Food Science and Applied Biotechnology, 2024, **7**(2), 182-194

UFT Academic Publishing House, Plovdiv

Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2024.v7.i2

Research Article

Histological structure and physicochemical indicators of frozen crocodile meat

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Abstract

The objective of the present study was to determine the changes in the histological structure and some physicochemical indicators of frozen crocodile meat. Vacuum packs of frozen crocodile meat stored at -18℃ for 18 months were purchased from retail stores. Histological sections were stained by hematoxylin-eosin and Masson's trichrome staining methods. Physicochemical indicators and fatty acid composition were determined by standard methods. The histological study proved clear morphological changes in the structural components of the muscles. Physicochemical analysis showed 21.90% protein content, 6.09% lipid content, 69.56% water content, and 1.09% ash content. The largest was the amount of monounsaturated fatty acids (47.45%), followed by saturated (36.39%) and polyunsaturated fatty acids (16.53%). More studies on the histological structure and physicochemical parameters of other members of the order Crocodilya are needed to gather data on the nutritional value and biological wholesomeness of the meat from these species.

Keywords

crocodile, frozen meat, morphological characteristics, physicochemical indicators, fatty acid composition

Abbreviations

H/E – hematoxylin and eosin; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – Saturated fatty acids; UFA – unsaturated fatty acids;

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Article history:
Received 23 February 2023
Reviewed 29 March 2024
Accepted 11 June 2024
Available on-line 10 October 2024

https://doi.org/10.30721/fsab2024.v7.i2.372
2024, UFT Academic publishing house, Plovdiv

Introduction

Interest in unconventional foods in developed countries has been growing over the past 50 years. In an effort to attract new customers and high profits, restaurants are changing their strategy and increasingly offering non-traditional delicacies (Černíková et al. 2015). Countries where crocodile meat is available as a delicacy are known and these are the USA, Ethiopia, Cuba, Australia, South Africa, Argentina, Brazil, Mexico (Cossu et al. 2007; Gamboa-Delgado et al. 2022; Hoffman and Cawthorn 2012; Morais et al. 2013). Crocodiles are mainly farmed for their skin, but in recent years crocodile meat has become available for consumption in many countries (Mdhluvu et al. 2021). According to Commission Regulation (EU) 2021/405, "reptile meat" means the edible parts, processed or not, obtained from farmed reptiles belonging to the species *Alligator mississippiensis*, *Crocodylus johnstoni*, *Crocodylus niloticus*, *Crocodylus porosus*, *Timon lepidus*, *Python reticulatus*, *Python molurus bivittatus* or *Pelodiscus sinensis*, authorized in accordance with Regulation (EU) 2015/2283 and included in the Union list of novel foods. According to EFSA (2007), crocodile meat intended for human consumption should be obtained from the back fillets and the tail. Balowski et al. (2015) reported that the interest in the tail part is probably due to its more tender consistency compared to the rest of the carcass.

In Europe, the consumption of crocodile meat is very limited, therefore the data on its consumption are common to all reptiles and combined with those for other marine animals. EFSA presents only one study on the consumption of fish, seafood, amphibians, reptiles, and invertebrates, which shows that the consumption of these species amounts to an average of 31,66 g/day in Romania (https://www.efsa.europa.eu/en/microstrategy/foodex2-level-7). Crocodile meat in the EU is mainly imported from other countries. The main part of reptile meat imports into the EU is from South Africa, the USA, and Zimbabwe, with a value increasing from €80,000 in 2002 to €475,000 in 2005. The majority of trade in Europe is via Belgium, France, Germany, the Netherlands, and the UK (EFSA 2007). Under the current legislative framework, the third countries from which consignments of reptile meat are allowed to enter the EU are Switzerland, Botswana, Vietnam, South Africa, and Zimbabwe (Commission Regulation (EU) 2021/405). World crocodile meat production statistics show that 1,000 t of crocodile meat were traded globally in 2015. This is meat intended for human consumption, which is usually tail meat and accounts for 33% of the carcass weight (Mdhluvu et al. 2021).

To our knowledge, there is a lack of information on the histological structure and physicochemical indicators of frozen crocodile meat intended for human consumption. For this reason, we set out to determine the morphological changes due to low temperatures, as well as to determine the protein, lipid, water, ash content, and fatty acid composition of crocodile meat in the frozen state.

Materials and Methods

Sampling. Eight vacuum packs of 500 g each with frozen crocodile tail meat stored at -18°C for 18 months were purchased from retail stores. The meat was produced and packed on a crocodile farm in South Africa. It was transported in a cooler bag to the laboratory and then thawed at 4°C overnight in a refrigerator. Samples for histological and physicochemical analysis were taken from each pack.

