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preliminary communication

Quality Factors of Commercial Snail Fillets as Affected by Species

Running title: Quality of Snail Fillets

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SUMMARY

Research background. This study fulfils a need for investigation of quality profile of snail fillets. Edible snails are a famous food product consumed worldwide and treated as delicacy. Nutritional value, color and textural properties, such as hardness, are critical factors that impact consumer acceptance of the product. Hardness of snail meat is affected by its native original microstructure.

Experimental approach. Fresh snails of the species farmed *Cornu aspersum maximum*, wild and farmed *Cornu aspersum aspersum* and wild *Helix lucorum* were used in order to investigate the qualitative profile of snail meat. Proximate composition, hardness and color measurements were conducted to fillets of all species. The histological structure of fillet was conducted to fillets of *Cornu aspersum maximum*.

Results and conclusions. Quality parameters of snail fillets were studied. A novel method of hardness analysis was proposed where the cylindrical part of snail fillets from the mid-posterior region with specific geometry 6 mm diameter and 6 mm height was used. The suitability of the mid-posterior region was enhanced by the uniform structure proved by the histological analysis. *Helix lucorum* snail

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fillet had the highest energy content and the highest hardness but the lowest carbohydrate content. The species *Cornu aspersum maximum* was evaluated with the highest values in a^* (redness), b^* (yellowness) and Chroma compared to other species. Parameter L^* (lightness) in wild snail fillets was lower compared to the farmed ones due to age, diet, farming or environmental conditions but also it could be related to snails' carbohydrate content.

Novelty and scientific contribution. This study yielded notable results on qualitative characteristics of snail fillets as food and important information is given on its meat properties. Furthermore, a novel methodology of hardness is provided in order to minimize natural, breeding and environmental influences. Finally, the research outcomes could lead to proper handling methods for further fabrication of snail meat.

Keywords: snail fillets; hardness; composition; histological structure

INTRODUCTION

Invertebrates constitute an important component of the diet worldwide and many molluscan species are known for their culinary value (1,2). Among gastropods, the helicine species (*Helix pomatia*, *Helix lucorum*, *Cornu aspersum*) consumed extensively in Europe, have been the principal subject of studies related to proximate composition (3-5), while the organoleptic and mechanical properties were studied to a lesser extent.

There is no terminology about the edible parts of the snail. Namely the whole snail is usually referred to as snail meat (3,6) and the foot-head mass which is the main edible part (7) is mentioned as foot (8,9), foot muscle (7) and pedal mass (4). The body of the edible terrestrial snails is invested with epithelium and is protected by a shell secreted by specialized epithelial cells. Underlying the epithelial layer of the gastropod's integument, complex arrays of muscle and connective tissue complete its basic structure (10). In literature, typical histological structure was reported for many species (10). The subepidermal connective tissue was traversed by different types of cells such as rhogocytes, glycogen cells and secretory cells which contain proteins, calcium, pigments, fat globules and mucus. In the lower layer of subepithelial tissue, between muscle and connective tissue, there are empty spaces, the haemocoelic sinuses where hemolymph is gathered. Foot mucus functional role is affected by proximate composition including 90–99.7 % weight of water and a glycoprotein complex (11). Greistorfer *et al.* (11) reported four types of mucus glands in the foot of the species *C. a. aspersum*. Histology of snail fillet might present differentiations in size of muscle cells (12) and collagen fiber diameter (13) related to species, age, diet and farming conditions. The histological

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analysis of meat products could also guarantee the authenticity in fraud cases (14) and is used for texture optimization after various treatments in order to meet consumer needs (15).

Snail edible tissue is a potential source of protein and essential nutrients such as vitamins and minerals, especially calcium, potassium, sodium and trace elements such as iron and selenium (3,4). Protein and carbohydrates and play major roles in providing the desirable rheological and textural attributes in meat and fish (16). Hardness is an important factor to the food industry as this texture parameter could be related with the way of food proceed and the acceptance by the consumers. In nowadays the textural parameters, have been studied extensively in various meat such as beef and lamb and it is well known that postharvest handlings, breeding, and product preservation are also parameters that affect hardness of the final meat products (17-19). Texture profile analysis (TPA) is a popular method for determining textural properties of foods and evaluating quality factors when sensory experiments are expensive, time - consuming and no easy to take place as for example for raw meat samples (20). In other studies, tissue parts of specific dimensions were used for the texture assessment for invertebrates and vertebrates (21). Mizuta *et al.* (21) used a part after the head of the prawn in order to estimate muscle firmness and Cimmino *et al.* (17) obtained pieces of goat meat parallel to the longitudinal orientation of the muscle fibers assessing meat tenderness. Schubring and Meyer (22) noticed significant differences between the values of hardness and chewiness of the species *C. a. aspersum* and *Achatina fulica*. The authors reported that *C. a. aspersum* assessed the lowest values and *A. fulica* the highest values of the aforementioned parameters compared with *H. pomatia* and *H. lucorum*. Nevertheless, the above approach mainly focusses on whole snail meat properties and particular cooked or do not take into account the variability in raw snail fillet quality properties. Texture analysis of raw meat of snail fillets could give further information on the technical and economic aspects of food processing.

Another important sensory attribute of food is color which also meet sales and profitability. Many researches showed that snail's visual selection is based on shell size and shell coloration diversity (23,24). Also, high frequencies of dark cells were mentioned in shaded environments and pale ones in open climatic selection is sufficient to maintain of dark shells in shaded environments and pale ones in open areas (25,26). Except for shell, snail meat color is also very important. Edible tissue of fresh wild snails *C. a. aspersum* presented high values of L^* lightness and b^* yellowness and low value of a^* redness (3). Also processed snail meat color parameters were important quality characteristics for snails collected in Lithuania after processed with deep freezing (27). The explorations among chemical composition in snail meat and color parameters are very important to give the magnitude of impact on variations among different snail species.

