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Nutrient Content and Antioxidant Properties of Eggs of the Land Snail *Helix aspersa maxima*

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Abstract

This paper describes selected biochemical and analytical composition of *Helix aspersa maxima* eggs. Analyses determined the percentages of proteins, lipids, sugars, the composition of water and mineral elements, also antioxidant capacity. *H. aspersa maxima* eggs contain the high percentage of protein and are low in lipids (cholesterol content average 0.69 mg%), and are also found rich in Ca. The results showed that the eggs possess antioxidant activity and do not lose the antioxidants during short term storage in salt solution. The results make it possible to evaluate nutritional quality of eggs land snails as a good food source.

Keywords: *Helix aspersa maxima;* Eggs; Antioxidants; Nutrients

Introduction

A *Helix aspersa maxima (Taylor) is* one of the two forms *H. aspersa*, typically antropochorus, widespread through the world in many zones, including temperate and subtropical climates [1]. For commercial breeding, *H. aspersa* is known as *H. aspersa maxima*, which is hermaphrodite snail with determinate grown pattern in many regions, colonizing new habitats [2]. The time for snails to mature and reproduce is 4-7 months [3]. The length of breeding period is between April and October. In some controlled condition [4], like farming system, with control of feed [5], snails can growth in a shade-cloth covered greenhouse giving protection from the sun, wind and predators. So it is possibility to control land snail eggs in March and May (data in present paper). The number of eggs is not significant among the different generation, but reproductive success increased with a number of generations [6]. And therefore *H. aspersa maxima* is the most suitable snail for farming owing it to its adaptability to different environmental conditions, high reproduction and growth rates [7].

The estimated global annual consumption of snail meat is approximately 30 thousand tons, but its fivefold increase is predicted in the next twenty years. The limited opportunities for collecting snails from the natural habitats contributed to the development of helix culture. Increasing number of snail farms in Poland is observed since the late nineties of XX century. Snail farms are being established to produce good quality snails for consumption. One of the most above area of using snail meat and eggs is food industry. Fish roes are consumed for special dinners and invitations all over the world and caviar is a well-known example. The caviar is an expensive product from fish processing industry which is considered as a high-price-flavor with a high nutritional value in international and domestic markets [8]. The rows of sturgeon are known as the original caviar. Land snails eggs are a source in food industry, food processing, also because they are a potential source of healthy food due to its low fat content and presence of nutrients required for a well-balanced diet. The snail meat is mainly consumed as delicacy characterized by excellent nutritious traits and a high dietetic value [9]. However, no studies were published on the nutritional and antioxidant properties of land snail eggs. In that case it is useful to make a basic analyzes for biochemical composition.

Material and Methods

Eggs sample

The sampling of land snail's eggs was performed from snail Farm in Brudnice, Poland. Snails had received the special supplementary for fed 70% rapeseed cake and 30% fodder chalk. Snails were cultivated on peaty soil with 6.5 pH. The samples of eggs were collected as an average laboratory sample from a few thousand snails *H. aspersa maxima.* The samples were collected on the same day of embryonic stage (March–sample 1, 3 and May–sample 2). After sampling, the eggs were refrigerated for 24 h (+4°C), then analyzed. Sample described as 3, was refrigerated in 10% salt solution.

In this study water content (moisture content), reducing sugars, protein, fat, cholesterol content, mineral analysis were determined. Also tests for total antioxidant capacity ($ABTS^{\bullet+}$ reduction and Fe^{3+} reduction) were used.

Water content in eggs

The eggs were washed with deionized water, weighed and dried at 105°C to constant weight, then weighted.

Determination of reducing sugars

Determining the amount of reducing sugars was performed using Somogyi-Nelson assay [10] with a slight modification, using UV-VIS Helios Gamma spectrophotometer (Thermo Spectronic, Great Britain), at the wavelength of 520 nm.

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Protein content

Protein content was determined by the colorimetric method by Bradford [11], using an UV-VIS Helios Gamma spectrophotometer (Thermo Spectronic, Great Britain), at the wavelength of 595 nm.

Cholesterol content

The determination of cholesterol was performed according to Chiocchio and Matković [12]. The samples (2 g of each) were saponificated with 2 g KOH in 25 mL of methanol (HPLC grade). After the saponification (1 h reflux to 70°C), they were left to cool. The supernatant was placed in a funnel and 20 mL of methanol was added twice over the precipitate. The three aliquots in the funnel with 30 mL of dry n-hexane were joined (previously filtered the 60 mL of methanol though Millex-HV 0.45 µm (Millipore S.A., Molsheim, France). This extraction was repeated twice. Fractions were joined and taken to dryness in a Rotavapor (Büchi Labortechnik AG, Switzerland).

