Bulgarian Chemical Communications, Volume 56, Special Issue D2 (pp. 66-70) 2024 DOI: 10.34049/bcc.56.D.S2P42

Chamelea gallina from the Black Sea (Bulgaria) as a sustainable source of macronutrients and fat-soluble vitamins

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Received: November 3, 2023; Revised: April 11, 2024

Marine bivalves are characterized as highly nutritional, easily digestible food, low in calories but high in proteins. Seafood nutrition data is useful for assessing the contribution to nutrient intake and for to the development of dietary guidelines. The aim of the present study is to determine the chemical composition: crude proteins, total lipids, carbohydrates, energy value and fat-soluble vitamins in white sand clam (*Chamelea gallina*). The samples were harvested from the Bulgarian coast of the Black Sea in the summer and autumn periods. Crude protein, carbohydrates and total lipids were determined using standard procedures. The fat-soluble vitamins were analysed simultaneously using high-performance liquid chromatography with ultraviolet and fluorescence detectors (HPLC-UV/FL). Analysed samples were characterised by high protein (16.4±0.2 to 18.2±0.1 g.100g^{-1} ww) and low lipid content (2.26±0.06 to 2.67±0.13 g.100 g^{-1} ww). Lipid levels showed greater variations compared to proteins. Carbohydrates varied between 1.72 and 2.85 g.100g^{-1} ww and the energy values – between 99.3 and 101.3 kcal.100g^{-1} ww. Higher amounts of all-trans retinol, cholecalciferol and vitamin K were found in the autumn samples, whereas α-tocopherol content decreased from summer to autumn. The present study reveals new data on the chemical composition of *C. gallina* harvested from the Bulgarian Black Sea coast. Despite the variations in their composition, *C. gallina* clams could be a healthy choice of low energy dense food due to high protein and fat-soluble vitamin levels and low lipid, carbohydrate and calorie contents.

Key words: *Chamelea gallina***,** macronutrients, fat-soluble vitamins, Black Sea

INTRODUCTION

One of the most important resources of basic and essential nutrients for human health are marine bivalves. According to FAO [1] the seafood is easily digestible, a valuable source of dietary proteins, polyunsaturated fatty acids, fat-soluble vitamins and several biologically active components. Data on the nutritional composition of native marine species are useful for assessing their contribution to nutrient intake and for developing national dietary guidelines. Since 2012 striped venus clam *Chamelea gallina* is commercially exploited in the Bulgarian Black Sea part, maximum catches reaching 506 tonnes in 2019 [2]. It is well known that in bivalve organisms the proteins have a structural role, whereas lipids act as basic energy sources for sustaining mussel growth and development. Essential elements such as K, Na, Ca and Mg are crucial for vital physiological functions in organisms. Insufficient intake of macronutrients and essential elements can affect human health and lead to the development of different non-chronic diseases [3]. The high content of fat-soluble vitamins, especially vitamin D_3, is a specific characteristic of marine lipids and significantly increases their quality from nutritional point of view. Moreover, the differences in proximate composition and fat-soluble vitamins content in bivalve tissues may be important not only for its optimal survival and reproductions, but for its nutritional quality. Thus, the evaluation of the chemical composition, macroelements and fat-soluble vitamins content may increase consumer’s interest and predict the market feasibility of the white clam *Ch. gallina* in Bulgaria. Limited information on *Ch. gallina* nutritional quality characterized through proximate composition, macroelements (Na, K, Ca and Mg) and fat-soluble vitamins (A, E, D and K) content was found in the scientific literature. Moreover, studies of seasonal changes in the macronutrient contents of the white sand clam (*Ch. Gallina)* from the Bulgarian part of the Black Sea are lacking. The aim of the present study is to determine the chemical composition: crude proteins, total lipids, carbohydrates, energy value, macroelements and fat-soluble vitamins in white sand clam (*Ch. gallina*).