Histological analysis. From each pack, 4 samples were taken for histological assessment. Two of the samples were used to study the transverse projection, while the other two samples were used to study the longitudinal projection of the muscle fibers. After sampling the material (1 x 1 x 0.5 cm in size) was fixed immediately in 10% buffered formalin (pH 7.4), washed in running water, dehydrated in an ascending alcohol series, cleared twice in xylene and embedded in paraffin. Sections with a thickness of 6 µm were obtained on a rotary microtome (YD-335A, China). Histological sections were stained with standard protocol hematoxylin-eosin and Masson's trichromic staining. Weigert`s iron hematoxylin kit for nuclear staining (Merck, Germany) and Masson-Goldner staining kit for the visualization of connective tissue with trichromic staining (Merck, Germany) were used for the Masson method. Entelan was used to obtain permanent histological preparations. Histological assessment was made by using an N-200M microscope (Hangzhou Sumer Instrument Co., Ltd, China). Photo documentation was done by camera OptikamB5 Digital Camera (OPTIKA MICROSCOPES, Italy) and software PROVIEW (Optika Srl, Ponteranica, Italy). The histological analysis was performed in the laboratory of the Department of Veterinary Anatomy, Histology and Embryology, Trakia University, Bulgaria.

Physicochemical analysis. The physicochemical analysis was performed in an accredited testing laboratory for food in Bulgaria. For each analysis to determine the physicochemical indicators, samples were taken from the packs of frozen crocodile meat. Table 1 lists the analysis methods. Lipid indices were calculated using the following formulas according to Chen and Liu (2020):

(1) Polyunsaturated fatty acid/saturated fatty acid ratio (PUFA/SFA) = ΣPUFA/ΣSFA,

(2) Omega-6 fatty acids/Omega-3 fatty acids ratio (n-6/n-3) = Σn-6/Σn-3,

(3) Index of atherogenicity (IA) = [C12:0 + (4 × C14:0) + C16:0]/ΣUFA,

(4) Index of thrombogenicity (IT) = (C14:0 + C16:0 + C18:0)/[(0.5×ΣMUFA) + (0.5 ×Σn-6 PUFA) + (3×Σn-3 PUFA) + (n-3/n-6)],

(5) Hypocholesterolemic/ hypercholesterolemic ratio (HH) = (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0).

Table 1. Physicochemical indicators and methods for determining them

Physicochemical indicator	Method	References
Protein content	BDS 9374:1982	(BDS 9374, 1982)
Fat content	BDS 8549:1992	(BDS 8549, 1992)
Water content	BDS 5712:1974	(BDS 5712, 1974)
Ash content	BDS 9373:1980	(BDS 9373, 1980)
Saturated fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)
Unsaturated fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)
Omega-3 fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)
Omega-6 fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)
Omega-9 fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)
Cholesterol	BDS EN ISO 12228-1:2015	(BDS EN ISO 12228-1, 2015)
Trans-fatty acids	BDS EN ISO 12966-4:2015	(BDS EN ISO 12966-4, 2015)

Statistical processing. The results from the physicochemical analysis were processed by computer software GraphPad Prism (v.8) and presented as mean values with standard error of mean.

Results and Discussion

Histological changes in frozen crocodile meat. All components of skeletal muscle tissue structure were affected (Fig. 1-9). The detailed examination of the prepared histological slides showed areas of muscle in which some of the muscle fibers were relatively well preserved. Their sarcolemma was preserved intact without tears or other type of damage (Fig. 1 A and B; Fig. 6 B). Numerous basophil-stained nuclei were also observed. The nuclei had an oval shape and were recorded both around the sarcolemma and in their central part. The number of nuclei categorized the fibers as a multinucleated skeletal muscle symplast (Fig. 1 A and B). The connective tissue component was represented by endomysium and perimysium internum (Fig. 1 A and B; 2 B; 4 A and B; 5 A and B; 6 A; 7 A and B; 8 A and B). The endomysium was found surrounding the muscle fibers, which had a well-preserved histostructure, but it was disrupted. It was visible as a broken thread around the fibers, but recognizable (Fig. 1 A and B; 6 A). In other histological preparations, the endomysium was not prominent due to more pronounced disruption of the muscles and destruction of the muscle fibers (Fig. 5 A; 7 A; 8 B). Perimysium internum was a clearly recognizable component (Fig. 1 A and B; 4 A and B; 5 A and B; 6 A; 7 A and B; 8 B). Its condition was affected by freezing and was torn in the studied areas, leading to disruption of the structure of skeletal muscle bundles and disorganization of muscle fibers (Fig. 1 B; 4 A and B; 5 A and B; 6 A; 7 A and B). Freezing had caused a change in the shape of the muscle fibers and they appeared shrunken and dehydrated (Fig. 3 A and B; 4 A and B; 7 A and B; 8 A). Enlarged void spaces were prominent between the shrunk myofibers (Fig. 3 A; 4 A and B; 5 A; 6 A; 8 A), which in some cases were filled with interstitial proteinaceous material (Fig. 1 A and B; 2 A and B; 5 B; 6 B; 7 B; 9 A and B). A significant amount of white adipose tissue was found near perimysium internum and the shrunk muscle fiber bundles (Fig. 7 B).

