Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1564496

JAES
Year: 9, No: 4, 2024 (616-622)
AÇEH
Yıl: 9, Sayı: 4, 2024 (616-622)

ARAŞTIRMA MAKALESİ RESEARCH PAPER

Extraction and Characterisation of Type I Collagen from the Scales of Redcoat *Sargocentron rubrum* (Forsskål, 1775)

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Received: *10.10.2024* ***Accepted:*** *28.11.2024* ***Published:*** *31.12.2024*

How to cite: **Doğdu, S.A. Turan, C. & Depci, T. (2024).** Extraction and Characterisation of Type I Collagen from the Scales of Redcoat *Sargocentron rubrum* (Forsskål, 1775). *J. Anatolian Env. and Anim. Sciences,* ***9***(4), 616-622. https://doi.org/10.35229/jaes.1564496

Atıf yapmak için: **Doğdu, S.A. Turan, C. & Depci, T. (2024).** Asker balığı *Sargocentron rubrum* (Forsskål, 1775) Pullarından Tip I Kolajen Ekstraksiyonu ve Karakterizasyonu. *Anadolu Çev. ve Hay. Dergisi*, ***9***(4), 616-622. https://doi.org/10.35229/jaes.1564496

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Abstract: Collagen, one of the most important biopolymers, is widely used in the food and pharmaceutical industries due to its functional and technological properties. Alien species, especially of Indo-Pacific origin, entering Mediterranean waters can exert pressure on native species and cause ecological and economic effects. In this study, we produced collagen from the scale of *Sargocentum rubrum* to bring this species to the economy and to reduce the pressure on our infested marine ecosystem as a surplus value. Acid-soluble collagen was extracted; a characteristic sodium dodecyl SDS-PAGE gel electrophoresis profile for type I collagen was obtained from the *S. rubrum* scales. The yield of collagen extracted from the scales of *S. rubrum* by the ASC method was calculated as 11.2%. The results of the analyses show that the collagen obtained from *S. rubrum* scales was Type I collagen with high yields. It has been proved that non-economic alien species as *S. rubrum* used in our study can be used as an alternative source instead of terrestrial animal collagen.

Keywords: Collagen extraction; *Sargocentron rubrum*; invasive fish species; type I collagen.

Asker balığı *Sargocentron rubrum* (Forsskål, 1775) Pullarından Tip I Kolajen Ekstraksiyonu ve Karakterizasyonu

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Öz: En önemli biyopolimerlerden biri olan kolajen, fonksiyonel ve teknolojik özellikleri nedeniyle gıda ve ilaç endüstrilerinde yaygın olarak kullanılmaktadır. Akdeniz sularına giren özellikle Hint-Pasifik kökenli yabancı türler, yerli türler üzerinde baskı oluşturarak ekolojik ve ekonomik etkilere neden olabilir. Bu çalışmada, bu türleri ekonomiye kazandırmak ve istila edilmiş deniz ekosistemimiz üzerindeki baskıyı azaltmak için *Sargocentum rubrum* pulundan kolajen üretimi gerçekleştirilmiştir. Kolajen asitte çözünür kolajen yöntemi ile ekstrakte edilmiş; *S. rubrum* pullarından tip I kolajen profili için karakteristik bir sodyum dodesil SDS-PAGE jel elektroforezi kullanılmıştır. ASC yöntemi ile *S. rubrum* pullarından ekstrakte edilen kolajen verimi %11.2 olarak hesaplanmıştır. Analiz sonuçları, *S. rubrum* pullarından elde edilen kolajenin yüksek verime sahip Tip I kolajen olduğunu göstermektedir. Çalışmamızda kullanılan *S. rubrum* gibi ekonomik olmayan yabancı türlerin karasal hayvan kolajeni yerine alternatif bir kaynak olarak kullanılabileceği kanıtlanmıştır. Elde edilen sonuçlar, *S. rubrum* pullarından elde edilen kolajenin biyomedikal ve diğer kozmetik endüstrileri için iyi bir alternatif kaynak olabileceğini göstermektedir.