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In our research is the first time that quality properties of snail fillets of different commercial species, farmed and wild, were evaluated. The primary aim is to understand the association of histology, chemical analysis, hardness and color parameters of each kind of four commercial snail fillets, farmed *Cornu aspersum maximum*, farmed and wild *C. a. aspersum* and wild *H. lucorum*. Additionally, a new method for texture analysis is also proposed based on the shape and size of specific part snail fillet sample in order to optimize hardness assessment. Elucidating quality factors for commercial snail fillets we aimed to help standardize quality parameters and give further information on the technical aspects of snail food processing.

MATERIALS AND METHODS

Snail samples and preparation

For the experimental procedure, we used market-size snails of commercial four species consumed worldwide: farmed *Cornu aspersum maximum*, farmed *Cornu aspersum aspersum*, wild *C. a. aspersum* and wild *Helix lucorum* (Fig. S1). Farmed *C. a. maximum* snails were supplied from a net covered greenhouse (Volos, Magnesia, Thessaly, Greece) and *C. a. aspersum* from an open field farm (Kontarriotissa, Pieria, Central Macedonia, Greece). Wild *C. a. aspersum* snails and wild *H. lucorum* were purchased from local retailers in Heraklion (Crete, Greece) and Serres (Central Macedonia, Greece) respectively. All snails were transported to lab 2 days after their collection, in April 2019.

A total of 238 fresh snails of the four aforementioned species were used for the analyses (Fig. S1). The mass (M) of whole raw snails and three morphometrical characteristics of each snail shell were measured in each specimen: shell diameter (D), shell height (H) and shell aperture diameter (d) using a precision balance (EMB 200-2, Kern & Sohn, Balingen, Germany) and a digital caliper (Fowler, USA) both with two decimal places. Additionally, mass of raw fillet (M_i) was recorded after shell removal and anatomy in order to separate fillet from visceral mass (Fig. S1) and to conduct histological, compositional, textural and colorimetric analyses.

The histological analysis was conducted only to *C. a. maximum* (10 raw fillets), while the other analyses were performed to all species. Color was assessed to 15 fillets/species, textural analysis to 12 fillets/species and 30 fillets/species were used for proximate composition (Fig. S1). Apart from whole fillets, cylindrical parts with 6mm diameter and 6mm height from the mid-posterior region of fillets were used for textural and histological analysis. The entire procedure of analysis is shown in Fig. S1.

Histological analysis

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The histological analysis was carried out in 10 snail fillets of farmed *C. a. maximum*, after anesthesia using twenty drops of *Eugenia caryophyllus* oil (clove oil, CHEMCO, Germany) diluted in 50 ml water (28). Snails were kept in this emulsion for 2 h at room temperature in order to relax and extend their body.

The specimens of fresh fillets, removed from the mid-posterior region of the fillet (Fig. S1), were fixed in 10 % neutral buffered formalin (Neutral Buffered Formalin 10 %, Thermo Fisher SCIENTIFIC, USA) and were placed in cassettes and inputted in histokinette (Leica TP 1020, Germany) for dehydration (immersion in ethanol solution of increasing concentrations), clearing with immersion in xylene solutions (Thermo Scientific, USA) to replace ethanol with an organic dissolvent and embedding in liquid paraffin wax (Histoplast PE, Thermo Scientific, USA) using heated paraffin embedding station (Leica EG 1159H, Germany). Paraffin blocks were left for cooling (Leica EG 1150C, Germany); then, the mold was removed and the blocks were mounted on a microtome (Slee Mainz Cut 5062, SLEE medical GmbH, Mainz, Germany) for sectioning (5 μ m sections). The sections were stained with the haematoxylin (Haematoxylin Harris Acidified, Thermo Scientific, USA)–eosin (Eosin Y Alcoholic, Thermo Scientific, USA) regressive staining procedure, covered with Canada balsam mounting medium, and observed under Carl Zeiss Light Microscopy (Carl Zeiss Ltd, Gottingen, Germany) connected with a ProgRes C10 digital camera (Berlin, Germany), and subsequently processed through image analysis using the software ProgRes Capture Pro 2.1 (Berlin, Germany). Some sections were additionally stained using the Masson's trichrome staining methods (Masson's trichrome kit with aniline blue DC, Parneac Quimica SAU, Spain).

Proximate composition

Proximate composition was assessed to 30 raw fillets per species according to AOAC (29). For moisture content determination (% *m/m*), 3 g was dried at 105 °C in an oven (TS 8056, Termaks, Bergen, Norway) until constant mass, and the water content was determined gravimetrically (29). Dry matter of fillets of each species was pooled and 10 g of dry matter were used for the following analyses. Crude protein (% *m/m*) was tested by the Kjeldahl method ($N \times 6.25$; Behr Labor-Technik, Germany) as it is referred in literature about proximate composition of snail meat (5) using 0.2 g dry matter and then expressed in net basis and crude fat by Soxhlet method (Sox-416 Macro, Gerhardt, Germany). With regard to ash content (% *m/m*), a water-free sample was combusted in a muffle furnace (Nabertherm L9/12/C6, Lilienthal, Germany) by heating at 600 °C for 3 h and the ash content was measured gravimetrically. Gross energy (KJ/g) was evaluated using an adiabatic IKA oxygen bomb calorimeter (C7000, IKA Werke, Staufen, Germany). Crude protein, crude fat and ash content results of each group were expressed in net basis. Carbohydrate content (% *m/m*) was calculated by

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the difference between 100 and the sum of the crude protein, crude fat and ash content in net basis (30). All measurements were carried out in triplicate and the values were averaged.

