayfish (*Pacifastacus leniusculus*) are used as a food source (Nonaka, 2012), One of the reasons for the lack of utilization of red swamp crayfish may be the lack of nutritional information about this species as a nutritious food source. Further, red swamp crayfish were imported to be utilized as feed for bullfrogs, unlike the signal crayfish which were imported as a food source for human consumption. While the red swamp crayfish is a familiar pet in Japan, its potential for use as a food source is low due to its tendency to inhabit "unhygienic ecosystems" such as muddy wetlands. The image of red swamp crayfish living in dirty places compounds the lack of nutritional information about the species.

One of the valued components of the exoskeleton of the red swamp and signal crayfish is astaxanthin, a carotenoid pigment with a high antioxidant capacity. Astaxanthin is also known to be a factor in the red and blue body color of red swamp crayfish (Nakagawa et al., 1974). Carotenoid pigments have also been reported to be involved in the variation of body coloration in signal crayfish, which changes depending on environmental factors (Sacchi et al., 2021). However, it seems no study has compared the morphology of pigments present in the normal (red and brown) and blue colors of red swamp and signal crayfish, respectively. Regarding the composition of tail muscle meat, several studies focused on the nutrients found in red swamp crayfish (Huner et al., 1988; Dabrowski et al., 1966), and others used inductively coupled plasma mass spectrometry to confirm heavy metal accumulation in the body (Suárez-Serrano et al., 2010; Bian et al., 2023). Some studies analyzed changes in body composition (nutritional value) due to growth environment and food composition (Tan et al., 2018; Miao et al., 2020; Zhang et al., 2023). However, comprehensive comparisons of the components of the meat of tail muscles, focusing on the nutritionally valued components in the red swamp and signal crayfish, are lacking.

OBJECTIVE

This study aimed to determine the nutritional value of red swamp crayfish as a food source by comparing the pigments present in the exoskeletons of red swamp and signal crayfish, and by making a comprehensive comparison of the nutrients in the tail muscles.

METHODOLOGY

Analysis of Astaxanthin in Red Swamp and Signal Crayfish in the Exoskeleton

Red swamp crayfish from the Kokai River in Ibaraki Prefecture and signal crayfish from Lake Akan in Hokkaido, both of which are sold frozen were purchased from dealers and used as experimental samples. We experimented to analyze astaxanthin derived from crayfish exoskeletons. Astaxanthin was identified by comparison of Rf values using thin-layer chromatography (TLC) (Higuchi et al., 2023). As a control, a krill containing astaxanthin and astaxanthin esters (monoesters and diesters) was used (Takaichi et al. 2003). For pigment extraction, the abdominal segment of each exoskeleton was shredded to approximately 1 mm, and 100 μL of acetone was added to 0.01 g of the material. The samples were lightly mixed, allowed to stand for 15 minutes, and then centrifuged for 20 seconds. Each sample was spotted on a TLC plate (TLC silica gel 60 F_{254} [4 × 8 cm]; Sigma-Aldrich), which was then placed in a solvent (petroleum ether/acetone = 7:3) in the deployment tank to separate the pigments.

Comparison of the Three Major Nutrients of Red Swamp and Signal Crayfish

Three sets of samples were prepared, each consisting of 8 frozen red swamps and 8 frozen signal crayfish, with three replicates for each group. They were weighed and their tails were harvested. The tails were weighed after shelling, and the yield was calculated. The tails of the samples were then homogenized using a homogenizer. Moisture content was determined after oven-drying at 135°C for 2 hours. The protein content was analyzed by the Kjeldahl method using each sample after the moisture measurement. A nitrogen coefficient of 6.25 was used. Fat was analyzed by ether extraction using the rapid fat extraction method. Ash was considered as an inorganic residue obtained by combustion method using an electric furnace at 550°C. Carbohydrates were obtained as the sum of moisture, proteins, fats, and ashes subtracted from a total of 100. The data were expressed as mean ± SD.

Comparison of Fatty Acids Between Red Swamp and Signal Crayfish

Fatty acids were measured in freeze-dried samples composed of 11 red swamp crayfish and 31 signal crayfish which were pooled and homogenized. Approximately 2 grams of the freeze-dried sample were placed in a 50-mL test tube. Twenty milliliters of methanol/chloroform (1:2) and 2 mL of saline were added, and the tube was shaken for 5 minutes. The supernatant was discarded, the lower layer was collected, and 10 mL of methanol/chloroform was added and extracted with shaking. The supernatant was discarded again and centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded, and the lower layer was collected. The recovered test solution was volatilized in a rotary evaporator, and lipids were recovered using diethyl ether. The extracted lipids were methyl-esterified and analyzed by gas chromatography (Shimadzu GC-2014). The column was ZS-FAME (length, 30 m; inside diameter, 0.25 mm; film thickness, 0.20 µm), and the carrier gas was helium. Qualitative analysis was performed, and the detected substances were identified and quantified using Supelco 37 Component FAME Mix (CRM47885) as the standard for measurement, and the fatty acid composition was calculated.