Freezing caused destruction of the muscle tissue to a great extent. In the areas studied, there were prominent spaces in which the presence of muscle fibers was absent and the tissue appeared incomplete and loose (Fig. 2 A and B; 3 A and B; 4 A and B; 7 B; 8 B; 9 A). In addition to the shrinking of muscle fibers, which is a partial change, completely destructured muscle fibers with impaired integrity were also observed, which is a complete change in these morphological objects (Fig. 2 A and B; 5 A and B; 7 A; 8 A and B). Meat quality is determined by a number of factors, of which morphological changes play an important role. Microstructure provides a qualitative assessment of meat condition before and after freezing (Rinwi et al. 2023). A significant number of studies have been performed to track the morphological changes and how they lead to muscle damage after freezing. It results in strong and irreversible changes, some of which associated with destruction of the muscle fiber sarcolemma. This causes substances that are intracellular components to enter the intercellular spaces. As a result of osmotic phenomena, the result is dehydration and shrinking of muscle fibers. Muscle bundles also undergo conformational changes. In addition, visual fields with presence of optically large voids between muscle fibers and muscle bundles are established. These void spaces are assumed to be imprints of the ice crystals present before thawing (Li et al. 2022; Shui et al. 2022; Strateva et al. 2021a, 2021b; Strateva and Penchev 2020; Strateva and Penchev 2021). This is because upon freezing, the large ice crystals formed in the extracellular spaces lead to an increase of void spaces between the fibers (Shui et al. 2022). This means that due to the mechanical force of the ice crystals exerting pressure on the myofibers, they change their polygonal shape, deform, shrink, and some of them are completely destroyed (Li et al. 2022).

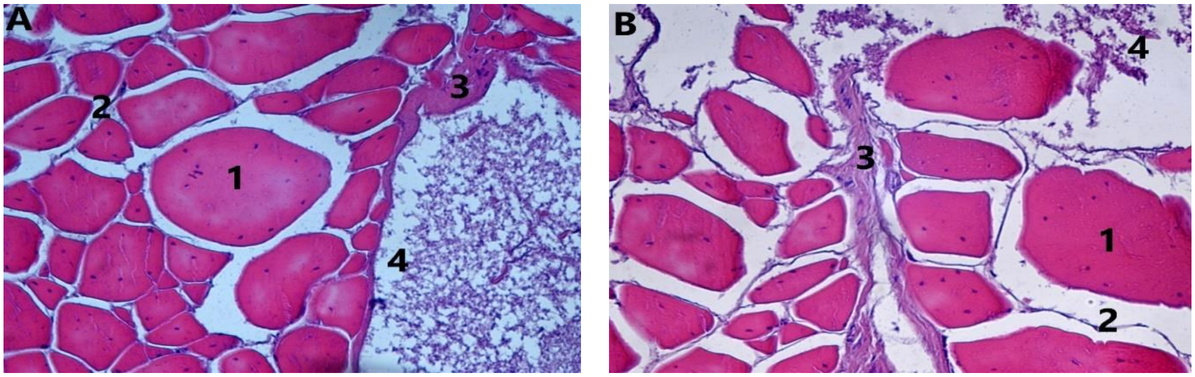

Figure 1. Crocodile tail muscles frozen at -18℃ for 18 months (A and B, 20x, H/E, scale bar=50 µm) (transverse projection). Key: 1: muscle fibers with preserved histostructure; 2: endomysium; 3: perimysium internum; 4: interstitial proteinaceous material

Interstitial proteinaceous material appears in intercellular spaces after freezing (Tinacci et al. 2018). The results of our study showed the presence of basophil-stained and granular proteinaceous material in the spaces between the muscle fibers. Its origin, in our opinion, is related to the destruction of myofiber sarcolemma under the action of ice crystals, as a result of which the integrity of muscle fibers and myofibrils is violated. Other authors also reported similar changes (Pavlov et al. 2008; Strateva et al. 2021a, 2021b; Tinacci et al. 2018). According to Lv et al. (2021), myosin and sarcoplasmic proteins could be detected in the interstitial spaces between myofibers after freezing.

Our results about the condition of muscle fibers and

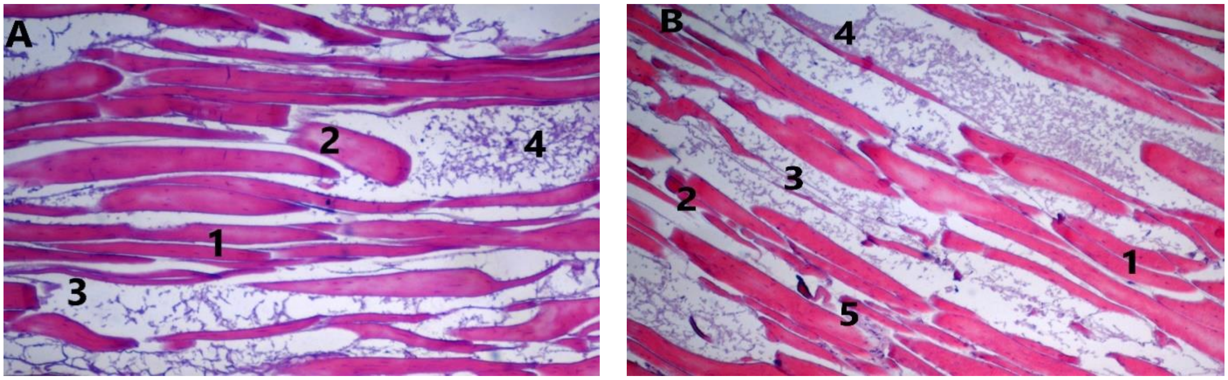

Figure 2. Crocodile tail muscles frozen at -18℃ for 18 months (A and B, 10x, H/E, scale bar=100 μm) (longitudinal projection). Key: 1: shrunk muscle fibers; 2: destructured muscle fibers; 3. enlarged space between fibers; 4. intersitial proteinaceous material; 5. endomysium