The samples were stored (4°C in darkness) until HPLC analysis. Each sample was re-dissolved in 200 µL methanol. For HPLC analysis we used Dionex HPLC system, a flow rate of 1.0 mL/min. The wavelength was set at 290 nm for determinate cholesterol and 254 nm for corticosterone. Thus, each sample was injected twice in the HPLC. The used column was a Hypersil GOLD (5 µm, 250 × 4.6 mm). Methanol: acetonitrile, 80:20 v/v (HPLC-grade) was the mobile phase.

Mineral analysis

The eggs were washed with deionized water, dried at 105°C, weighed and mineralized. The samples were mineralized by treatment with 10 ml of 65% HNO_3 (Suprapur, Merck KGaA, Germany) in two steps working program: heating temperature 200°C, 15 minutes followed by heat at 200°C, 35 min in microwave digestion system (Ethos One, Milestone Srl., Italy). The mineralized sample quantity was about 1 g. After mineralization the samples were diluted with double deionized water (Milli-Q Millipore 18.2 MΩ/cm resistivity). The analysis of selected elements of eggs was performed using ContrAA700 Atomic Absorption Spectrometer (Analityk Jena AG, Germany) with air/acetylene burner. Standards solution were prepared from 1000 mg/L stock solution (CertiPUR, Merck KGaA, Germany) of Fe, Mg, Ca, Zn, Mn, Cr and Cu by dilution with 0,5% HNO_3 (Suprapur, Merck KGaA, Germany). Determination of the investigated components was carried out in three replicates, average results (±SD) being calculated per g dry weight.

Total antioxidant capacity

Antioxidant properties of the snail eggs were assayed with a spectrophotometric method with the use of $ABTS^+$ (synthetic cationradicals) test and the results were presented as Trolox [13] (standard; µmol of Trolox per 1 g of fresh eggs). The absorbance was recorded at 734 nm (UV-VIS Helios Gamma spectrophotometer, Thermo Scientific, Great Britain)

FRAP assay (Ferric ion reducing antioxidant power) was performed according to the methods of Benzie and Strain [14]. Trolox was used as a standard. The absorbance of the samples was determined against blank at 593 nm. The values obtained were expressed as µmol of Trolox per gram of fresh sample.

Statistics analysis

The results were expressed as means +/- standard deviation (SD).

Results and Discussion

Heliculture is an alternative culture in many countries. Also in Poland, the area of snail farm increases. Although the production of good quality snails is favorable a little is known about nutritional exigency [10]. And still the research on snail cultures *Helix aspersa maxima* is rare, despite that these are species with high economic value and a luxury food. The aim of this paper was to present basic analytical and biochemical analyses of the land snail eggs. Some details about eggs, data collection, the storage conditions, eggs weight and moisture content are summarized in Table 1. Wet mass of eggs was between 5.45-6.2 g and dry almost seven times less than wet mass. The moisture content was significant in samples collected in different months (March and May), but in the same embryonic stage (sample 1, sample 2). The percentage of water content in sample storage in 10% salt solution in +4C is lower than in samples storage in +4°C without salt. These results are very similar to studies for snails *H. pomatia* and *H. aspersa* presented by Fontanillas [15] and Bonnet et al. [16].

Rapeseed cake is a high-energy and high-protein feed. It is consisting of heavy-pressed rapeseed and is suitable as a food source for many animals. Total protein content in snail eggs of *H. aspersa maxima* was determined (Figure 1). The content of protein in dry mass was from 34.6% (sample 3) to 42.2% (sample 1), what confirms high content of protein and the fact, that the snails were fed with concentrated high-energy and high-protein rapeseed cake. Researchers confirm that the snail meat contains very high level and quality protein [17]. The data confirm that the protein content found in *H. aspersa maxima* meat was 12% in fresh mass [18], 16,3% in fresh mass [12] and 14.8–18.4% in dry mass [10]. In our study the high content of eggs protein was noticeable. The dry mass of *H. aspersa* contains 10% lipids [19], including phospholipids and cholesterol. Cholesterol in animals is the predominant sterol and in slugs and snails are almost 90% of

Number of sample	Day of collection	Wet weight [g]	Dry weight [g]	Water content [%]
1.	March Sample storage in +4°C	6.1909	0.92361	85.1
2.	May Sample storage in +4°C	5.4511	0.7712	85.6
3.	March Sample storage in 10% salt solution, in +4°C	5.6313	0.8759	84.4

Table 1: Collection of eggs from land snail.