Anahtar kelimeler: *Kolajen ekstraksiyonu, Sargocentron rubrum, istilacı balık türleri, tip I kolajen.*

INTRODUCTION

Biomaterials are distinguished by their distinctive properties, which make them ideal for use in conjunction with living tissues. Their versatility has led to an expanding role within the biomedical industry (Puad et al., 2019; Doğdu et al., 2024). Biomaterials are defined by their ability to meet specific criteria, including the following: biocompatibility, serializability, functionality, and manufacturability (Doğdu et al., 2021; Alemu Reta et al., 2024). In recent years, biomaterials have been used effectively in the treatment of damaged tissues, prolonging the life of the affected body part and tissue regeneration (Billiet et al., 2012).

Collagen is one of the most important biomaterials due to its wide range of industrial applications and is the most widely used biomaterial (Gorgieva and Kokol, 2011; Schmidt et al., 2016; Meyer, 2019; Zeng et al., 2024). Collagen is an important protein found in animal dermal tissue and in bone connective tissue. It constitutes approximately 30% of the total protein content of these tissues (Ogawa et al., 2003; Li et al., 2013; Khong et al., 2016; Stoilov et al., 2018; Lu et al., 2023). So far, researchers have identified at least 29 different forms of collagen, each characterised by its unique molecular composition and function (Shenoy et al., 2022; Ata et al., 2025). Type I collagen is the most prevalent protein in the human body, comprising approximately 90% of total protein (Chowdhury et al., 2018). It is predominantly present in the skin, bones, organ capsules, tendons, cornea, and fascia, with minimal expression in cartilaginous tissues (Naomi et al., 2021). The most prevalent designation of type I collagen is that of fibril-forming collagen; it is this form that is most widely utilised as a biomaterial in the field of tissue engineering, largely due to its abundance in biological systems (Sheehy et al., 2018). The resulting type I collagen is used in numerous sectors, including the food, cosmetics, biomedical and pharmaceutical industries (Felician et al., 2018; Cherim et al., 2019).

Originally derived from the skins of terrestrial animals such as cows and pigs, collagen has been replaced by alternative collagen sources such as fish in recent years due to its potential to transmit numerous diseases, including transmissible spongiform encephalopathy (TSE), foot-and-mouth disease (FDM) and avian influenza (Muyonga et al., 2004; Coppola et al., 2020). Marine organisms are seen as promising alternative sources of collagen due to the lack of religious restrictions and the absence of documented infectious diseases (Coppola et al., 2020). In particular, bycatch organisms obtained from fisheries activities are shown to be an important biomass, yet underutilised source of collagen (Lim et al., 2019; Rahman, 2019). By using this underutilised biomass as a source of biomaterial, it is thought that it can be one of the important methods of combating issues such as the management of invasive species as well as bringing species with no economic contribution to the economy (Doğdu et al., 2019; Doğdu et al., 2023).

The redcoat *Sargocentron rubrum* (Forsskal, 1775) is a member of the Holocentridae family. It is found in great numbers in coastal reefs and is typically found in the crevices of rocks at depths between 1 and 84 metres, forming dense aggregations (Randall, 1998; Kabaklı and Ergüden, 2022). *S. rubrum* is a species with a broad geographical distribution, occurring in the Red Sea and the western Pacific Ocean, from southern Japan to Vanuatu and New Caledonia in the southwest Pacific and extending east to New South Wales in Australia. In addition, it has been present in the Eastern Mediterranean Sea since its first record in 1947 (Haas and Steinitz 1947; Froese and Pauly, 2024). Although *S. rubrum* seems to be a successful representative of the lessepsian migration, it has not been studied other than biological studies because of its low commercial value (Taskavak and Bilecenoğlu 2001; Can et al. 2002; Türker et al. 2020; Kabaklı and Ergüden, 2022).

In this study, it was aimed to reduce the pressure on our invaded marine ecosystem by extracting and characterising collagen from *Sargocentum rubrum* scales for the first time and bringing a species with no economic value to the economy.