the connective tissue component in the crocodilian muscles are consistent with those found by Strateva and Penchev (2020), Strateva and Penchev (2021), Strateva et al. (2021a), Strateva et al. (2021b), Li et al. (2022a), Shui et al. (2022). The histological characteristics of muscle fibers and connective tissue sheaths in our study correspond to those found by Lin et al. (2009), namely that each skeletal muscle is divided into bundles by perimysium, which is connective tissue. Furthermore, we are in agreement with Lin et al. (2009) about muscle fiber shape, too. We also established polygonal shape of the muscle fibers unaffected by the low temperatures and having maintained their integrity. We established the location of endomysium around the myofibers, which coincided with the histological finding described by Lin et al. (2009).

According to Poudyal et al. (2023), large ice crystals damage muscle structure. We also consider remarkable the role of crystals in damaging crocodile tail muscles. The presence of preserved muscle fibers and the greater number of shrunk fibers in our study compared to destroyed and torn ones can be explained by the large amount of white adipose tissue and connective tissue (perimysium) in crocodile muscles. It is likely that these structures helped to limit the mechanical force and the pressure exerted by the ice crystals on the fibers and thus managed to preserve their integrity. The amount of fat in crocodile meat is low (Hoffman and Cawthorn 2012). Fat content in fresh meat amounts to 1.9% in *Crocodylus porosus*, 6.23% in *Crocodylus niloticus* (Saadoun and Cabrera 2008), 1.9% in *Crocodylus johnstoni* and 5.5% in *Crocodylus siamensis* (Nongtaodum et al. 2005).

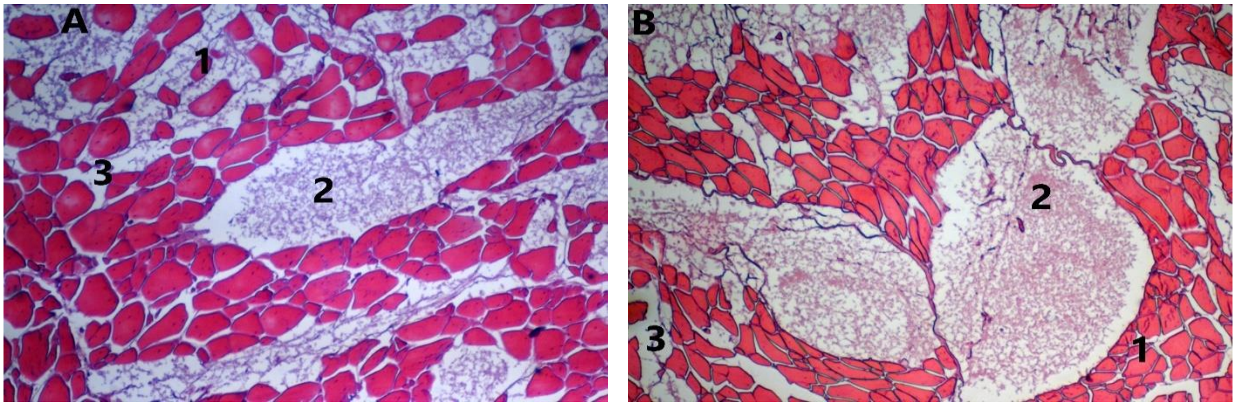

Figure 3. Crocodile tail muscles frozen at -18℃ for 18 months (A and B, 10x, H/E, scale bar=100 μm) (transverse projection). Key: 1: shrunk muscle fibers; 2: interstitious proteinaceous material; 3. enlarged space between fibers

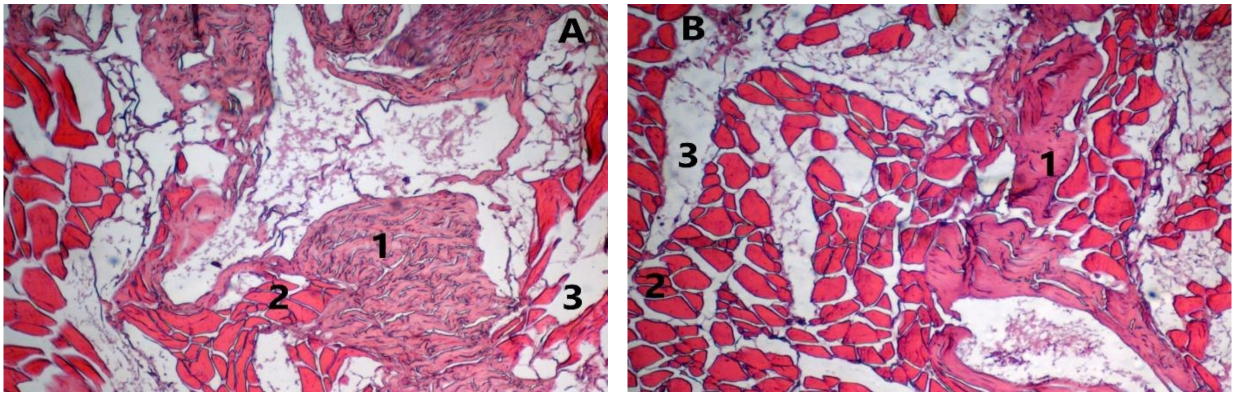

Figure 4. Crocodile tail muscles frozen at -18°C for 18 months (A and B, 10x, H/E, scale bar=100 µm) (transverse projection). Key: 1: perimysium internum; 2. shrunk muscle fibers; 3: enlarged space between fibers