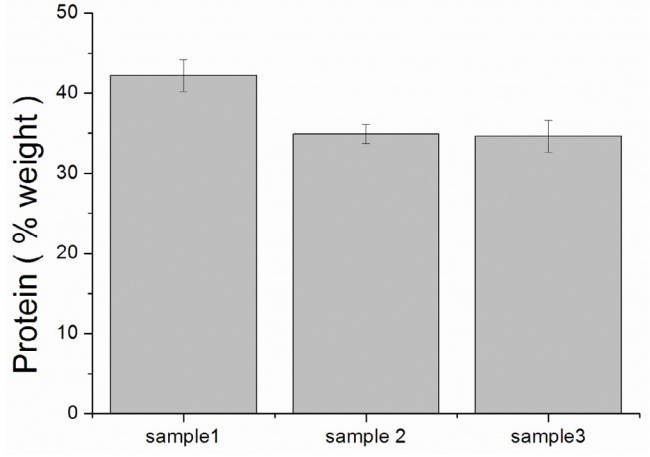

Figure 1: Protein of *Helix aspersa maxima* eggs. Vertical bars represent standard deviations. Sample 1: Eggs collected in March and stored in +4°C; Sample 2: Eggs collected in May and stored in +4°C; Sample 3: Eggs collected in March and stored in 10% salt solution in +4°C.

total lipids [20,21]. In our results average cholesterol content is 0.0077 mg/g. It confirms that cholesterol content in eggs of land snail (0.56 mg%-sample 1) is relatively low comparing to the literature data [22]. In the case of sample 2 and 3 similar results were obtained (Figure 2), so there is no significant difference between time of eggs deposition and preparation of samples (May) and adding eggs to the 10% salt solution (0.77 mg% and 0.75 mg%, respectively). The content of cholesterol was higher for the sample obtained in May (sample 2) and March (sample 3) than for eggs obtained in March and stored only in +4°C. Moreover in human nutrition dietary lipids are indicated as important, also health professionals recommend low cholesterol, low energy and low SFA (saturated fatty acid) diets [10]. According to Murphy [5] snail meat contains little fat. *H. aspersa* eggs in this study do not contain glucose.

Snails are prepared for human consumption in different ways. In *H. aspersa maxima* the visceral hump and foot is removed, head and mantle edge are eaten. Some example of mineral and trace elements from eggs are presented in Table 2. Claeys and Demeyer [18] point richness of *H. aspersa* in mineral salts, nutritive value of relatively low

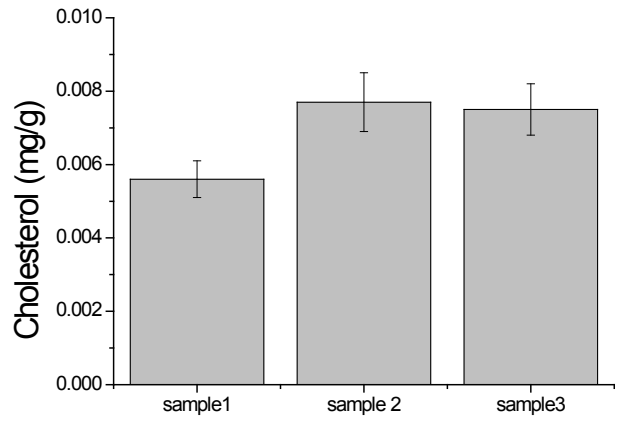

Figure 2: Cholesterol content (mg/g) of dry mass. Sample 1: Eggs collected in March and stored in +4°C; Sample 2: Eggs collected in May and stored in +4°C; Sample 3: Eggs collected in March and stored in 10% salt solution in +4°C.

Species	Part of body	Minerals (mg.100 g^{-1})/DM			Minerals (mg.kg^{-1})/DM			References
		Ca	Mg	Mn	Fe	Cu	Zn	
Helix aspersa maxima	Foot	1390	575	-	112	164	64	[15]
	Viscera	1370	700	-	392	48	1660	[15]
Helix aspersa aspersa	Foot	1620	425	-	84	252	80	[15]
	Viscera	1120	625	-	220	60	1580	[15]
	Meat	-	-	-	5.21	-	-	[20]
Helix lucorum	Foot	1840	400	-	60	228	88	[15]
	Viscera	1040	375	-	720	68	1420	[15]
Helix pomatia	Foot	4580	375	-	144	300	88	[15]
	Viscera	1340	550	-	312	44	2480	[15]
	Meat	-	-	-	17.1	-	-	[21]

DM: Results expressed as % in dry mass; #: Data from analysis described in this paper

Table 2: Mineral content of selected species of snails.

bodyweight. So far there is no data about nutritional value and mineral concentration of snail eggs. But it is known that mineral element in food is important for human health [23]. The potential contribution of 100 g of *H. aspersa eggs* to the Recommended Dietary Allowances (RDA) (US Department of Agriculture, 2004) is presented in Table 3. Likewise to our study, calcium was determined the highest in *H. aspersa* [24,25].