Hardness measurement

For hardness measurements, 6 whole fresh fillets and 6 cylindrical (6 mm diameter and 6 mm height) parts from the mid-posterior region of fillets of each species (Fig. S1) were analyzed at 5 °C. The instrumental texture measurement was performed using texture analyzer (Admet Texture Analyzer eXpert 5601, AdMEt, Inc., USA) and cylindrical probe of 18 mm diameter. The instrumental texture measurements were performed as texture profile analysis (TPA) at 75 % compression and the jog rate was 100 mm/min. According to Schubring and Meyer (22) and Ruiz De Huidobro *et al.* (18), the texture attribute “hardness” is defined as the maximum force of the 1st compression (Fmax). For each group of whole fresh snails and cylindrical parts of farmed *C.a. maximum*, farmed and wild *C.a. aspersum* and wild *H. lucorum*, average Fmax values were evaluated.

Color measurement

Color measurements were performed at 5 °C on the surface of the ventral region of fresh, *C.a. maximum*, farmed and wild *C.a. aspersum* and *H. lucorum* fillets using colorimeter (HunterLab MinScan XE Plus, USA) in the CIELAB color space (31). Lightness (L^*), redness (a^*), and yellowness (b^*) were recorded per each kind of snail fillets (Fig. S1). L^* or lightness express dark to light scale of 0 to 100. The parameters a^* or redness shows green to red and b^* or yellowness represents blue to yellow both on a scale of -60 to +60. Also Hue or Hue angle was determined by the equation: $\arctangent(b^*/a^*)$ and Chroma or the color saturation index was calculated manually by the $\sqrt{(a^{*2} + b^{*2})}$ according to the method of (32). Three replicate measurements were obtained and averaged. The 15 fillets were divided in five groups of three snail fillets.

Statistical analysis

The data were analyzed using SPSS Statistics 23 (33) and expressed as mean values \pm standard deviations (S.D.). One-way analysis of variance (Anova) followed by Tukey's Multiple Comparison Test at the significant level of 0.05 were used to compare the data of morphometrical characteristics of snail shell and mass of whole snails and snail fillets of different species (34).

RESULTS AND DISCUSSION

Morphological characteristics of snails

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The snail species of this study belonged to the list of edibles farmed and wild species used also in food manufacture (5,35,36). The snail species reported differences in size, mass and shell morphometrical characteristics (Table 1). The aforementioned differences were explained by the species, the age of snails, the period of collection, the breeding conditions and diet (4,5,37,38).

As it concerns our samples, shell diameter (D), a parameter of snail size, ranged from 30.93 to 40.03 mm. *Cornu aspersum maximum* (40.0±3.3) mm and *Helix lucorum* (35.1±3.3) mm had significant higher D value from farmed (30.9±1.8) mm and wild (31.6±2.4) mm *Cornu aspersum aspersum* ($p < 0.05$). Similar statistical differences with D was also reported to mass of snails (M). More specifically, wild *C. a. aspersum* weighted (7.8±1.2) g and farmed *C. a. aspersum* (8.4±1.4) g were significantly ($p < 0.05$) lower than *H. lucorum* snails (12.7±2.5) g and *C. a. maximum* (21.3±3.6 g) (Table 1).

Even though, fillet is the main edible part of snails and is used in food manufacture, consumers might eat also a part of visceral mass. *C. a. maximum* snail fillet weighted (2.1±2.1) g and *H. lucorum* (1.6±0.6) g. Only, M_f of farmed (1.1±0.4) g and wild (1.0±0.3) g *C. a. aspersum* snails were the same ($p > 0.05$) (Table 1). In literature, there are studies presented histology and proximate composition of snails, but mass of snail fillet was firstly assessed.

Microstructure profile of snail fillet

The histological analysis was conducted to the cylindrical part (Fig. S1) of middle-posterior region of fillet used for textural assessment which had specific shape and size. The suitability of middle-posterior region is explained by the absence of parts of digestive, reproductive and nervous system which are in the anterior region. Histological structure of fillet of farmed *C. a. maximum* is illustrated in Fig. 1.

According to Fig. 1a, the integument in the ventral region presented a flat rough surface and was covered by an epithelium formed macroscopically visible infoldings. Ventral epithelium of fillet was thicker than the dorsal region as it was also mentioned in a recent study. According to the last, the ventral epithelium of the snail *Cepaea hortensis* was found to be twice thicker than the epithelium on the dorsal side (9).

Mucus glands were embedded in epithelium and subepithelial matrix of fillet (Fig. 1b). Most of the secretory cells in pedal sole were of a distinct kind that produced mucus combined with protein (10,11).

Moreover, based on the histological analysis of *C. a. maximum* fillet, we identified the subepithelial matrix of muscle cells and connective tissue (Fig. 1c). The connective tissue was traversed by different types of cells such as rhogocytes, glycogen cells including packed glycogen

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and secretory cells which contain proteins, calcium, pigments, fat globules and mucus (10). In terms of muscle tissue, muscle cells presented different orientation and formed bunches (Fig. 1c). Czarnoleski *et al.* (12) studied the histological structure of farmed *C.a. aspersum* and *C.a. maximum* kept at 15 and 20 °C, and fed with food included plants, minerals and vitamins, enriched with dry soil and an additive of CaCO₃. Even though higher temperature led to smaller muscle and epithelial cells in both species, the overall conclusion of this study was that farmed *C.a. aspersum* consisted of larger muscle and epithelial cells compared with farmed *C.a. maximum*, regardless of breeding temperature, 15 or 20 °C (12).

The matrix of muscle and connective tissue was interwoven by numerous capillary processes of the system of haemocoelic sinuses where hemolymph is gathered (Fig. 1c). Gastropods has open circulatory system and hemolymph circulates throughout the body and serves also as a hydroskeleton (12).

We indicated that muscle cells were surrounded by collagen fibers after Masson trichrome stain in sections of mid posterior region of fillet (Fig. 1d). Collagen fiber size might present differentiation in farmed and wild populations. According to Berillis *et al.* (13), collagen fibers of the farmed snails *C.a. aspersum* were bigger (38.8±7.6) nm than those of the wild snails of the same species (32.7±7.2) nm.