MATERIAL AND METHOD

Materials: A total of 58 *Sargocentron rubrum* specimens were collected from local fishermen in May 2023. The samples averaged 17.7 cm in length and weighed 118.08 g. Fish samples were stored at -21˚C until scales were obtained. After the scales were removed from the samples, they were washed with distilled water and dried in an incubator at 36˚C. A total of 50.2 g of dry fish scales were obtained from these samples.

Demineralization Process: The scales were washed on two occasions in solutions of 10 wt% NaCl to eliminate any extraneous proteins on their surface. To remove non-collagenous proteins, the prepared scales were mixed with NaOH. The mixture was stirred continuously at +4ºC for 2 days. At the end of 48 hours, pure water was added to the mixture at a ratio of 1:5 (w:v) and pH was neutralized. Then the mixture was centrifuged at 5000 rpm for 20 min and the solution was filtered and made ready for the next step. 10% butanol was added to the sample at a ratio of 1:5 (w:v) and kept at 4˚C for 24 hours. Then the solution was centrifuged at 5000 rpm for 20 min. The centrifuged solution was filtered to remove butanol and the extraction step was started (Senaratne et al., 2006; Alves et al., 2017).

Collagen Extraction: Acid-soluble collagen (ASC) method was used for collagen extraction (Nagai and Suzuki, 2000; Senaratne et al., 2006; Li et al., 2013; Alves et al., 2017). Acetic acid (CH_3COOH), an organic acid, was used for ASC extraction. The red coat scales samples were extracted in 0.5 M CH_3COOH solution for 3 days, the solution was changed at 24 hour intervals and the filtrate was collected in a separate container. After each solution change, the accumulated filtrates were combined and subjected to precipitation with NaCl with a final concentration of 2.5 M to precipitate the collagen in the extract. The precipitated collagen samples were centrifuged at 10000 rpm for 1 hour in a cooled centrifuge at +4°C. The collagen samples precipitated at the bottom of the centrifuge tubes were dissolved in 0.5 M CH_3COOH and dialysed first against 0.1 M CH_3COOH and then against distilled water. After dialysis, the collagens obtained were lyophilized.

Proximate analyses and Collagen Yield: Proximate analyses were performed according to the methods of the Association of Official Analytical Chemists (Helrich, 1990). Also, we used the following formula to determine the collagen yield, considering the dry weight of the material (Nagai and Suzuki, 2000; Senaratne et al., 2006; Li et al., 2013; Alves et al., 2017). This approach accurately determined collagen quantification and provided a reliable indicator for the extraction efficiency of collagen from scales.

$$Collagen\,Yield\;(\%) = \frac{Weight\;of\;collagen\;(g)}{Weight\;of\;dry\;scales\;(g)} \times 100$$

Amino Acid Content Analyses: The amino acid content analyses in *S. rubrum* scales collagen were determined following the procedures set forth by Antoine et al. (1999). Also, Moisture, ash, fat, and protein levels in all collagen samples were calculated. A guard column was not used for the HPLC column. A Spectroflow 980 programmable fluorescence detector equipped with a 5 µL flow cell was used, with an excitation monochromator set at 330 nm and an emission cutoff filter of 418 nm. Other detector settings include a 0.1 PMT signal, 10% zero offsets, 1.0 s response (rise time units), and 10-3 A full-scale output range.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): To evaluate the molecular weight (MW) of the protein fractions released during the extraction step, SDS-PAGE was employed for the analysis of collagen samples. The electrophoresis procedure was conducted by Laemmli's method (Laemmli, 1970), employing a stacking gel with a concentration of 4%, and a resolving gel with a concentration of 12.5%. The lyophilised sample obtained as collagen was dissolved in 1% (w/v) SDS at a concentration of 2 mg/mL and the sample buffer was 0.5 M Tris-HCl, pH 6.8, containing 4% (w/v) SDS, 20% glycerol (v/v) and 10% (v/v) beta-mercaptoethanol in a 1:4 (v/v) ratio. Subsequently, 25 mL aliquots of the aforementioned samples were subjected to a heating process at 100°C for a period of 10 minutes. This was conducted in a pool, after which they were placed on a polyacrylamide gel and subjected to vertical electrophoresis. The gel was then stained with Coomassie blue (0.05% w/v in 15% v/v methanol and 5% v/v acetic acid) for ten minutes and decolourised with a solution of 30% methanol and 10% acetic acid for a period of 12 hours.