Comprehensive Comparison of Hydrophilic Compounds in the Tails of Red Swamp and Signal Crayfish

Hydrophilic compounds in the edible parts of crayfish meat, especially those related to taste and functionality, were analyzed. Using untreated frozen samples of signal and red swamp crayfish, 10 samples (n=10) were collected from the edible portion of each solid sample and freeze-dried overnight. The lyophilized sample (10 mg) was crushed and homogenized, and a mixed solvent containing ribitol at a final concentration of 100 µg/mL, as internal standard (methanol/water/ chloroform = 5:2:2), and zirconia beads were added to the sample. Hydrophilic compounds were extracted by shaking and stirring for 90 minutes. Methoxyamine hydrochloride (Sigma-Aldrich) dissolved in pyridine was then added and reacted at 37°C for 30 minutes to oxime, followed by *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Sigma-Aldrich) and trimethylsilylation in Ultrapure pyridine (Wako), which were used as analytical samples. The obtained samples were analyzed using a GCMS-TQ8040 NX triple GCMS-TQ8040 NX triple quadrupole mass spectrometer (Shimadzu) connected to a GCMS-TQ8040 NX quadrupole mass spectrometer (GL Science). Pure helium was used as carrier gas. The column temperature was 50°C for 3 minutes, increased to 320°C at 15°C/min, and maintained at 320°C for 6 minutes. Mass spectrometry was performed by electron ionization, with an ionization voltage of 70 eV, vaporization chamber temperature of 230°C, transfer line temperature of 250°C, detector temperature of 250°C, and scan m/z of 50-500. The gas chromatography-mass spectrometry (GC/MS) data were analyzed using the NIST 17 library and a GC/MS solution (Shimadzu). The relative values for each sample were standardized using the peak area of the internal standard, ribitol.

RESULTS AND DISCUSSION

Analysis of Astaxanthin in Red Swamp and Signal Crayfish Exoskeleton and Separation of Astaxanthin

The red and blue colors seen in the red swamp crayfish and the brown and blue colors of the signal crayfish were visually different (Fig. 1A). The TLC results of the crayfish exoskeletons are shown in Fig. 1B. The exoskeletons of the four crayfish species show bands at the same positions. The results confirm that the same three types of astaxanthin - free-formed, monoesters, and diesters- are present in the exoskeletons of both red swamp and signal crayfish, despite their very different apparent colors. It has already been reported that the pigment factor of blue lobster is astaxanthin (Buchwald and Jencks, 1968). It has been reported that the factor pigment of blue-red swamp crayfish is a carotenoid pigment. On the other hand, the factorial pigment of the blue signal crayfish is not known. This study clarified that the pigment responsible for both species is astaxanthin and the mode of its presence in the blue crayfish. Astaxanthin in the exoskeletons is a substance used as an antioxidant. Therefore, it can be used as a food resource.

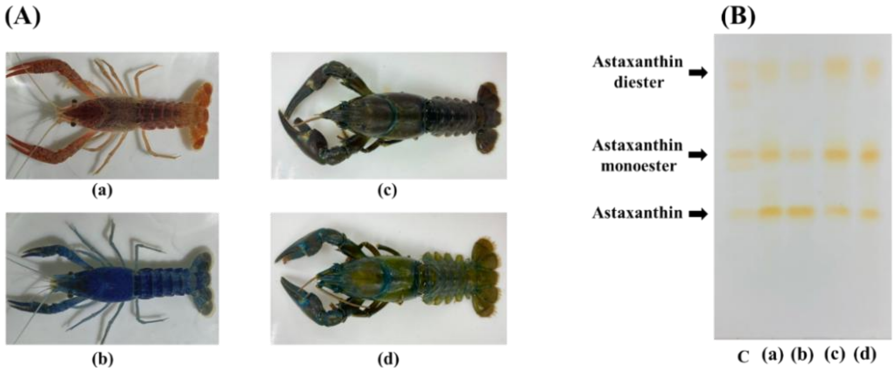

Fig. 1 Comparison of color variation and astaxanthin between red swamp crayfish and signal crayfish

(A) Color variation of red swamp and signal crayfish: (a) red-colored red swamp crayfish, (b) blue-colored red swamp crayfish, (c) brown-colored signal crayfish, and (d) blue-colored signal crayfish. (B) Comparison of astaxanthin by thin-layer chromatography between the red swamp and signal crayfish (from left to right): C control, (a) red-colored red swamp crayfish, (b) blue-colored red swamp crayfish, (c) brown-colored signal crayfish, and (d) blue-colored signal crayfish.

