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Sterol and Mineral Profiles of the Common Sea Snail *Hinia reticulata* and the Long Sea Snail *Nassarius mutabilis* (Gastropods) Collected from the Middle Adriatic Sea

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Abstract

Sea snails represent a common food in the world as a source of sterols, such as cholesterol and phytosterol, and minerals. Sterols play important roles in body functions and also minerals are important for human health, so the intake of these nutrients into human diets should be known. The aim of this study was to examine the sterol and mineral profiles of the long (*Hinia reticulata*) and the common (*Nassarius mutabilis*) sea snails. Samples of both species were collected at different catch times from November 2019 to March 2020 and transported to the University of Camerino (UNICAM) for the evaluation of their sterol and mineral profiles, including toxic elements (Cd, Cr, Pb). The results of the study showed that the average content of total lipid were 57 mg/100 g, 38 mg/100 g for cholesterol and 19 mg/100 g for phytosterol in the long sea snail, and the values were respectively 68, 48, and 20 mg/100 g in the common sea snail, without significant differences in the two examined sea snails. Additionally, the result of the mineral analysis showed that both species were significant sources of minerals, with negligible levels of toxic metals and metalloids. Therefore, the Long and the Common sea snail are suitable and safety sea products for human nutrition.

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Introduction

Snails are an essential part of dishes across the world because of their palatability and nutritional content. In European countries such as Italy, Portugal, Spain, and Turkey, snails, along with other shellfish such as mollusc and oyster, are a major constituent of seafood diets. In Italy in particular, they attract more attention for their use in traditional gastronomic dishes, in which sea snail meat is used as the main ingredient, and its organoleptic characteristics are stood out. In 2011, the estimated annual consumption in Italy was 306 million snails[1] and the number has increased considerably in recent years; according to a 2017 Coldiretti (National Federation of Direct Farmers) survey, the consumption of snails by Italians has increased by 325% in the last 20 years.

For a long time, snails were considered to be a traditional dish in Mediterranean cuisine; however, the growing standard of living and the popularity of European cuisine in Asian countries have made sea snails a famous meat in the Asiatic continent, thus increasing its global demand. Similarly, snail meat is consumed in African countries, especially in West Africa, where they are included in the staple diets.

Snails are univalves, and belong to the class Gastropoda of the phylum Mollusca. The phylum Mollusca is one of the largest phyla of animals, it includes more than 80 000 species, most of them aquatic, and very vary in size, from giant squids and clams to little snails. Inside the phylum Mollusca the class Gastropoda is divided into three groups: univalves, which includes sea snails, that has got only one single shell; bivalvia, which includes mussels and oysters, characterized by two shells connected by a flexible ligament; and cephalopoda, which includes octopus and squid, characterized by a set of arms or tentacles. A considerable amount of literature has been published on the nutritional composition of some molluscs such as oysters[2 - 3] and mussels[4 - 5]; however, studies on the nutritional composition of sea snails are limited.

The general knowledge concerning sea snails as food shows that snail meat has a high protein content, a low fat and carbohydrate content, and is an important source of minerals and vitamins, such as sodium, calcium, potassium, phosphorus, and vitamin E.[1 – 6 – 7 – 8] Additionally, similar to shell fishes such as mussels[3 - 4] and oyster,[2 - 3] snail meat is a good source of polyunsaturated fatty acid (PUFA),[6] which is an important nutritional element. Therefore, snail meat makes an excellent high-protein low-fat diet. Among snail species, the long sea snail (*Hinia reticulata*) and the common sea snail (*Nassarius mutabilis*) have been used as food in Europe, particularly in Italy. A recent study showed that the long sea snail and the common sea snail could be considered a valuable source of nutrients such as protein and fatty acids.[8]

In Italy, sea snails are a common product caught from the Adriatic sea. A search detected at the port of San Benedetto del Tronto estimated the annual catches of sea snails caught by the fishermen in Center Italy, showing that the catch of the common sea snail were about kg 780 ± 210 / year and the catch of the long sea snail were about kg 705 ± 190 / year.[8]