Compositional analysis of snail meat

According to Table 2, wild *C.a. aspersum* snails presented the highest moisture content (83.3±1.0) % and farmed snails of the same species presented the lowest value (81.8±1.5) %. *C. a. maximum* snails reported moisture content (83.0±1.6) % and *H. lucorum* snails (82.2±1.6) %. Based on our analysis, crude protein ranged from 10.3 to 13.5 % and wild species *H. lucorum* presented the richer protein content (13.5±0.1) % than farmed species *C. a. maximum* (10.3±0.3) %. Farmed and wild snails *C. a. aspersum* had the same value (11.0 %) (Table 2). Similarly, wild species *C.a. aspersum* (0.7±0.3) % and *H. lucorum* (0.6±0.1) % assessed higher fat content than farmed species *C.a. aspersum* (0.4±0.1) % and *C. a. maximum* (0.1±0.0) %. As it is illustrated in Table 2, raw fillets of wild and farmed *C. a. aspersum* presented the highest value (1.5 %) of ash content while *C.a. maximum* snails presented the lowest (1.1±0.0) %. *H. lucorum* snails had (1.3±0.1) % ash content. The energy content ranged from (20.0±0.2) KJ/g to (21.1±0.2) KJ/g (Table 2). According to the results in the present study, even though *H. lucorum* snails reported the highest energy content, they indicated the lowest carbohydrate content (2.4±0.1) %. Raw fillets of the other wild species, *C.a. aspersum*, contained (3.4±0.4) % and snails of the farmed species *C.a. maximum* and *C.a. aspersum* (5.5±0.2) % and (5.4±0.5) % respectively.

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Based on the results of our study, raw fillets of all the species presented similar proximate composition which is reported in literature (3-5,38). Proximate composition of farmed snail species is affected by farming system and diet. According to Gomot (4), fillet of *C.a. maximum* snails which ate an artificial feed presented higher fat content 0.8% than the fat content 0.1 % reported in this study in fillets of snails of the same species which were supplied from a net covered greenhouse in Central Greece. Milinsk *et al.* (5,39) reported that proximate composition of *C.a. maximum* snail body might change based on diet. Even though, the feed of the farmed *C.a. maximum* used in this study, was supplemented with calcium, the ash content of their fillet was the lowest. Moreover, Gomot (4) found a slightly lower ash content 1.3 % in fillet of *C.a. aspersum* snails fed with E3-2 than the ash content 1.5 % of our fillets from snails derived from an open field farm and fed with plants. On the contrary, the ash content of the *H. lucorum* snail fillets was the same 1.3 % in both studies. In the present study, artificially fed *C.a. maximum* snails indicated richer carbohydrate content 5.5 % than the content 2.0 % reported by Gomot (4).

Hardness evaluation of snail fillets

Hardness is a texture-related property which describes a product which displays substantial resistance to deformation or breaking (40). Many foods are processed and formulated with a large number of ingredients, but it is not difficult to control overall textural properties (41,42). On the other hand, there are foods with texture characteristics connected with their native original microstructure. Raw snails are native foods and their structures are complete naturally. Processing to fillets, size reduction takes place and hardness is changing. There are also many factors which affects hardness in raw food such as chemical composition, breeding, environmental factors and usually there is no direct correlation among composition and hardness (40). Hardness of snails was only assessed by Schubring and Meyer (22). The authors used whole body treated snails for texture assessment and reported the highest value in *Achatina fulica* 50.2 N, 27.4 N and 23.5 N in *H. lucorum* and *Helix pomatia* respectively, and the lowest value of hardness in *C.a. aspersum* 18.5 N.

Whole shapes and sizes snail fillets according to nature variations could not be completely identical. In order to eliminate these dissimilarities in this research except of using the whole snail fillet samples for hardness detection we also deformed cylindrical parts of snail fillets of specific dimensions 6 mm diameter and 6 mm height. The chosen part according to the histological analysis has a more uniform microstructure.

As the chosen cylindrical part have smaller size than the complete fillets, hardness values of fillets were higher than hardness values of cylindrical parts of fillets and the same finding were for standard deviations (Fig. 2). Fmax values of whole fillets ranged from 16.7 to 42.2 N, while hardness

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of cylindrical parts ranged from 2.6-12.7 N. More specifically, hardness values of *H. lucorum* showed the highest value of hardness among fillet groups 42.2 N and cylindrical part groups 12.7 N. The fillet hardness of this species was higher from *C.a. maximum* 16.7 N and wild *C.a. aspersum*. Fillets of farmed *C.a. aspersum* were harder 21.4 N than the fillets of wild *C.a. aspersum* 17.0 N (Fig. 2). *C.a. maximum* cylindrical parts were not the softest among groups 6.1 N. Based on our results, cylindrical parts of farmed and wild *C.a. aspersum* were less hard and reported the same hardness value (2.6 N). The highest hardness values derived from cylindrical parts was found on *H. lucorum* sample which had the highest nitrogen content compare to the other snail species. We found the same one for whole fillet analysis. It is the first time that a part of specific size and shape of snail fillet used to evaluate hardness in order to minimize natural, breeding and environmental influences.

Color assessments of snail fillets

Color measurements of each type of snail fillet were recorded in Table 3. Snail fillets were exposure to air at the chiller temperature (5 °C), before the surface of the ventral region color was evaluated using a HunterLab Miniscan XE plus in the CIELAB color space (31). The highest L^* parameters (lightness) (49.2) was assessed by farmed *C.a. aspersum*. *C.a. maximum* presented also the highest a^* (redness) and b^* (yellowness) parameters (5.4 and 14.2 respectively). Snail fillets of species *C.a. maximum* were lighter (45.9) than wild *C.a. aspersum* (35.2), while the lowest mean value of L^* was assessed by fillets of *H. lucorum* (32.7). We reported almost the same value of a^* (1.0 and 1.1 respectively) in fillets of farmed and wild *C.a. aspersum*, while wild snails of this species were more yellow (9.4) than the farmed (6.5). *H. lucorum* were more red (4.6) but less yellow (7.8) than the other wild species, *C.a. aspersum*. Differences in Chroma are meaningful but for hue no big differences could be detected among snail samples. *C.a. maximum* has the highest Chroma value (15.2) and farmed *C.a. aspersum* the lowest. Wild species, *C.a. aspersum* and *H. lucorum*, have similar values of Chroma.