Comparison of the Three Major Nutrients of Red Swamp and Signal Crayfish

The results of the nutrient analysis of the red swamp and signal crayfish are shown in Table 1. The components are water, proteins, fats, carbohydrates, and ash. Moisture accounted for approximately 80% of the total, and ash content was approximately 1.5%. The organic matter in the composition consisted mostly of proteins, with only a small amount of fats and carbohydrates.

Table 1 Comparison of the three major nutrients of red swamp crayfish and signal crayfish

	Red swamp crayfish (%)		Signal crayfish (%)	
Moisture	80.13	±0.23	83.29	±0.16
Protein	17.33	±0.23	14.87	±0.07
Fat	0.20	±0.01	0.36	±0.04
Carbohydrate	0.86	±0.44	0.17	±0.12
Ash	1.47	±0.06	1.30	±0.04

The muscle components are composed of moisture, protein, and fats, of which fats tend to be inversely proportional to moisture and protein. This is due to most of the moisture in muscles existing within the muscle fibers and being bound together. However, in the results of this experiment, signal crayfish had lower protein and higher water content than red swamp crayfish. From this, it was inferred that the moisture in the tail of the signal crayfish consists of a large amount of free moisture that is not bound to muscle fibers. This suggests that the signal crayfish, which has a strong salt tolerance and can live in brackish water, adjusts its osmotic pressure by retaining free water between the muscle and exoskeleton.

Comparison of Fatty Acids between Red Swamp and Signal Crayfish

Table 2 shows the fatty acid composition of the lipids extracted from the tails of red swamp and signal crayfish. Palmitic (C16:0), oleic (C18:1), linoleic (C18:2), arachidonic (C20:4), and eicosapentaenoic acids (C20:5) were abundant, accounting for approximately 70% of the total content in both species. In terms of fatty acid composition, red swamp crayfish had more saturated fatty acids, whereas signal crayfish had more polyunsaturated fatty acids. The fatty acids varied between the two crayfish, with the largest differences observed across each fatty acid. Eicosapentaenoic (C20:5) and arachidonic acids (C20:4) were more abundant in signal crayfish, whereas linoleic and oleic acids (C18:1) were more abundant in red swamp crayfish. Based on the widely known functions of fatty acids, it can be inferred that oleic acid is strongly perceived in red swamp crayfish. Eicosapentaenoic, linoleic, and arachidonic acids are expected to have health effects as eicosanoids such as prostaglandins, which are essential bioactive substances in organisms. This effect is presumed to be beneficial for crayfish fats.

Table 2 Comparison of fatty acids between the red swamp crayfish and signal crayfish

Composition			
Compound		Red swamp crayfish (%)	Signal crayfish (%)
C14:1	Methyl myristoleate	0.4	0.2
C15:0	Methyl pentadecanoate	1.4	0.4
C15:1	Methyl cis-10-pentadecenoate	1.7	1.6
C16:0	Methyl palmitate	12.4	13.3
C16:1	Methyl palmitoleate	4.1	5.5
C17:0	Methyl heptadecanoate	1.9	0.6
C17:1	cis-10-Heptadecanoic acid methyl ester	0.7	0.6
C18:0	Methyl stearate	7.1	6.2
C18:1n9c	cis-9-Oleic acid methyl ester	3.6	4.1
C18:1n9t	trans-9-Elaidic acid methyl ester	19.2	17.1
C18:2n6c	Methyl linoleate	0.4	0.2
C18:2n6t	Methyl linolelaidate	10.2	3.9
C18:3n3	Methyl linolenate	0.4	0.2
C18:3n6	Methyl γ-linolenate	3.1	1.5
C20:0	Methyl arachidate	0.1	0.2
C20:1	Methyl cis-11-eicosenoate	0.7	0.9
C20:2	cis-11,14-Eicosadienoic acid methyl ester	0.8	1.4
C20:4n6	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	12.2	14.3
C20:5n3	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	13.4	21.5
C22:6n3	cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	4.6	5.4
C24:0	Methyl lignocerate	1.2	0.3
C24:1	Methyl nervonate	0.3	0.5
Saturated fatty acid		24.1	21.0
Mono-unsaturated fatty acid		30.8	30.6
Poly-unsaturated Fatty acid		45.1	48.4
Total (%)		100.0	100.0

Fatty acids, like sugar, play a role in supplying energy after consuming crayfish. Among fatty acids, saturated fatty acids and unsaturated fatty acids differ in their stability against oxidation, with unsaturated fatty acids being more easily oxidized and consumed. For these reasons, it is inferred that the tail of the red swamp crayfish contains more carbohydrates than the signal crayfish and has a superior energy supply derived from sugar and less energy supply derived from fatty acids.