Snails are also in high demand because of their beneficial sterol profile. Sterols such as cholesterol play an important role in human nutrition, such as in the maintenance of cell structure, in the synthesis of steroidal hormones, and in the production of vitamin D; it is therefore necessary that sterols are included in the diet of humans at appropriate levels.[3 - 9] According to the World Health Organization (WHO 2003), the maximum recommended intake of cholesterol is 300 mg/day.[6] Shellfish are low in saturated and trans fats, and are a good source of healthy polyunsaturated fats.[3 - 10] Some shellfish also contain significant amounts of other non-cholesterol sterols that can decrease the absorption of cholesterol.[3 - 10] Apart from zoosterols such as cholesterol, sea snails are also rich in phytosterols, of which the main ones are sitosterol, stigmasterol, and campesterol. This is because sea snails feed mainly on algae and vegetables, which are rich in these phytosterols. Phytosterols are precious elements contained in foods of plant origin, and play an important role in reducing the LDL-cholesterol level in human blood,[11] and reducing the risk of coronary heart disease.[6] According to some research, they may also promote the health of the prostate and urinary system, and in general, prevent the development of cancer.[12 - 13] The main source of phytosterols are vegetable oils and dried fruit, mushrooms, and algae, which represent the main food for sea snails, thus improving their phytosterol profile. Additionally, as a result of their nutrition and environment, sea snails may also serve as a mineral source; however, their mineral content is dependent on their environment. As aquatic animals, apart from algae and vegetables, snails also feed on dead animals, and their health and the chemical characteristics of their meat directly reflect the quality of the aquatic environment in which they live. The accumulation of nitrogenous catabolites and heavy metals have been recorded in the tissues of sea snails living in polluted seas and poor-quality waters.[14 – 15 - 16]

Previous studies on the long sea snail and the common sea snail have been focused on their PUFA profiles and amino acid content. Therefore, to enrich the knowledge of the two species, the present study aimed to examine the sterol profile (total lipid, cholesterol, and phytosterol) and the mineral profile (iron, calcium, zinc, magnesium, and potassium) of the long sea snail and the common sea snail collected from the Adriatic Sea. Additionally, the level of toxic metals and metalloids in the two studied snails was also investigated in order to ascertain the consumer safety eating their meat. For this purpose, the level of cadmium, chromium and lead were evaluated in the common and in the long sea snail.

Materials and Methods

Samples and Sampling Areas

Samples of the two species of sea snails, *H. reticulata* and *N. mutabilis*, were collected at different catching times from November 2019 to March 2020 from the middle Adriatic Italian Sea, and processed according to the procedure of Felici *et al*. (2020).[8] At each time of catching, 50 specimens for each specie were sampled and transported in an icepack to the Chemistry and biochemistry laboratory of the School of Biosciences and Veterinary Medicine at the University of Camerino (UNICAM) for the determination of total weight (meat + shell) and the separated fractions of meat and shell, after having extracted the edible part. From the edible fractions, pools of each group were homogenised and subjected to analysis. To measure the weight, an electronic scale (mod. CP224S Sartorius, Gottigen, Germany) was used.[8] The moisture content of the snails was determined following the procedure of the Association of Official Analytical Chemists (AOAC 1990).[17] The edible portion was used for the sterol profile and mineral profile analyses and for the heavy metals and metalloids evaluation.