Today there are not many investigations in snail color. For fresh *C.a. aspersum*, Cagiltay *et al.* (3) reported higher values of L^* (54.7 ± 1.8) and b^* (19.5 ± 1.5) and a^* close to zero. Schubring and Meyer (22) reported color parameters of minced treated snails. *C.a. aspersum* fillets were the less bright (31.0) but the reddest (4.0) among species. *H. lucorum* snails presented the following values of L^* , a^* and b^* 39.6, 2.3 and 7.4 respectively.

In our research color parameters were studied for wild and farmed snail fillets. The species of farmed snails (*C. a. maximum* and *C. a. aspersum*) showed higher values in L^* and higher carbohydrate concentrations compare to the wild ones (*C. a. aspersum* and *Helix lucorum*). Although the farmed snails have lighter color skin due to their feeding, L^* parameter could also be related to

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their meat carbohydrate content as these species have higher carbohydrate concentrations. *Helix lucorum* fillet skin is dark brown and it has the lowest carbohydrate concentration (2.4 ± 0.1) and the lowest L^* value (32.7 ± 4.2). Differences detected in color parameters b^* , Chroma and hue were less essential. The species *C.a. maximum* farmed was evaluated with the highest values in a^* , b^* and Chroma compared to other species.

CONCLUSIONS

This study gives qualitative characteristics and important information on chemical composition, hardness and color of wild and farmed snail fillets. It is the first time that a part of specific size and shape of snail fillet proposed to evaluate hardness and minimize influences such as age, diet, farming and/or environmental conditions. Histological analysis supports and describes the uniform structure of the specific part of snail fillet. In chemical analysis *H. lucorum* snail fillet was reported with the highest energy content and the highest hardness but with the lowest carbohydrate content. Farmed snail fillets provide significant higher brightness and had higher carbohydrate content compare to the wild species. Finally, the quality characteristics of wild and farmed snail fillets and the novel measurement methodology for hardness could give information for further processing of snails meat in food industry.

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CONFLICT OF INTEREST

The authors declare not to have any conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at <https://www.ftb.com.hr>.

AUTHORS' CONTRIBUTION

E. Kougiagka performed investigation, data analysis, writing and prepared the original draft. C. Apostologamvrou conducted the histological analysis. P. Giannouli and M. Hatzioannou designed the investigation and performed methodology, data analysis, writing, review and editing.

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REFERENCES

1. Barbosa I, Brito G, Santos G, Santos L, Teixeira L, Araujo R, Korn M. Multivariate data analysis of trace elements in bivalve molluscs : Characterization and food safety evaluation. Food Chem. 2018;273.
<https://doi.org/10.1016/j.foodchem.2018.02.063>
2. Miliou H, Fintikaki M, Tzitzinakis M, Kountouris T, Verriopoulos G. Fatty acid composition of the common octopus, *Octopus vulgaris*, in relation to rearing temperature and body weight. Aquaculture. 2006;256:311-22.
<https://doi.org/10.1016/j.aquaculture.2006.02.050>
3. Cagiltay F, Erkan N, Tosun D, Selcuk A. Amino acid, fatty acid, vitamin and mineral contents of the edible garden snail (*Helix aspersa*). J Fishscicom. 2011;5(4): 354-63.
<https://doi.org/10.3153/jfscom.2011040>
4. Gomot A. Biochemical composition of Helix snails: Influence of genetic and physiological factors. J. Molluscan Stud. 1998;64:173-81.
<https://doi.org/10.1093/mollus/64.2.173>
5. Milinsk MC, Padre R, Hayashi C, D Oliveira CC, Visentainer JV, D Souza NE, Matsushita M. Effect of feed protein and lipid contents on fatty acid profile of snail (*Helix aspersa maxima*) meat. J. Food Compos. Anal. 2006; 19:212-6.
<https://doi.org/10.1016/j.jfca.2004.09.011>
6. Emelue GU, Evivie SE, Edmowande P. Processing and preservation of snail meat in Benin city, Nigeria. NJAEF. 2013;9(2):15-9.
7. Bracchi PG. Differentiation between *Helix* and *Achatina* Snail Meat by Gel Electrophoresis. J. Food Sci. 1988;53(2):652-5.
<https://doi.org/10.1111/j.1365-2621.1988.tb07778.x>
8. Tonar Z, Markos A. Microscopy and Morphometry of Integument of the Foot of Pulmonate Gastropods *Arion rufus* and *Helix pomatia*. Acta Veterinaria Brno. 2004; 73:3–8.
<https://doi.org/10.2754/avb200473010003>