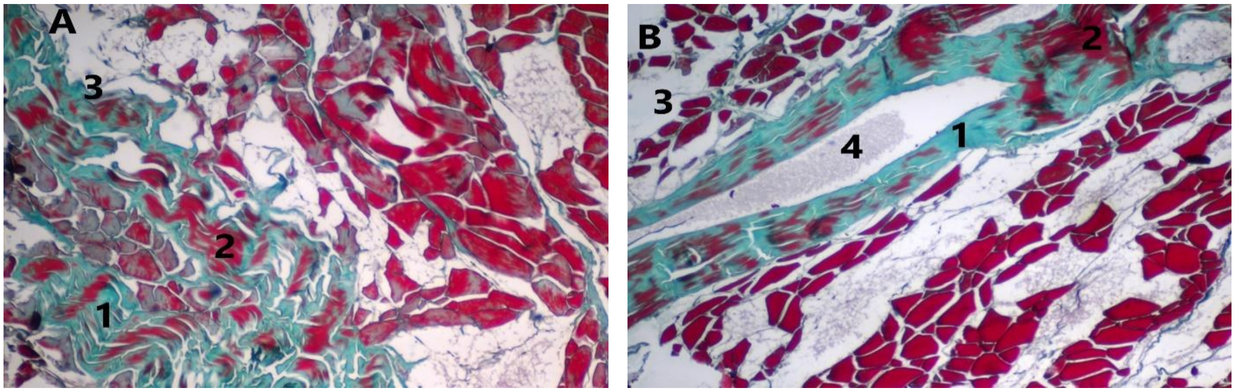

Figure 5. Crocodile tail muscles frozen at -18°C for 18 months (A and B, 10x, Masson staining, scale bar=100 µm) (transverse projection). Key: 1: perimysium internum; 2. destructured muscle fibers; 3. enlarged space between fibers; 4: interstitial proteinaceous material

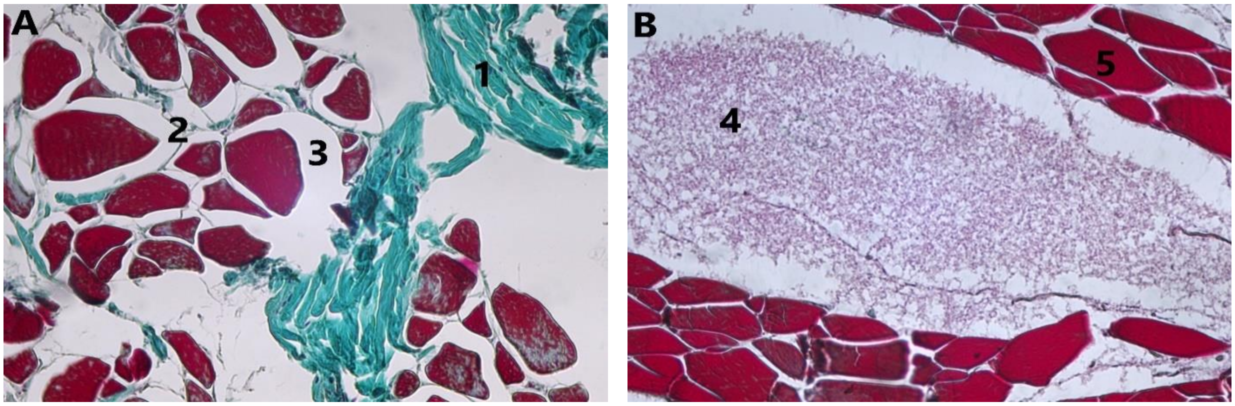

Figure 6. Crocodile tail muscles frozen at -18°C for 18 months (A and B, 20x, Masson staining, scale bar=50 µm) (transverse projection). Key: 1: perimysium internum; 2. endomysium; 3. enlarged space between fibers; 4: interstitial proteinaceous material; muscle fibers with preserved histostructure