In our research, in breeding snails has been used a fodder chalk. Fodder chalk is a source of concentrated calcium (Ca) and it is used for producing fodder mixtures or for direct feeding in mineral mixtures. This chalk has been used for years in feeding animals as a supplement offering necessary minerals, so indispensable for good health of breeding stock. In our research Ca content was 88.4-117 mg/g dry mass and % of RDA from 107 to 239 in eggs stored in salt solution and refrigerate, respectively. Also the data summarized in Tables 3 and 4 shows that land snail eggs are good source of Ca, Cu and Fe.

Current study presents new data, on the antioxidant capacity of *H. aspersa* eggs using a novel and common methods like ABTS $^{\bullet+}$ test and FRAP assay [26]. The results presented in Table 5 indicate that eggs have high antioxidant capacity. Also the data show no loss of antioxidants during short term storage in salt solution, but comparing the two methods, it is obvious that ferreric reducing assay indicate the higher antioxidant power of eggs. Comparing to the plant extracts the eggs have almost ten times less antioxidant capacity [27].

Element	RDA (mg)	% of RDA supplied by 100 g eggs		
		Sample 1	Sample 2	Sample 3
Fe	10	3.04	5.11	2.57
Mg	420	1.60	2.08	1.23
Ca	1000	239	165	107
Zn	11	2.10	2.41	1.50
Mn	2.3	2.59	3.52	2.88
Cu	0.9	43.01	48.28	35.74

Sample 1: Eggs collected in March and stored in +4°C;
Sample 2: Eggs collected in May and stored in +4°C;
Sample 3: Eggs collected in March and stored in 10% salt solution in +4°C.

Table 3: Contribution of 100 g of *Helix aspersa eggs* and the Recommended Dietary Allowances (RDA).

Element	Nutrient mineral (mg/g dry weigh)		
	Sample 1	Sample 2	Sample 3
Fe	0.0201	0.0365	0.0165
Mg	0.455	0.625	0.325
Ca	88.4	117.4	105.5
Zn	0.017	0.019	0.011
Mn	0.0043	0.0058	0.005
Cu	0.0268	0.0311	0.0223
Cr	0.0014	0.0014	0.0016

Sample 1: Eggs collected in March and stored in +4°C;
Sample 2: Eggs collected in May and stored in + 4°C;
Sample 3: Eggs collected in March and stored in 10% salt solution in + 4°C.

Table 4: Mineral element composition of eggs.

Methods for antioxidant capacity	Sample 1	Sample 3
FRAP assay	2.98 ± 0.09	2.99 ± 0.36
ABTS test	1.17 ± 0.15	0.89 ± 0.25

Sample 1: Eggs collected in March and stored in + 4°C;
Sample 3: Eggs collected in March and stored in 10 % salt solution in + 4°C.

Table 5: Antioxidant capacity of snail eggs.

The results presented by Cagiltay et al. [24] showed that *H. aspersa* is a good source of essential amino acid, fatty acid, vitamin and minerals. Noteworthy, in the nutritional composition of snail are high in protein quality, but low in fat contents, which also coincides with the data of the investigations. In that case snail eggs are an alternative food for people with low fat diet requirements [24,28,29].

Conclusion

Many snail local farms are being established to produce good-quality snail for consumption. Researchers suggested using meat of *Helix aspersa maxima* as a source of food ready for consumption and recommended snails as advantageous foodstuff from a dietary point of view and as a good source of proteins energy value and low in calories [19].

The chemical composition of samples of *H. aspersa* eggs was investigated to determine their nutrition value. The results show that the eggs are high in the water (84.4-85.6% of total) and low in cholesterol (0.77 mg% of total), do not contain glucose. The protein content is high, above 40% of dry mass and is noticeable. Moreover, the eggs are also found rich in Ca and Cu and Fe. The FRAP assay show antioxidant capacity of raw material. Salt solution is a good source to store snail eggs. The results indicate that the *H. aspersa* eggs could be taken as an alternative food source.

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Author Contributions

A.G.: Conceived and designed the experiments, performed the experiments, analyzed the data. MD wrote the paper, analyzed the data. BO performed the experiments.

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