RESULTS AND DISCUSSION

The ratios of the nutritional components of collagen extracted from the *S. rubrum* scales were found as water 6.8%, ash 0.9%, fat 0.6% and protein 91.7%. From 50.2 g dry-weight fish scales obtained from *S. rubrum* samples, 5.65 g collagen was extracted by the ASC method. The yield of the extracted collagen was calculated as 11.2%. Nagai et al. (2001) found that the collagen yield extracted from the outer skin of cuttlefish (*Sepia lycidas*) by ASC method was 2%. Kittiphattanabawon et al. (2005) reported the yield of collagen extracted from the skin and bone of *Priacanthus tayenus* by ASC method as 10.94%. Duan et al. (2009) reported the yield of collagen extracted from *Cyprinus carpio* skin, scales and bone using ASC method as 41.3%, 1.35% and 1.06%, respectively. Kaewdang et al. (2014) reported the yield of collagen extracted from the swim bladder of *Thunnus albacares* fish by ASC and PSC methods as 1.07% and 12.10%, respectively. Sionkowska et al. (2015) reported that the yield of collagen extracted from the skin of *Brama australi* fish by ASC method was 1.5%. Wahyu and Widjanarko (2018) found that the collagen yield extracted from the outer skin of milkfish (*Chanos chanos*) by the ASC method was 0.73%. Yu et al. (2018) reported the yield of collagen extracted from the skin of *Nibea japonica* by the PSC method as 84.8%. Doğdu et al. (2019) reported the collagen yield extracted from the skin of the pufferfish *Lagocephalus sceleratus* using the ASC method was 50.9%. Rodrigues et al. (2023) reported that the yield of collagen obtained from Atlantic Codfish (*Gadus morhua*) skins using the ASC method was between 2.87% and 4.80%. Ampitiya et al. (2023) reported that the collagen yield obtained from the skins of *Thunnus albacares*, *Scomberomorus commerson* and *Lates calcarifer* species by ASC method was 61.26%, 58.21% and 59.31%, respectively. The differences in collagen yield in the studies may be due to different collagen structures between species, by-products or variability of extraction procedures as parameters of extraction i.e. acid concentration, the ratio of raw materials to acid volume, extraction temperature, time (Wu et al., 2016; Pal and Suresh, 2016). When we look at the amounts of collagen obtained by acid (ASC) or enzymatic (PSC) extraction from the same species in previous studies, it is seen that the collagen extracted by enzymatic reaction is generally more efficient. In our study, 11.2% collagen yield obtained from *S. rubrum* scales is an acceptable yield value for collagen obtained from fish species and confirms that the study is an effective extraction method.

Amino acid analysis helps us to understand the quantitative composition of collagen. There are important properties that make collagen unique (Sharma et al., 2019). Type I collagen has a high content of the amino acids glycine, alanine, hydroxyproline and proline. The standard amino acid sequence of the triple helix structure is Gly-X-Y since the amino acid in every third position of the polypeptide chains forming the repeat structure is glycine, it is considered to be the main amino acid in type I collagen (Muyonga et al., 2004; Yousefi et al., 2017). In our study, the amino acid composition of collagen extracted from *S. rubrum* scales was found to consist of 35.10% glycine, 14.10% alanine, 12.90% proline, 9.80% hydroxyproline, 8.82% glutamic acid, 5.41% arginine, 3.41% aspartic acid, 3.01% lysine and 7.45% other amino acids (Figure 1). These results are close to collagen obtained from marine organisms by other researchers (Eastoe, 1957; Berillis, 2015; Sotelo et al., 2016; Nasri, 2019; Son et al., 2022). Although small differences are observed between the studies, glycine constitutes more than one-third of the structure of collagen obtained from fish. In addition, the high hydroxyproline obtained indicates increased stability of the triple helix of collagen due to hydrogen bonds between polypeptides (Sotelo et al., 2016; Alves et al., 2017; Li and Wu, 2018; Wahyu and Widjanarko, 2018; Akita et al., 2020).