Sterol Profile Determination

Determination of the sterol profile was performed according to the method of Felici *et al*. (2020).[8] The lipid extract of the samples was used for the determination of the sterol profile after a saponification process. Total lipid content was determined using the modification of the chloroform:methanol procedure described by Folch *et al*. (1957).[18]

In the Chemistry laboratory of the University of Camerino (UNICAM), the total sterol content was determined gravimetrically and the individual sterols were determined after derivatisation in trimethyl-silyl derivatives using Agilent 6890 N gas chromatographer (Agilent Palo Alto, CA, USA) equipped with a db5 (60 m x 0.25 mm) with helium (1 ml / min) as carrier gas at a constant flow of 1.0 mL/min. The temperature of the gas chromatographer was 170°C for 15 min at the beginning, then it increased by 1°C/min until reaching the temperature of 190°C, to increase again by 5°C/min until 220°C; finally, it remained at the same temperature for 17 min.[8]

Determination of Mineral Profile, Heavy Metals and Metalloids In the Meat of the two Sea Snail Species

Following the procedure reported by Felici *et al*. (2020),[8] the quantity of iron, calcium, zinc, magnesium and potassium in the two snail species was determined using Agilent Agilent inductively coupled plasma with mass spectrometer (ICP-MS) 7800 model (Agilent, Palo Alto, CA, USA), an inductively coupled plasma with mass spectrometer (ICP-MS) system. With the same procedure the quantity of heavy metals and metalloids (cadmium, chromium and lead) were investigated.

The calibration curve profiles were obtained using standard element solutions obtained by diluting the mother solution in 3% HNO3 + 0.05% HCl with yttrium, scandium, terbium, and bismuth as internal standards.[8]

Statistical Analysis

Data obtained from the study, including data of the mineral content of the samples were subjected to one-way analysis of variance (ANOVA) using SPSS 25 (Version 25.0, Armonk, NY, USA) and significant means were compared using Student–Newman–Keuls (SNK) test of the same software. Means were considered significant at $p < 0.01$.

Results

Results of the total lipid, cholesterol, and phytosterol contents of the sea snails are presented in Fig. 1. The total lipid content of the long sea snail ranged from 47 to 67 mg/100 g (mean: 57 ± 10 mg/100 g), whereas that of the common sea snail ranged from 58 to 78 mg/100 g (mean: 68 ± 8 mg/100 g). No significant difference was recorded in the values of total lipids between the two species. In addition, the cholesterol and phytosterol contents of the sea snails were evaluated. The result showed that the cholesterol contents of the long sea snail ranged from 32 to 44 mg/100 g (mean: 38 ± 6 mg/100 g) and the phytosterol content from 14 to 24 mg/100 g (mean: 19 ± 5 mg/100 g), whereas the values for the common sea snail ranged, respectively for cholesterol and phytosterol, from 43 to 53 mg/100 g (mean: 48 ± 5 mg/100) and from 16 to 24 mg/100 g (mean: 20 ± 4 mg/100 g). No significant difference was recorded between the two species.

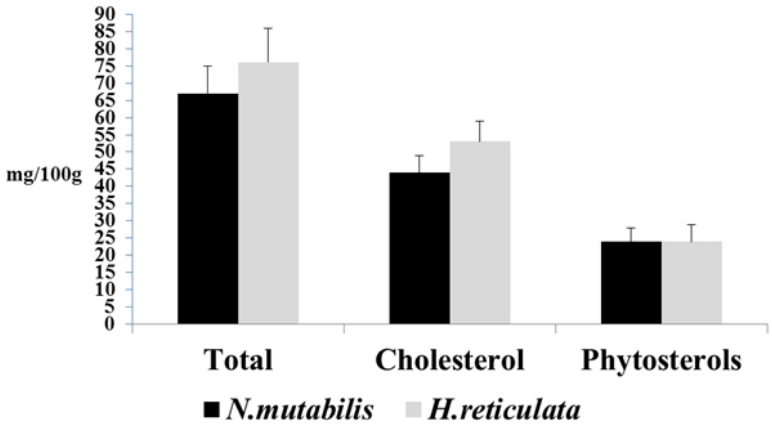

Fig. 1: Total lipid, cholesterol and phytosterols of *Nassarius mutabilis (N. mutabilis)* and *Hinia reticulata (H. reticulate)* expressed in mg/100 g. The black blocks refer to the long sea snail *(N. mutabilis)*, while the grey blocks refer to the common sea snail *(H. reticulata)*. The blue lines mark the minimum registered quantity. Standard deviations are also reported on the top of the blocks

Table 1: The Fe, Ca, Zn, Mg, and K contents of the two sea snail species *Hinia reticulata* and *Nassarius mutabilis* expressed as mean values and the standard deviations.