Comprehensive Comparison of Hydrophilic Compounds in the Tails of Red Swamp and Signal Crayfish

Comprehensive analysis of hydrophilic compounds in crayfish muscle using GC/MS detected 549 peaks and identified 107 components common to both red swamp and signal crayfish. These results indicate that both crayfish muscles share common compounds.

The 107 identified components were analyzed in detail. Functional and tasting compounds were included as described below. Amino acids, such as threonine, serine, proline, glycine, glutamine, asparagine (sweet taste), glutamic acid, and aspartic acid (typical umami compounds). Nucleic acid-related substances such as inosine, inosinic acid, adenine, adenosine, guanine, guanosine, ribose, and others. Sugars, such as glucose, mannitol, inositol, galactitol, sucrose, and fructose. Vitamins, such as pantothenic acid, nicotinic acid, nicotinamide, and ascorbic acid. Various functional substances such as taurine, ornithine, creatine, citrulline, and gamma-aminobutyric acid.

The contents of these identified substances were examined for differences between red swamp and signal crayfish. The data showed that hypotaurine, trehalose, creatine, sorbitol, aspartic acid, inositol, and glutamic acid were more than twice as abundant in red swamp crayfish than in signal crayfish. In signal crayfish, amino acids such as alanine, threonine, isoleucine, proline, leucine, and valine were more than twice as abundant as those in red swamp crayfish, such as taurine, tryptamine, and other materials.

Next, the component data obtained by GC/MS analysis were subjected to principal component analysis. Red swamp and signal crayfish were divided into two major clusters, reflecting the differences in the components (Fig. 2). The components that characterized this difference were amino acids and sugars such as glucose, hypotaurine, inositol, sucrose, and trehalose. These results indicate that despite sharing many common components, red swamp, and signal crayfish have differences in the component content of the tail muscles.

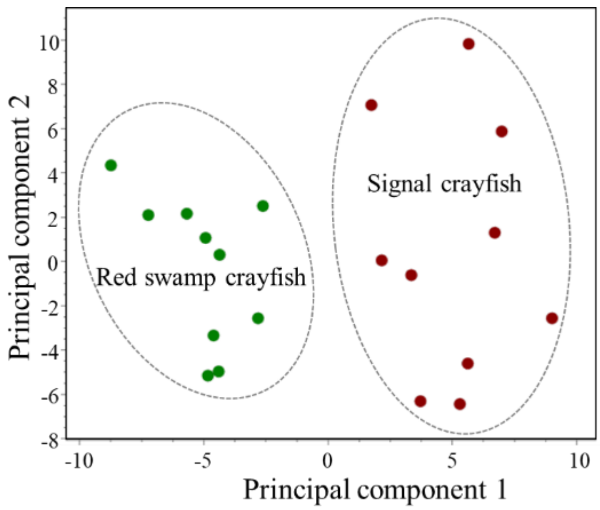

Fig. 2 Principal component analysis of the tails of red swamp and signal crayfish

CONCLUSION

TLC comparison of the pigments in the exoskeletons of red swamp and signal crayfish showed bands at the position of the ester forms of astaxanthin as well as the free form of astaxanthin in all samples. This data indicates that astaxanthin is a factor pigment in signal and red swamp crayfish, regardless of body color, and that astaxanthin is present in the exoskeleton in three different forms in both species. Astaxanthin in the exoskeleton is a substance used as an antioxidant and as a food source.

A comparison of the three major nutrients in the tail muscle between the red swamp and signal crayfish showed differences and that red swamp crayfish had more saturated fats, whereas signal crayfish had more polyunsaturated fatty acids. The protein contents were the same across species. Furthermore, a comprehensive analysis of hydrophilic compounds in the tail muscle, especially those related to taste and functionality, using a triple quadrupole GC/MS identified 107 components common to each sample, including a variety of sugars, nucleic acid-related substances, vitamins, and functional compounds. Principal component analysis using these data showed that red swamp and signal crayfish were divided into two major clusters, reflecting differences in component content. The components that characterized this compositional difference were amino acids and sugars. These results indicate that despite sharing many common components, there are differences in the component content in the tail muscle of red swamp and signal crayfish. Therefore, those components with greater amounts in red swamp crayfish than in signal crayfish may lead to a new added value of red swamp crayfish as a food resource. Further research should be conducted to further define the characteristics of red swamp crayfish through the rearing environment and feeding to increase the number of nutritionally valued components.

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