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9. Von Byern J, Cyran N, Klepal W, Rudoll L, Suppan J, Greistorfer S. The structure of the cutaneous pedal glands in the banded snail *Cepaea hortensis* (Muller, 1774). *J. Morphol.* 2017; 279:187–98.
<https://doi.org/10.1002/jmor.20763>
10. Luchtel DL, Deyrup-Olsen I. Body Wall: Form and Function In: Barker GM, editor. *The biology of terrestrial molluscs*. Wallingford, UK: CABI Publishing, 2001; pp.147-178.
<https://doi.org/10.1079/9780851993188.0000>
11. Greistorfer S, Klepal W, Cyran N, Gugumuck A, Rudoll L, Suppan J, Von Byern, J. Snail mucus—Glandular origin and composition in *Helix pomatia*. *Zoology.* 2017; 122:126–38.
<https://doi.org/10.1016/j.zool.2017.05.001>
12. Czarnoleski M, Labecka AM, Kozłowski J. Thermal plasticity of body size and cell size in snails from two subspecies of *Cornu aspersum*. *J. Molluscan Stud.* 2016; 82:235–43.
<https://doi.org/10.1093/mollus/eyv059>
13. Berillis P, Hatzioannou M, Karapanagiotidis IT, Neofitou C. Morphological study of muscular tissue collagen of wild and reared *Cornu aspersum* (Müller, 1774). *Molluscan Res.* 2013;33 (1):6–13.
<https://doi.org/10.1080/13235818.2012.754143>
14. Malakauskiene S, Alioniene I, Dziugiene D, Babrauskiene V, Riedel C, Alter T, Malakauskas M. Histological analysis for quality evaluation of cured meat sausages. *Vet Med Zoot.* 2016;74(96): 23-6.
15. Matsuguma M, Takahashi M, Matsukuma N, Fujita M, Fujita S, Wada K. Evaluation of Palatability, and Physicochemical and Histological Properties of Papain-treated Minced Fish for Consumption by the Elderly. *Food Sci Technol Res.* 2014;20 (1):115-20.
<https://doi.org/10.3136/fstr.20.115>
16. Jamilah B, Mohamed S, Abbas KA, Abdul Rahman R, Karim R, Dalya H. Protein-starch interaction and their effect on thermal and rheological characteristics of a food system: A review. *J Food Agric Environ.* 2009;7(2):169-74.
<https://doi.org/10.1234/4.2009.1593>
17. Cimmino R, Barone C, Claps S., Varricchio E, Rufrano D, Caroprese M, Albenzio M, D Palo P, Campanile G, Neglia G. Effects of dietary supplementation with polyphenols on meat quality in Saanen goat kids. *BMC Vet. Res.* 2018;14:181.
<https://doi.org/10.1186/s12917-018-1513-1>
18. Ruiz De Huidobro F, Miguel E, Blazquez B, Onega E. A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Sci.* 2005; 69:527–36.

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<https://doi.org/10.1016/j.meatsci.2004.09.008>

19. Simitzis PE, Deligeorgis SG, Bizelis JA, Dardamani, A, Theodosiou I, Fegeros K. Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Sci.* 2008; 79:217–23.

<https://doi.org/10.1016/j.meatsci.2007.09.005>

20. Schreuders KG, Schlangen M, Kyriakopoulou K, Boom MR, Van der Goot A. Texture methods for evaluating meat and meat analogue structures: A review. *Food Control.* 2021;127.

<https://doi.org/10.1016/j.foodcont.2021.108103>

21. Mizuta S, Yamada Y, Miyagi T, Yoshinaka R. Histological Changes in Collagen Related to Textural Development of Prawn Meat During Heat Processing. *J. Food Sci.* 1999;64 (6):991-5.

<https://doi.org/10.1111/j.1365-2621.1999.tb12266.x>

22. Schubring R, Meyer C. Quality factors of Terrestrial Snail Products as affected by the species. *J. Food Sci.* 2002;67(8):3148-51.

<https://doi.org/10.1111/j.1365-2621.2002.tb08874.x>

23. Heller J, Gadot M. Shell polymorphism of *Theba pisana* - the effects of rodent distribution. *Malacologia.* 1984;25:349-54

24. Rosin ZM, Olborska P, Surmacki A, Tryjanowski P. Differences in predatory pressure on terrestrial snails by birds and mammals. *J. Biosci.* 2011;36:691–9.

<https://doi.org/10.1007/s12038-011-9077-2>

25. Cowie RH. Climatic selection on body colour in the land snail *Theba pisana* (Pulmonata: Helicidae). *Heredity.* 1990; 65:123–6.

<https://doi.org/10.1038/hdy.1990.78>

26. Burla H, Gosteli M. Thermal advantage of pale coloured morphs of the snail *Arianta arbustorum* (Helicidae, Pulmonata) in alpine habitats. *Ecography.* 1990; 16:345-350.

27. Zymantiene J, Zelvyte R, Jukna C, Jukna V, Jonaitis E, Sederevicius A, Mazeikiene, Z, Pampariene I, Zinkeviciene J. Selected features of vineyard snails shell, their movement and physico-chemical composition of foot meat. *Biotechnol. Equip.* 2006; 20:82-7.

<https://doi.org/10.1080/13102818.2006.10817310>

28. Araujo R, Remon JM, Moren, D, Ramos MA. Relaxing techniques for freshwater molluscs: trials for evaluation of different methods. *Malacologia.* 1995;36:29–41.

29. AOAC Official methods of analysis. 16th ed.; AOAC: Arlington, VA, USA; 1995.

30. Erkan N, Selcuk A, Ozden O. Amino Acid and Vitamin Composition of Raw and Cooked Horse Mackerel. *Food Anal. Methods.* 2010; 3:269–75.