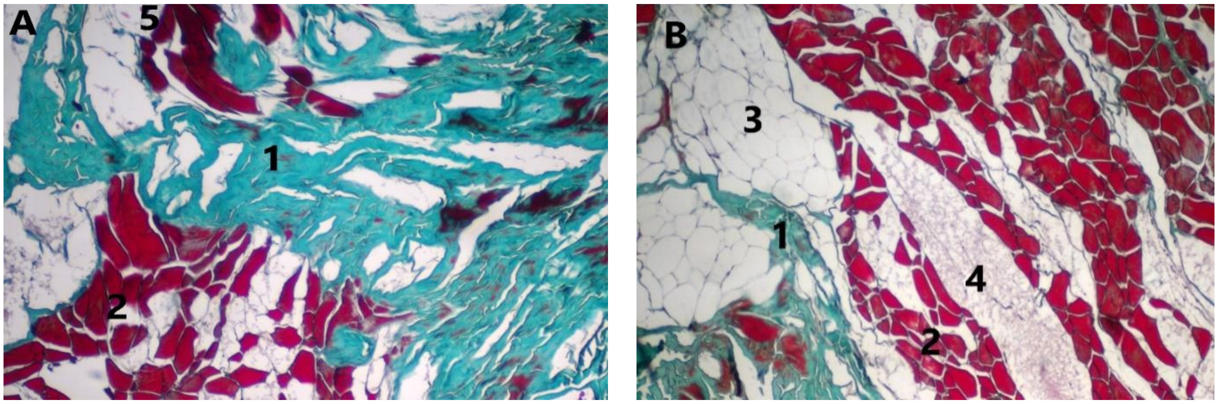

Figure 7. Crocodile tail muscles frozen at -18℃ for 18 months (A and B, 10x, Masson staining, scale bar=100 µm) (transverse projection). Key: 1: perimysium internum; 2. shrunk muscle fibers; adipose tissue; 4: interstitial proteinaceous material; 5. destructured muscle fibers

Our results are similar, but clear species differences are observed.

Physicochemical indicators of frozen crocodile meat. The results of protein, fat, water and ash content are summarized in Table 2. The water content of frozen crocodile meat was 69.56%, while the amount of protein was 21.90%. The fat content was 6.10% and the ash content was 1.09%. Crocodile meat is high in protein (Zhang et al. 2021). The demand for alternative sources of animal protein will increase by 2050 (Luthada-Raswiswi et al. 2019), placing crocodile meat as a likely source for their supply. Protein content in our study is close to values reported by other authors for fresh meat from *Crocodylus porosus* (22%) (Saadoun and Cabrera 2008) and *Crocodylus johnstoni* (21%) (Nongtaodum et al. 2005).

The amount of fat in crocodile meat is low (Hoffman and Cawthorn 2012). Fat content in fresh meat amounts to 1.9% in *Crocodylus porosus*, 6.23% in *Crocodylus niloticus* (Saadoun and Cabrera 2008), 1.9% in *Crocodylus johnstoni* and 5.5% in *Crocodylus siamensis* (Nongtaodum et al. 2005). Our results are similar, but clear species differences are observed. The amount of fat can be affected by the way the animals are kept, and Junior et al. (2015) considered that differences were found between farmed specimens and wild representatives. In addition, changes in the quantitative profile of fat can also be expected due to the intensity of movement, nutrition and seasonal changes. There is a lack of information on the lipid content of frozen meat from crocodiles or other representatives of the order Crocodilya. Nongtaodum et al. (2005) reported that the water content of *Crocodylus porosus* meat was 75.9% and that of *Crocodylus johnstoni* was 75.9%. Our results showed that the water content of frozen crocodile meat was 69.56%. This result indicates a good water-holding capacity of the muscle fibers after freezing and thawing. Furthermore, the preserved secondary myofibrillar proteinaceous structure could also be suggested as a likely reason for the relatively high water content. In addition, the morphological characteristic of fibers can also be a probable cause. From the histological finding, it is clear that fibers with preserved integrity are also present, which have probably reabsorbed thawed water during the thawing process and it was not lost on the surface in the form of drops.

According to Altemio et al. (2021), ash content is influenced by the composition of the raw material. Data about the ash content of fresh meat from *Caiman yacare* (1-1.05%) (Saadoun and Cabrera 2008) and Spectacled caiman (0.88-1.10%) (Huang et al. 2018) have been published. Leak et al. (2003) proved that the ash content of alligator meat was 1%. In our study, the ash content of frozen crocodile tail meat was 1.09%.

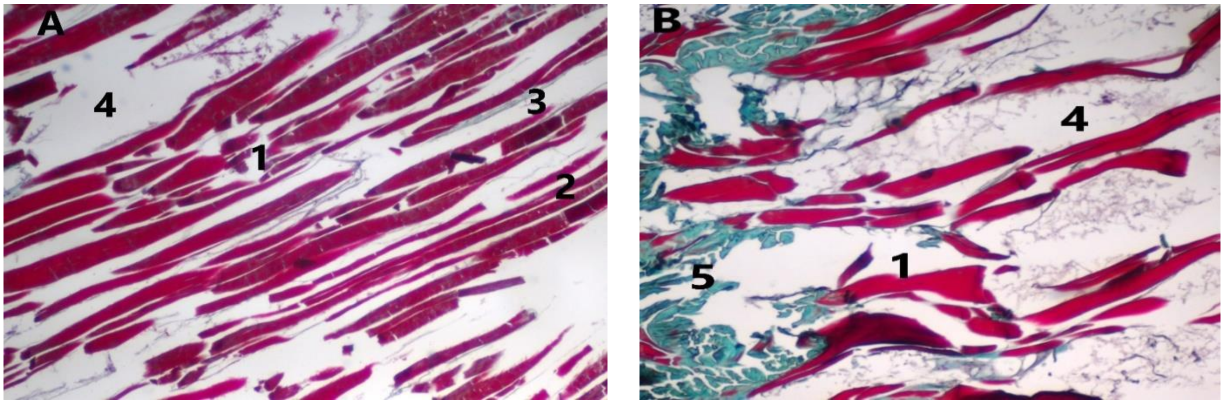

Figure 8. Crocodile tail muscles frozen at -18°C for 18 months (A and B, 10x, Masson staining, scale bar=100 µm) (longitudinal projection). Key: 1: destructured muscle fibers; 2: shrunk muscle fibers; 3: endomysium; 4: enlarged space between fibers; 5. perimysium internum