MATERIALS AND METHODS

Sample collection and preparation

Samples of white sand clam *Ch. gallina* were collected from the Northern part of Bulgarian Black Sea coast and were harvested in June and October 2021. For each specimen, the main biometric parameters were measured individually. One

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kilogram of white clam was brought to the laboratory. Only clams of medium size were taken for analyses. The specimens were washed, shucked and placed on a filter paper to absorb the excess moisture and then the flesh was removed.

Proximate composition analysis

The homogenized clam tissue (2.000±0.005g) was dried at 105±2°C in an air oven for 18-20 hours to a constant weight [4]. The crude protein content was determined by Kjeldahl's method [5]. The total lipids (TL) were estimated according to the method of Bligh & Dyer [6]. The carbohydrates were determined according to BDS 13488:1976 [7]. The energy values based on fat, protein and carbohydrate contents were calculated according to WHO/FAO [8] Atwater specific coefficients (4.0 kcal.g^{-1} for proteins and carbohydrates, 9.0 kcal.g^{-1} for lipids).

Macroelements analysis

1 g wet weight (ww) of homogenized *Ch. gallina* tissue was mixed with 8 cm^3 HNO_3 (65% w/v) and 2 cm^3 H_2O_2 (30 %w/v), placed in Teflon vessels and digested in a microwave closed-vessel digestion system MARS 6 (CEM Corporation, USA). A 3-stage program was used according to the procedure given by Peycheva *et al*. [9]. The concentrations of Na, K, Ca and Mg in the samples were determined using ICP-OES spectrometer (Optima 8000, Perkin Elmer, USA).

Fat-soluble vitamin analysis

The astaxanthin, β-carotene and cholesterol contents in the edible clam tissue were evaluated. An aliquot of the homogenized sample (1.000±0.005 g) was weighed and 0.3M methanolic KOH was added. Six parallel samples were prepared and subjected to saponification at 50°C for 30 min. The fat-soluble components of interest were extracted with two portions of n-hexane: dichloromethane = 2:1 solution. The combined extracts were evaporated and the dry residue was dissolved in methanol: dichloromethane and injected (20 µl) into the HPLC/UV/FL system. All fat-soluble compounds were analysed simultaneously by an HPLC system, equipped with an RP analytical column (Synergi Hydro-RP (80 Å, 250×46 mm; 4 µm). The results were expressed as µg per 100 g wet weight (µg.100 g^{-1}ww).

Statistical analysis

Analyses for the chemical composition, macroelements and fat-soluble vitamins were performed in six replicates and the results were expressed as mean values ± standard deviation (SD). Mean values were compared by one-way ANOVA followed by a post-hoc Tukey's test. Statistical significance was considered at $p \leq 0.05$ (Graph Pad Prism 6).

RESULTS AND DISCUSSION

Proximate composition

The specific abiotic factors across the water basins significantly affect the proximate composition of bivalve species. According to FAO [1,8] the macronutrients proportions are species-specific and usually greater intra- and interspecies variations in chemical composition are observed. In this study considerable differences in carbohydrate and protein content in sand clam *Ch. gallina* between seasons were found. The seasonal changes of proximate composition of *Ch. gallina* tissues are presented in Table 1. It is well known that bivalves contain higher protein levels compared to fish species. Venogupal and Gopakumar [10] reported that average protein content in clams from the Indian Ocean varied between 9-14.5 g.100 g^{-1}ww. Significant difference in protein content was reported for *Ch. gallina* from Marmara Sea: 7.28 - 7.70 g.100g^{-1} ww [11], from Azov Sea:15 g.100g^{-1} ww [12] and Adriatic Sea: 10.40 g.100g^{-1} ww [13]. Protein content of the Black Sea *Ch. gallina* was significantly higher (up to 18 g.100g^{-1} ww) compared to published results. Additionally, marine proteins are classified as rich of essential amino acids and good digestibility, which increase their nutritional quality. According to FAO [8] and Regulation (EC) No 1924/2006 [14] seafood which contains protein higher than 15 g.100g^{-1} ww can be classified as "protein-rich" source and insignificant seasonal variation in protein levels described *Ch. gallina* as a sustainable natural source of proteins.