<https://doi.org/10.1007/s12161-009-9108-x>

31. CIE Standard Psychometric Systems, Standard Colorimetry; 2015: 253-77.

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32. AMSA Meat Color Measurements Guidelines (2nd edition): American Meat Science Association; 2012.
33. SPSS, v. 23, IBM, New York, NY, USA; 2018.
34. Zar JH, editor. Biostatistical Analysis. (5th Edition). Upper Saddle River: Prentice-Hall/Pearson; 2010.
35. Apostolou K, Staikou A, Sotiraki S, Hatzioannou M. An Assessment of Snail-Farm Systems Based on Land Use and Farm Components. *Animals*. 2021;11(2):272.
<https://doi.org/10.3390/ani11020272>.
36. Borgo R, Souty-Grosset C, Bouchon D, Gomot L. PCR-RFLP Analysis of Mitochondrial DNA for Identification of Snail Meat Species. *J. Food Sci.* 1996;61(1): 1–4.
<https://doi.org/10.1111/j.1365-2621.1996.tb14712.x>
37. Barker GM. Gastropods on Land: Phylogeny, Diversity and Adaptive Morphology In: Barker GM, editor. *The biology of terrestrial molluscs*. Wallingford, UK: CABI Publishing; 2001. pp. 1-146.
38. Hatzioannou M, Exadactylos A, Panagiotaki P, Lazaridou M, Neofitou C. Setting Quality Standards for Farmed Snails *Helix aspersa*. Report, Ministry of Education and Religious Affairs, Greece; 2008.
39. Milinsk MC, Padre RG, Hayashi C, Souza NE, Matsushita M. Influence of feeds enriched with different vegetable oils on the fatty acids profiles of snail *Helix aspersa maxima*. *Food Chem.* 2003;82:553–8.
[https://doi.org/10.1016/S0308-8146\(03\)00010-4](https://doi.org/10.1016/S0308-8146(03)00010-4)
40. Bourne M, editor. *Food Texture and Viscosity*. Academic Press Inc; 2011.
41. Agoub AA, Giannouli P, Morris ER. Gelation of high methoxy pectin by acidification with D-glucono-d-lactone (GDL) at room temperature. *Carbohydr. Polym.* 2009;75:269–81.
<https://doi.org/10.1016/j.carbpol.2008.07.016>
42. Yang X, Li A, Li X, Su L, Guo Y. An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. *Trends Food Sci. Technol.* 2020;102:1–15.
<https://doi.org/10.1016/j.tifs.2020.05.020>

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Table 1. Morphological characteristics of the different snail species

Species	d/mm	h/mm	d/mm	m/g	m_f/g
<i>C. a. maximum</i> (farmed)	(40.0±3.3) ^a	(39.2±3.8) ^a	(23.1±3.5) ^a	(21.3±3.6) ^a	(2.1±0.6) ^a
<i>C. a. aspersum</i> (farmed)	(30.9±1.8) ^b	(30.5±2.2) ^b	(16.8±1.6) ^b	(8.4±1.4) ^b	(1.1±0.4) ^b
<i>C. a. aspersum</i> (wild)	(31.6±2.4) ^b	(30.8±2.2) ^b	(16.5±1.4) ^b	(7.8±1.2) ^b	(1.0±0.3) ^b
<i>H. lucorum</i> (wild)	(35.1±3.3) ^c	(31.9±3.2) ^b	(16.3±2.9) ^b	(12.7±2.5) ^c	(1.6±0.6) ^c

Results are expressed as mean value±S.D. ($N=57$). Data within the same column marked with different lowercase letters are significantly different at $p<0.05$ in ANOVA. D =snail shell diameter, h =snail shell height, d =snail shell aperture diameter, m =mass of whole raw snail, m_f =mass of raw fillet

Table 2. Proximate composition of fillets different snail species

Parameter	<i>C. a. maximum</i> (farmed)	<i>C. a. aspersum</i> (farmed)	<i>C. a. aspersum</i> (wild)	<i>H. lucorum</i> (wild)
w(moisture)/%	83.0±1.6	81.8±1.5	83.3±1.0	82.2±1.6
w(crude protein)/%	10.3±0.3	11.0±0.5	11.0±0.6	13.5±0.1
w(crude fat)/%	0.1±0.0	0.4±0.1	0.7±0.3	0.6±0.1
w(ash)/%	1.1±0.0	1.5±0.1	1.5±0.5	1.3±0.1
$E(kJ/g)$	20.5±0.15	20.3±0.2	20.0±0.2	21.1±0.2
w(carbohydrate)/%	5.5±0.2	5.4±0.5	3.4±0.4	2.4±0.1

Results are expressed as mean value±S.D. ($N=3$)

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Table 3. Color parameters of fillets of different snail species

Species	L^*	a^*	b^*	Hue	Chroma
<i>C. a. maximum</i> (farmed)	45.9±3.1	5.4±0.7	14.2±2.1	1.2	15.2
<i>C. a. aspersum</i> (farmed)	49.2±3.1	1.0±0.2	6.5±0.8	1.4	6.6
<i>C. a. aspersum</i> (wild)	35.2±2.2	1.1±0.2	9.4±1.3	1.5	9.5
<i>H. lucorum</i> (wild)	32.7±4.2	4.6±1.3	7.8±1.6	1.0	9.1

Results are expressed as mean value±S.D (N=5). L^* = lightness, a^* =redness and b^* =yellowness