		Common sea snail (Hinia reticulata)	Long sea snail (Nassarius mutabilis)
Fe	mg/100 g w/w	3.15 ± 1.1	4.40 ± 0.4
Ca	mg/100 g w/w	31.50 ± 1.5	32.50 ± 1.5
Zn	mg/100 g w/w	1.70 ± 0.2	1.45 ± 0.2
Mg	mg/100 g w/w	51.08 ± 2.8	54.00 ± 1.3
K	mg/100 g w/w	293.50 ± 2.5	296.44 ± 2.30

The results of the mineral contents of snail samples are presented as means and standard deviations (Table 1). The elements investigated were iron (Fe), calcium (Ca), zinc (Zn), magnesium (Mg), and potassium (K). In the common sea snail the mean content obtained was 3.15 ± 1.1 mg/100 g for Fe, 31.50 ± 1.5 mg/100 g for Ca, 1.70 ± 0.2 mg/100 g for Zn, 51.08 ± 2.8 mg/100 g for Mg, and 293.50 ± 2.5 mg/100 g for K. Whereas the contents in the long sea snail were 4.40 ± 0.4 mg/100 g for Fe, 32.50 ± 1.5 mg/100 g for Ca, 1.45 ± 0.2 mg/100 g for Zn, 54.00 ± 1.3 mg/100 g for Mg, and 296.44 ± 2.30 mg/100 g for K.

Concerning the amount of heavy metals and metalloids, the long sea snail showed the maximum registered concentration of 0.031 mg/100g for cadmium (Cd), 0.028 mg/100g for chromium (Cr) and 0.028 mg/100g for lead (Pb). For the common sea snail the maximum registered values were respectively 0.045 mg/100g, 0.041 mg/100g and 0.033 mg/100g.

Discussion

This study confirmed that sea snail can be included into human diet owing to the organoleptic and nutritional characteristics of the meat. As reported in previous studies, snail meat is rich in polyunsaturated fatty acids (PUFA),[21] essential amino acids, and minerals.[1 – 7 - 8 - 10] Our results showed that the both evaluated sea snail are valuable food.

Total Lipid Profile

Previous studies on snails focused mainly on their zoosterol and phytosterol composition,[22] fatty acid composition,[1 – 7 - 8] and cholesterol content.[10] To the best of our knowledge, this study is the first to evaluate the total lipid content of the long sea snail and the common sea snail; the lipid content of both species were similar and were generally low. Comparing our results for sea snails with those obtained for land snails,[1] it can be seen that sea snails contain lower lipid content compared to land snails. In comparison with other shellfish such as mussels[4 - 5] and oysters,[2] sea snails contain a relatively lower quantity of lipids.

Cholesterol

In the current study, cholesterol was the major sterol present in the two sea snail species studied. Studies have shown that cholesterol is the major sterol present in shellfish such as sea snails; however, other sterols such as stigmasterol and desmosterol are also present in limited quantities,[13]

Phytosterols

To the best of our knowledge, this study is the first to report the phytosterol profile of the common sea snail and the long sea snail. Similar to results in mussels[4] and in several mollusc species,[23] our results showed that the two studied sea snails had around 50% phytosterols in their sterol-pool. On the contrary, in land snail species, phytosterol content is lower: 6.3 mg/100 g in the northwest hesperian (*Vespericola columbiana*), 6.9 mg/100 g in Helix sp., and 3.8 mg/100 g in *Haplotrema sportella*,[22]