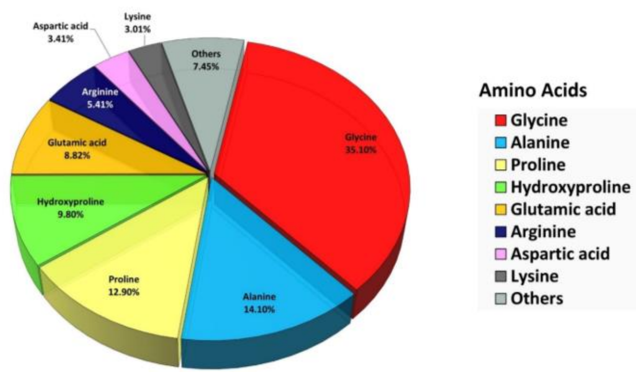

Figure 1. Amino acid composition of collagen obtained from *S. rubrum* scales.

SDS PAGE showed that collagen obtained from *S. rubrum* scales by the ASC method is composed of α 1 and α 2 chains and their dimers (β chains) (Figure 2). The α components showed two different types, varying in their mobility for both reducing and non-reducing conditions. Therefore, it can be concluded that the collagen obtained is composed of at least two α (α1 and α2). Also, the collagen exhibited high molecular weight components, specifically β components, as well as trace amounts of γ component. When we look at the molecular weight marker and Sigma Type-I collagen markers, it shows that the obtained collagen has molecular weights ranging from 116 to 200 kDa for the α 1 and α 2 chains. This is an indication that the collagen obtained is type I. This is similar to the pattern observed for some other fish species (Nagai et al., 2001, Gòmez-Guillèn et al., 2002) and is typical for type I collagen (Light and Bailey, 1985).

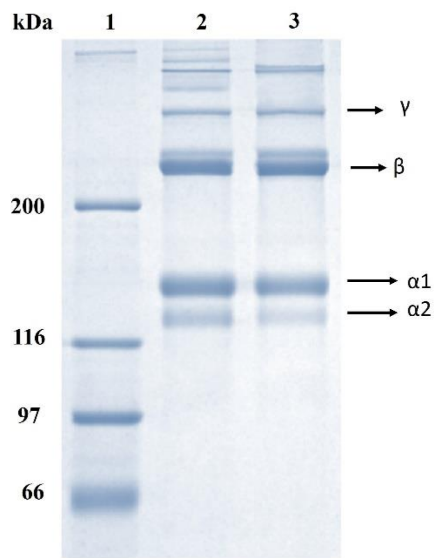

Figure 2. SDS-PAGE gel electrophoresis of *S. rubrum* scales collagen (1: Molecular weight marker, 2: Sigma Type-I collagen, 3: collagen obtained from *S. rubrum* scales).

CONCLUSION

In conclusion, collagen was extracted from *Sargocentron rubrum* species for the first time in the literature and characterised. As a result of the analyses, it was determined that the collagen obtained was Type I and had an acceptable yield of 11.2%. It has been proved that non-economic alien species such as *S. rubrum* used in our study can be used as an alternative source instead of terrestrial animal collagen used for industrial purposes. It was revealed that the wastes such as the scales or skin of alien and non-economically fish species. In this way, many alien species, which are considered invasive species in our country and the Mediterranean Sea, can be brought into the economy.

ACKNOWLEDGEMENTS

This study was supported by the İskenderun Technical University Scientific Research Projects Office

(2022YP05). The authors thank the İskenderun Technical University Scientific Research Projects Office.

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