Table 1. Seasonal variations in proximate composition (g.100 g^{-1} ww) and energy values (kcal.100 g^{-1} ww) of edible tissues of *Ch. gallina* from Black Sea coast

Compound / Month	Lipids	Proteins	Carbohydrates	Moisture	Energy values
June	2.54 ±0.22	17.30±0.54	2.40±0.25	75.70±1.50	101.62±3.50
July	2.67±0.30	16.40±0.37	2.80±0.50	74.80±0.95	100.83±3.15
October	2.26^{a,b}±0.25	18.10 ^{a,b}±0.95	1.85^{a,b}±0.20	76.10±2.05	99.82 ^{a,b}±2.80

Values with ^{a,b} are significantly different ($p < 0.05$) ^{a} – *June vs. October;* ^{b}- *July vs. October*

Total lipid (TL) content showed an opposite pattern compared to proteins, with the highest values in summer (July - 2.67g.100g^{-1} ww) and lowest in October (2.26 g.100g^{-1} ww). One possible reason is the accumulation of energy reserve prior to gametogenesis (June-July) in this clam species. Generally, no significant variation was observed for TL values in *Ch. Gallina.* The average TL content during the analysed months was below 3 g.100g^{-1} ww, thus white clam can be classified as "low lipid" species [14]. Significantly lower amounts for TL content were reported for Marmara Sea striped venus clam – 0.59 (June) to 1.06 g.100g^{-1} ww (October) (Özden *et al*., 2009) [11], for Azov Sea clam – 0.9 g.100g^{-1} ww (Bityutskaya *et al*., 2021) [12], whereas Adriatic Sea white clam showed a similar pattern- decrease of TL in summer: 1.55 g.100g^{-1} ww (June) to two-fold decrease in autumn: 0.73 g.100g^{-1} ww (September) (Orban *et al*., 2007) [13].

According to Venogupal and Gopakumar [10] the carbohydrate content in shellfish is low. In the present study, a seasonal pattern in the carbohydrate contents is observed and decrease in autumn period (up to 1.84 g.100g^{-1} ww), compared to summer months was found. One possible explanation is that the carbohydrate levels (as energy reserves) can vary- utilise or accumulate in response to changes in environmental conditions [13]. Similar seasonal decrease of carbohydrate content in autumn samples was reported for Adriatic Sea *Ch. gallina* [13], whereas Özden *et al.* [11] showed significantly higher levels for carbohydrates in the range of 4.44 (June) to 3.40 g.100g^{-1} ww (October). Bityutskaya *et al.*, [12] reported a higher level of carbohydrates in samples collected in Azov Sea (3.4 g.100g^{-1} ww). There is no comparable data for proximate composition of white clam *Ch. Gallina* from Bulgarian Black Sea coast in the scientific literature. The analysed venus clam demonstrated constant seasonal levels of the energy values which depend on primary metabolites content and showed a minor change mainly related to a decrease in lipid and carbohydrate amounts. The calculated energy levels of *Ch. gallina* edible tissues are in the range of 99.82 – 101.83 kcal.100g^{-1} ww. Based on the results obtained throughout the study period, the clam presented a low energy content. Significantly lower energy values are reported for Marmara Sea *Ch. gallina* – average 66.5 kcal.100^{-1} g ww [11] and for Azov Sea white clam – 58 kcal.100^{-1} g ww [12]. This difference is due to the significantly lower amounts of total lipids and proteins found for this species.