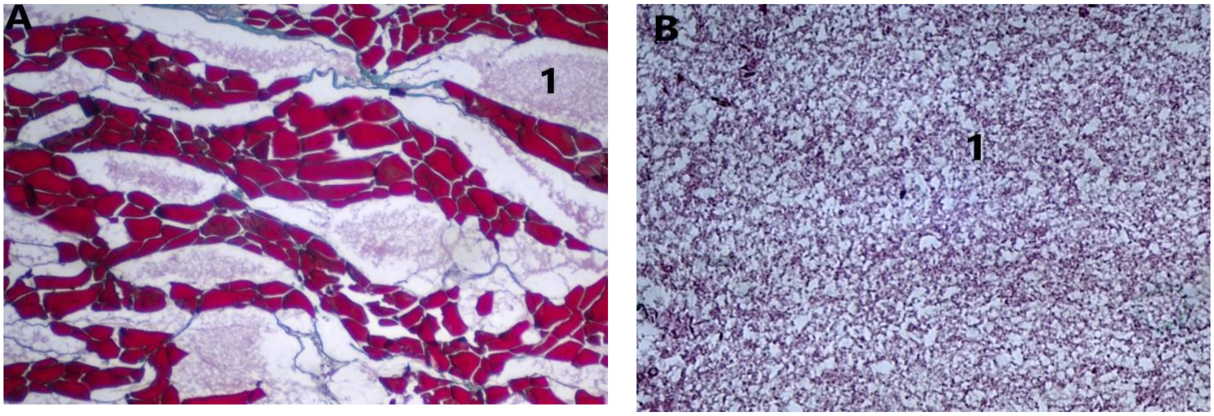

Figure 9. Crocodile tail muscles frozen at -18°C for 18 months (A:10x, scale bar=100 µm и B: 20x, scale bar=50µm, Masson staining) (transverse projection). Key: 1: interstitial protein material

Fatty acid composition of frozen crocodile meat. The results in Table 3 show the fatty acid composition of frozen crocodile meat. The saturated fatty acid content was 36.39%, while the unsaturated fatty acid values were 63.98%. The proportion of monounsaturated (47.45%) was greater than that of polyunsaturated fatty acids (16.53%). The most abundant of the saturated fatty acids was palmitic acid with 28.07%, followed by stearic acid with 6.89%. The highest amount of monounsaturated fatty acids was exhibited by oleic acid (46.79%), while linoleic acid (15.38%) was in the highest amount of polyunsaturated fatty acids. No trans-fatty acids were detected. The amounts of omega-6 (15.70%) and omega-9 (47.06%) fatty acids were significantly higher compared to omega-3 fatty acids (0.72%). The amount of cholesterol was 1.69%.

Table 2. Physicochemical indicators of frozen crocodile meat

Physicochemical indicators, %	**Mean ± SEM**
Proteins	21.90±0.38
Fats	6.10±0.56
Water content	69.56±0.71
Ash content	1.09±0.02

The fatty acid composition of crocodile meat is highly dependent on diet and differs among age groups and between species (Osthoff et al. 2010). Hoffman et al. (2000) found 37% SFA, 10% MUFA and 51% PUFA in Nile crocodile (*Crocodylus niloticus*) tail meat. In our study, we found 36.39% SFA, 47.45% MUFA and 16.53% PUFA.

Compared to the study by Hoffman et al. (2000), our results showed higher content of MUFA and, respectively, lower content of PUFA.

Another interesting fact is that Isberg (2008) found a difference in the fatty acid composition of wild and farmed Nile crocodiles. A higher amount of omega-3 fatty acids was found in wild crocodiles. In our study, the amount of omega-3 fatty acids was low (0.72%). Our results are consistent with the statement of Isberg (2008), since the samples were purchased from stores and the crocodile meat originated from a licensed breeding and trading farm. According to Vicente-Neto et al. (2010), differences in fatty acid composition can be expected due to food intake, genotypic characteristics and physiological characteristics of the gastrointestinal tract. This means that differences are observed between poly- and monogastric animals. The higher amount of SFA in ruminants compared to crocodiles is due to anaerobic microorganisms found in the rumen of ruminants. These microorganisms hydrogenate omega-3 and omega-6 PUFA in the saturated stearic acid. Subsequently, it is absorbed and increases the amount of saturated fatty acids.

The PUFA/SFA ratio is an index used to assess the impact of food on cardiovascular disease. A hypothesis has been assumed that all dietary PUFAs can suppress low-density lipoprotein cholesterol (LDL-C) and lower serum cholesterol levels, while all SFAs contribute to high serum cholesterol levels. Therefore, the higher the ratio, the more positive the effect on health (Chen and Liu 2020). In our results, this index was 0.45, which meets the recommendation it to exceed 0.4 (Stratev et al. 2017).