Owing to the importance of fishes in human nutrition and health, the sterol profiles of fishes have been studied extensively. Ozyurt *et al*. (2012) studied the sterol profiles of some Mediterranean fishes caught in different seasons,[6] Comparing the result of the present study to results obtained in fishes, the mean content of cholesterol in the two species of evaluated sea snails was similar to the lowest phytosterol content recorded in fishes; the phytosterol content ranged from 15.00 to 36.54 mg/100 g in seabream (*S. aurata*), 27.66 to 48.07 mg/100 g in mackerel (*T. trachurus*), and 14.37 to 28.65 mg/100 g in common sole (*S. solea*),[6]

Mineral Profile

The principal aim of this study was to investigate the sterol profile of the common sea snail and the long sea snail present in the Adriatic Sea. Moreover, the mineral profile analyses of the sea snails were necessary to verify the reports of Felici *et al*. (2020) 8, and the results obtained in the present study were in agreement with their findings.

A study on the mineral content of the Mediterranean marine snail, H. trunculus, was carried out by Zarai *et al* (2011),[21] and the most abundant electrolytes were potassium (224.80 ± 55.10 mg/100 g), sodium (196.10±30.00 mg/100 g) and magnesium (178.70 ± 54.30 mg/100 g). A comparison with the data obtained by Zarai *et al* (2011)[21] showed that the Fe, Ca, Zn, and Mg contents of the Mediterranean snail were higher than the values obtained in the long sea snail and the common sea snail in the present study; however, K was higher in the present study. On the contrary, the result of a study on *T. mitilis, L. torquata,* and *L. undulata* showed a different mineral concentration from what was recorded in the present study.[14] In addition, the mineral profile of the edible garden snail[1] and Pomacea canaliculate[7] were different from what was obtained in the present study; Pomacea canaliculate had a higher P content compared to that of the long and the common sea snails.

The results of the present study showed that the long sea snail and the common sea snail are sources of important minerals such as iron, zinc, magnesium, and calcium, and their inclusion into healthy human diet should be considered. In particular, the sea snails are an excellent source of potassium, which possesses several biological functions. Potassium contributes to the health of the heart and vessels, bone, and kidney;[24] and is involved in enzyme activities, protein synthesis, and insulin secretion.[25]

Toxic Metals and Metalloids

Sea water is constantly polluted by contaminants from industrial activities, such as micro-plastics and heavy metals. These pollutants are usually deposited in the meat of aquatic organisms. Residues of heavy metals were found in fish and sea food products in Bosnia and Herzegovina.[26] In the Black, Marmara, Aegean, and Mediterranean Seas, cadmium, arsenic, lead, and mercury were found in selected fish species and marine animals.[27] Therefore, for public health reasons and for the safety of sea snail food, it is important to investigate the presence and the possible accumulation of these toxic substances in the meat of sea snails.[8 – 19- 28]

In this study, results of the heavy metal and metalloid content in the two analysed sea snails showed that the content of toxic metals were below the safe recommended standards for human consumption.[19 - 20]

Conclusions

The sterol, mineral and toxic metals content in the soft tissue of the two marine snail species, *Hinia reticulata* and *Nassarius mutabilis*, were investigated. Both species of Gastropods were found to be a good source of nutrients and they resulted healthy and safety foods.

As reported in previous studies on shellfish, the organoleptic properties and chemical composition of sea snails in the Adriatic sea differed depending on the location and season; Different seasons influence the water temperature and the metabolism of aquatic animals, which may alter their composition. Differences based on different sea areas or different season were not assessed in the present study.

There are conflicting information on the mineral contents of sea snails, therefore, there is a need for detailed studies, particularly on the quality and characteristics of sea snail meat.

Moreover, studies on the metabolic processes and energy requirement of shellfish and their effect on the mineral profile and sterol content should be carried out. As shown in mussels,[6] seasonal differences in the quality of sea snail meat may be linked to sea water conditions and temperature. However, seasonal differences in phytosterol concentrations in the two sea snails (*H. reticulata* and *N. mutabilis*) studied were not assessed, and is therefore recommended for future studies.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

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