Macroelement contents

Macroelements play an important role in various metabolic processes. Their deficiency can lead to a number of chronic diseases; therefore, it is necessary to maintain an adequate intake through a balanced diet rich in these elements. Potassium is a very important, osmotically active inside cell element, acting together whit sodium in regulation of body's water balance. The recommended from EFSA sodium RDI is safe and can reduce risks of CVD development in adult population. In addition, magnesium and calcium can prevent CVD, osteoporosis and certain cancer forms. Bivalve species usually accumulate the physiologically active macroelements in their edible tissues [11,13]. This statement is well illustrated in the present study since the analyzed clam species is a good source of sodium and potassium, especially in the autumn months. The seasonal changes of macroelements content of *Ch. gallina* edible tissues are presented in Table 2. Significant seasonal differences (p<0.05) in macroelement contents were observed. The highest concentration of all analysed elements were found in autumn months. It is well known that sodium is naturally present in foods of animal origin such as white clams. This statement is confirmed by the obtained results, where, sodium has the highest levels (over 4000 mg.kg^{-1} ww), among all elements while magnesium has the lowest concentration. K, Ca and Mg have stable levels during the summer months, while the largest fluctuations (p<0.05) are determined for sodium.

Table 2. Seasonal variations in macroelements content (mg.kg^{-1} ww) of edible tissues of *Ch. gallina* from Black Sea coast

	K	Na	Ca	Mg
June	1605.20±180.16	2968.70±136.6	786.4±178.8	573.5±25.0
July	1527.30±112.47	1988.40±115.63	749.5±108.42	546.72±13.77
October	2171.5±229.40^{a,b}	4037.20±281.95^{a,b}	1759.40±190.50^{a,b}	958.5±85.50^{a,b}

Values with ^{a,b} are significantly different (p < 0.05) ^{a} *– June vs. October;* ^{b} *- July vs. October*

Table 3. Seasonal variations in fat-soluble vitamin content (μg.100^{-1}g ww) of edible tissues of *Ch. gallina* from Black Sea coast

	Vit A	Vit E	Vit D_3	Vit K
June	35.60±5.52	7673.00±350.50	40.10±5.20	52.00±6.50
July	40.50±8.35	8200.00±410.10	39.65±3.45	55.84±5.30
October	38.60±10.15	6377.00±280.24^{b}	41.70±6.10^{b}	56.30±5.27^{a}

It can be summarised that the macroelements show the following pattern of distribution during the study period: Na> K> Ca> Mg. The EFSA has developed the recommended daily intake (RDI) for these macroelements: for potassium 3500 mg.d^{-1} (2016)[15]; for sodium 2000 mg.d^{-1} (2019)[16]; for magnesium (2015)[17] 350 mg.d^{-1} and for calcium (2015)[18] 750 mg.d^{-1} for men and 700 mg.d^{-1} for women. The presented results show that a 100 g portion of the white clam edible tissues (autumn samples) can provide over 200% of the RDI for Na, Ca and Mg, while up to 62% of RDI for K. Significantly higher contents for all analysed macroelements were reported for Marmara Sea *Ch. gallina* [11], for Adriatic Sea [13] and Azov Sea [12] white clams. As filter-feeding species bivalves can intake macroelements through seawater and food. Observed differences compared to presented results may be due to specific abiotic environmental factors as salinity, temperature, available foods and other.

Fat – soluble vitamins

Fat-soluble vitamins are essential metabolites for the optimal homeostasis, reproductive cycle and growth of bivalves. Mussels are among marine animal species that are characterized as good sources of vitamins. In their edible tissues, these substances significantly vary depending on the season of catch, the size of the individuals, the reproductive period, the nutrients received through their food intake, *etc*. In this study fat soluble vitamins are expressed as an average and standard deviation (mean ±SD) and the results are shown as microgram per 100 grams wet weight (μg.100^{-1}g ww) in Table 3.

The average content of the three analysed vitamins over the studied months for white clam species showed insignificant seasonal changes in the levels of vitamins A, D_3 and K.