The large amount of omega-6 PUFA and the very high values of the n-6/n-3 ratio promote the development of cardiovascular diseases, cancer, inflammation and autoimmune diseases. The ratio between omega-6 and omega-3 should not exceed 4 (Simopoulos 2008), but in our study we obtained more than 5 times higher values.

Since the PUFA/SFA ratio is not suitable for determining the atherogenicity of foods, Ulbritcht and Southgate (1991) proposed a new index called the Atherogenicity Index based on PUFA/SFA ratio. This index shows the relationship between saturated and unsaturated fatty acids. The saturated fatty acids C12:0, C14:0, and C16:0 are considered proatherogenic because they favor the attachment of lipids to the cells of the circulatory and immune systems. On the other hand, unsaturated fatty acids are antiatherogenic because they suppress plaque accumulation and reduce levels of phospholipids, cholesterol, and esterified fatty acids. Therefore, consuming foods with a low atherogenic index can reduce the amount of total cholesterol and LDL-C in human blood plasma. This index varies between 0.21 and 1.22 for fish, 0.71 and 0.82 for shrimp, and 0.27 and 1.32 for meat (Chen and Liu 2020). We calculated the atherogenic index value of frozen crocodile tail meat to be 0.47.

Ulbritcht and Southgate (1991) suggested also a Thrombogenicity Index, which characterizes the thrombogenic potential of fatty acids, indicating the propensity to form clots in blood vessels. C12:0, C14:0, and C16:0 have prothrombogenic potential, while MUFA, omega-3, and omega-6 have a pronounced antithrombogenic effect. Therefore, consuming foods with low thrombogenic index values is beneficial for the cardiovascular system. We found the thrombogenic index of frozen crocodile tail meat to be 1.05. Other studies showed that this index varied between 0.14 and 0.87 for fish, 0.21 and 0.30 for shrimp, and 0.28 and 1.69 for meat (Chen and Liu 2020).

Hypocholesterolemic/Hypercholesterolemic ratio (HH) is an index first used by Santos-Silva et al. (2002) in analysing the fatty acid composition of lamb meat. This index determines the effect of fatty acid composition on cholesterol and characterizes the relationship between hypocholesterolemic fatty acid (cis-C18:1 and PUFA) and hyper-cholesterolemic fatty acid (C12:0, C14:0, and C16:0). The HH index is more accurate in determining the effect of fatty acid composition on cardiovascular disease than the PUFA/SFA ratio (Chen and Liu 2020). We found the HH index of frozen crocodile meat to be 2.21. Other studies reported similar results for fish (0.87-4.83) and meat (1.27-2.78) (Chen and Liu 2020).

Table 3. Fatty acid composition of frozen crocodile meat

Fatty acids, %	**Mean±SEM**
Caproic acid (C6:0)	0.01±0.00
Caprylic acid (C8:0)	0.17±0.08
Capric acid (C10:0)	0.09±0.03
Undecylic acid (C11:0)	0.04±0.02
Lauric acid (C12:0)	0.14±0.08
Myristic acid (C14:0)	0.49±0.07
Myristoleic acid (C14:1n-5)	0.15±0.02
Pentadecylic acid (C15:0)	0.12±0.01
Palmitic acid (C16:0)	28.07±0.69
Margaric acid (C17:0)	0.16±0.04
Stearic acid (C18:0)	6.89±0.25
Oleic acid (C18:1n-9)	46.79±0.45
Linoleic acid (C18:2n-6)	15.38±1.02
α-Linolenic acid (C18:3n-3)	0.70±0.10
γ-Linolenic acid (C18:3n-6)	0.10±0.03
Arachidic acid (C20:0)	0.16±0.03
Eicosenoic acid (C20:1n-9)	0.27±0.04
Gadoleic acid (C20:1n-11)	0.24±0.05
Arachidonic acid (C20:4n-4)	0.32±0.10
Eicosapentaenoic acid (C20:5n-3)	0.002±0.002
Docosanoic acid (C22:0)	0.06±0.01
Docosahexaenoic acid (C22:6n-3)	0.03±0.02
SFA	36.39±0.92
UFA	63.98±1.06
MUFA	47.45±0.48
PUFA	16.53±1.23
n-3	0.72±0.11
n-6	15.70±0.86
n-9	47.06±0.46
Trans-fatty acids	0
Cholesterol	1.69±0.66
PUFA/SFA	0.45
n-6/n-3	21.67
IA	0.47
IT	1.05
HH	2.21

*SFA-Saturated fatty acids; UFA-Unsaturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids; n-3 - Omega-3 fatty acids; n-6 - Omega-6 fatty acids; n-9 - Omega-9 fatty acids

Conclusions

The results from this study clearly show that freezing at -18°C for 18 months disrupts all components in the structure of crocodile skeletal muscle tissue. The physicochemical analysis proved that frozen crocodile meat is a wholesome source of protein and fat in the human diet. Frozen crocodile meat contains more monounsaturated than polyunsaturated fatty acids. The ratio between omega-6 and omega-3 fatty acids showed 5 times higher values than recommended. More studies on the histological structure and physicochemical indicators of other members of the order Crocodilya are needed in order to gather data on the nutritional value and biological wholesomeness of the meat of these species.

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