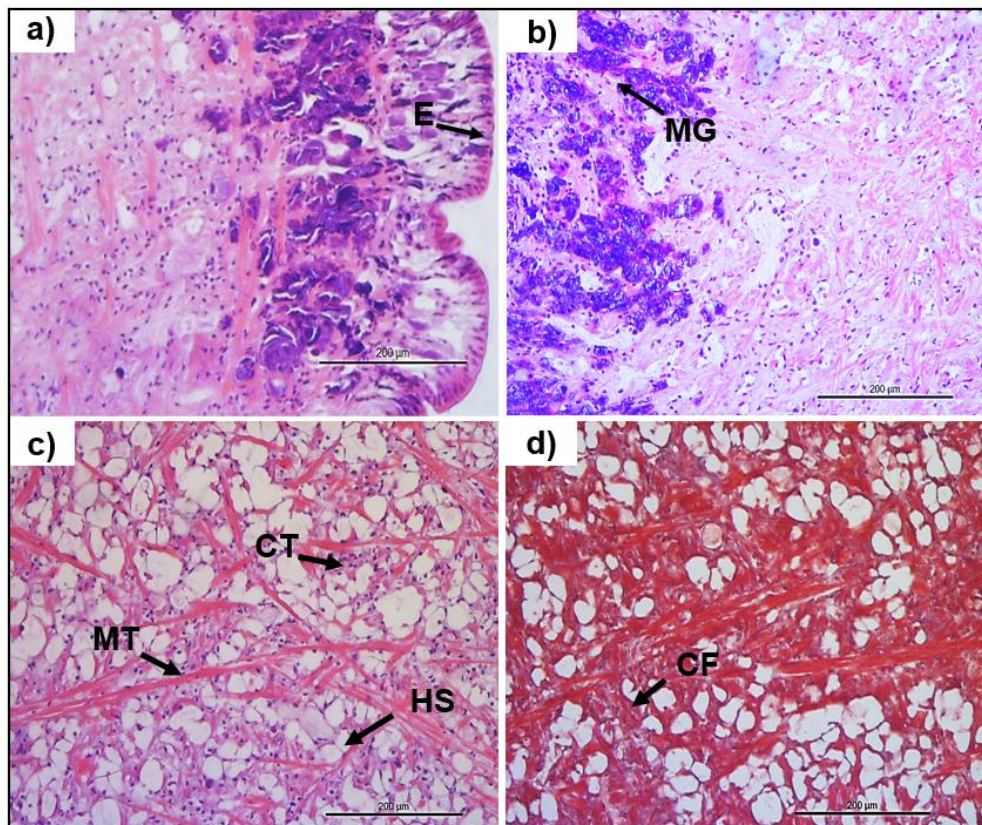


Fig. 1. Micrographs of longitudinal (a, c, d) and transverse section (b) of mid-posterior region of raw fillet of *C. a. maximum* snail with haematoxylin/eosin staining (a, b, c) and Masson's Trichrome Stain (d). Abbreviations: CF, collagen fibers; CT, connective tissue; E, epithelium; HS, system of haemocoelic sinuses (empty spaces); MT, muscle cells; MG, mucus gland. Scale bar: 200 µm

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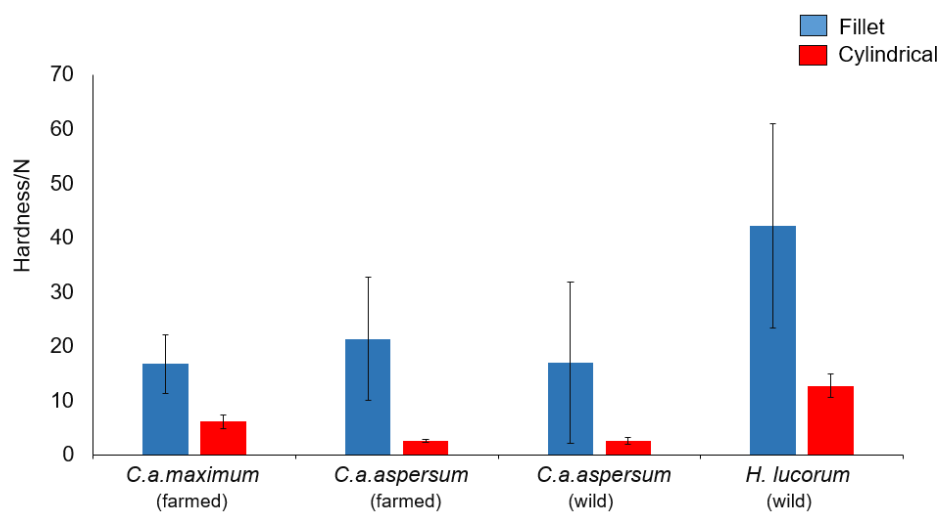


Fig. 2. Mean values \pm S.D. of hardness/N of whole raw fillets and cylindrical parts of fillets in farmed *C.a. maximum*, farmed and wild *C.a. aspersum* and wild *H. lucorum* snails.

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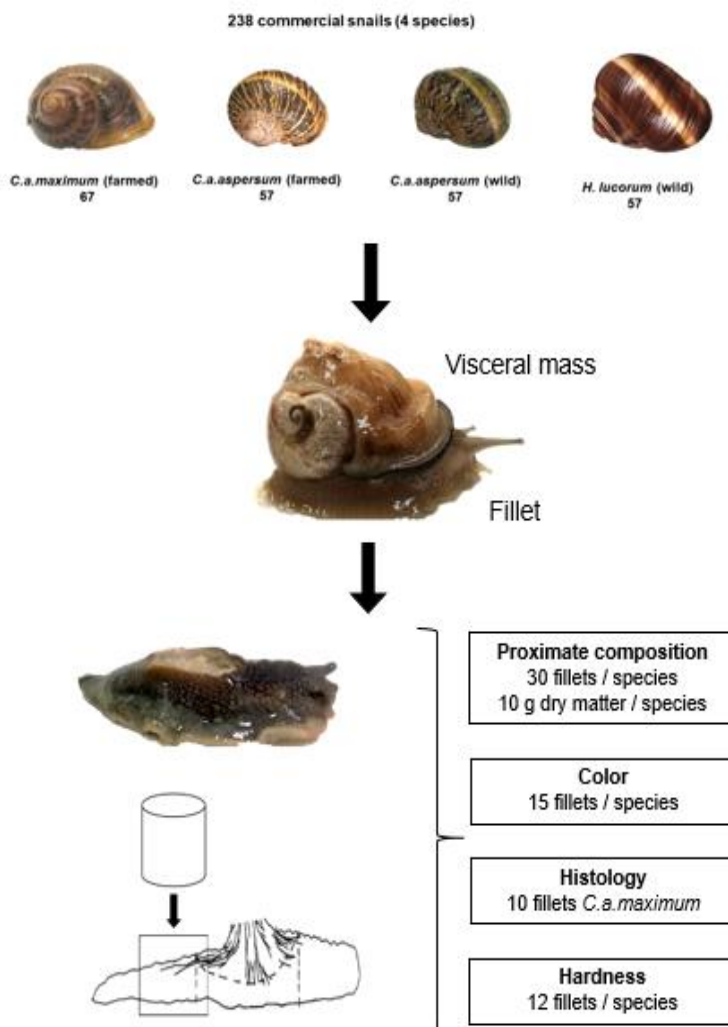


Fig. S1. Scheme of snail preparation before analyses