The analysed fat-soluble component with high antioxidant activity is vitamin E (α-tocopherol). Among studied vitamins α-tocopherol was found in highest amount – average 7417 μg.100^{-1}g ww, with peak determined in July (8200 μg.100^{-1}g ww, $p<0.05$). According to EFSA [19] and Ordinance No. 1/22.01.2018 of the Ministry of Health [20] the established amounts in 100 g of edible tissue of white clam *Ch. gallina* provide significantly good amounts of vitamin E compared to the recommended daily intake (RDI) - average 57% of RDI.

In this study vitamin A was detected in lowest values - average 38.20 μg.100^{-1}g ww, which confirms that the analysed species is not a good source of this vitamin. The RDI recommendations [19] for vitamin A are between 490 μg.d^{-1}g (for women) and 570 μg.d^{-1}g (for men). Based on the average amounts determined, consumption of 100 g portion in this species provides up to 6-8% of the RDI for vitamin A. Venogupal and Gopakumar [10] reported higher values for vitamin A in the mussel species (54 μg.100^{-1}g ww), but significantly lower amount for vitamin E (750 μg.100^{-1}g ww) compared to our results. MacDonald [21] reported similar results for vitamin A (38.7 μg.100^{-1}g ww) and lower for vitamin E (790 μg.100^{-1}g ww) for the New Zealand green-lipped mussel tissues. There is limited information on *Ch. gallina* fat-soluble vitamin content. Orban *et al.*[13] reported lower vitamin E values (920 μg.100^{-1}g ww) for Adriatic Sea white clam whereas vitamin A was detected in trace amounts.

Vitamin D_3 is essential for various physiological processes and has a vital role for maintenance of normal blood levels of calcium and phosphorus. This vitamin is usually presented in high concentrations in marine lipids. In this study high content of vitamin D_3 was found – average 40.46 μg.100^{-1}g ww, which supplies over 200 % of RDI [18]. Merdzhanova *et al*. [22] reported significantly lower levels (3.10 μg.100^{-1}g ww) for vitamin D_3 in black mussel *M. galloprovincialis* from Bulgarian Black Sea coast.

Vitamin K has anti-inflammatory activity, and can act as a vital component against various chronic aging diseases. In addition, this vitamin affects age-associated diseases due to its antioxidant and anti-inflammatory effects. Popa *et al.* [23] supposed that vitamin K, along with magnesium supplementations, can affect bone fractures especially in elderly population. In the present study insignificant seasonal variations were found in vitamin K content. Average level in *Ch. gallina* edible tissue is 54.6 μg.100^{-1}g ww, supplying 78% of RDI for this vitamin [18]. There is not comparable information for Black Sea *Ch. gallina* vitamins content.

CONCLUSION

Wild striped venus clam *Ch. gallina* from the Bulgarian Black Sea coast may be classified as a food whit high nutritional quality due to its proximate composition, macroelements and fat-soluble vitamin content. Regardless of the observed variations in the chemical composition, the white clam is rich in protein (average 17.30 g.100^{-1}g ww), lipid (average 2.5 g.100^{-1}g ww) and carbohydrate (average 2.35 g.100^{-1}g ww). High amounts of Na, K, Ca and Mg were determined in white clam edible tissues, which make it a valuable source of macroelements in human food. There is no available information for the seasonal changes in fat-soluble vitamin contents in striped venus clam *Ch. gallina* from the Bulgarian Black Sea coast. This species can supply over 270 % of RDI for vitamin D_3, 78 % of RDI for vitamin K and over 50% for vitamin E. Significant quantities of Ca (average 1100 mg.kg^{-1} ww) and Mg (average 690 mg.kg^{-1} ww) along with high levels of both vitamin D_3 and K demonstrated its promising potential in prevention osteoporosis and several non-chronic diseases.

Acknowledgement: *This study is financially supported by the National Science Fund of Bulgaria: Project # КП-06-ОПР03/11 from 18 Dec 2018.*

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