

## Microbiological and chemical analysis of land snails commercialised in Sicily

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### Abstract

In this study 160 samples of snails belonging to the species *Helix aspersa maxima* and *Helix aspersa muller* were examined for chemical and microbiological analysis. Samples came from Greece and Poland. Results showed mean concentration of cadmium ( $0.35 \pm 0.036$  mg/kg) and lead ( $0.05 \pm 0.013$  mg/kg) much higher than the limit of detection. Mercury levels in both species were not detected. Microbiological analysis revealed the absence of *Salmonella* spp. and *Clostridium* spp. in both examined species. *E. coli* and *K. oxytoca* were observed in *Helix aspersa maxima* and *Helix aspersa muller*. Furthermore, one case of fungi positivity in samples of *Helix aspersa muller* was found. The reported investigations highlight the need to create and adopt a reference legislation to protect the health of consumers.

### Introduction

Terrestrial gastropods, commonly named *chioccioline*, constitute a niche food product, traditionally appreciated by Sicilian consumers. These products have a seasonally marketing, with a peak in the summer period (Tedde *et al.*, 2009). Genus *Helix* (*H. aspersa*, *H. pomatia*, *H. lucorum* or Turkish snail) are the gastropods most commonly used for food production. They are usually considered as a minor food due to their low consumption. However, in some countries they are commonly consumed both in the breeding and capture forms (Sava, 2013).

*Chioccioline* production falls within the scope of EC Reg. n.852/04, while the transformation follows the EC Reg. n. 853/04 (European Commission, 2004a, 2004b). However, there is no legislation that lays down specific requirements for microbiological and chemistry of these products. Bivalve are the only molluscs class subjected to regulations (EC Reg. 1881/06; European Commission, 2006). These

regulations defines maximum levels of contaminants, including heavy metals, for food-stuffs, necessary insertion due to their accumulation ability. Numerous studies have been carried out using various species of terrestrial gastropods as bioindicators of environmental status (Regoli *et al.*, 2006). In consideration of the risk posed by the possible presence of potentially pathogenic micro-organisms in gastronomic preparations, which does not include cooking priority to consumption (Marongiu *et al.*, 1993), and in the absence of data relating to the possible contamination by heavy metals, it was deemed appropriate to gather information about microbiological and chemical properties of *chioccioline* samples commercialised in Sicily. *Chioccioline* can be considered as a pollutant vector due to their behaviour, as they live on soil and feed on plants with high heavy metals concentration (Scaffardi *et al.*, 2007). Furthermore, microbiological aspects seem to be very important for the hygienic aspects assessment because these snails are possible carriers of many parasitic and bacterial forms. In fact, predominant bacterial species such as *Enterobacteriaceae* (*Citrobacter*, *Morganella*, *Klebsiella* ed *Enterobacter*, *E. coli*, *Pseudomonadaceae*, *Proteus* spp., *Hafnia*, *Yersinia enterocolitica*), gram-positive *Staphylococcus* (*S. aureus*) and *Listeria* in the shell were detected. Frequently, the presence of fungi such as *Aspergillus* spp., *Chrysosporium* spp., *Fusarium* spp., *Rhizopus* spp. and *Mucor* spp. and parasites such as *Clonorchis sinensis* and *Fasciola* can be detected as well. *Aeromonas hydrophila*, *Escherichia coli* e *Listeria monocytogenes* are among the food-borne diseases agents that can be revealed (Cantoni, 2013). Stored methods such as freezing cannot be considered as a prevention mean for *Enterobacter cloacae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus* spp., *Listeria monocytogenes*, *Bacillus subtilis* and *B. cereus*. Microbiological risk is linked to the consumption of treated products (*ready to eat*) contaminated by *Listeria monocytogenes* and enterotoxic strain of *Staphylococcus aureus* and *Bacillus cereus* (Wallace *et al.*, 1975).

The aim of this study was to assess the presence of microbiological and chemical contaminations in gastropods samples from Poland (*Helix aspersa maxima*) and Greece (*Helix aspersa muller*).

### Materials and Methods

Of each species of gastropods, 80 samples were examined by the Veterinary Institute of Sicily laboratories in February 2014. The Chemistry and Food Technologies Area was responsible for the determination of heavy

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metals [cadmium (Cd), lead (Pb) and mercury (Hg)], while the Territorial Health Department was responsible of microbiological analysis. The validated methodology on heavy metals contamination in fish products has been retained the most analogous for the implementation of our analysis. The determination of heavy metals was performed by an inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 series; Agilent Technologies, Santa Clara, CA, USA), an ICP plasma torch analyser producing ionisation and a mass spectrometer for the ion separation and detection. Extraction of samples ( $\approx 1$  g for each one) was performed. Subsequently the extract was placed in a polytetrafluoroethylene (PTFE) microwave vessel with 2 mL of HNO<sub>3</sub> (65% ultrapure) and 5 mL of H<sub>2</sub>O for mineralisation. Vessels containing the samples were closed and placed in Multiwave digestion 3000 (Anton Paar) digester. After mineralisation the content was transferred into 50 mL PVC test tubes. Vessels were washed twice with 10 mL of ultrapure water, subsequently the content was transferred in 50 mL test tubes and diluted with ultrapure water for the ICP-MS detection. This method can reveal Cd, Hg and Pb concentration in mg/kg (ppm). Microbiological analysis was performed for the research of *Salmonella* spp., *Clostridium* (in particular *C. perfringens*), *E. coli*, *Yersinia*, *Listeria*, *Pseudomonas*, *Streptococcus*, *Klebsiella* and fungi. *Salmonella* spp. research has provided a pre-enrichment phase in buffered peptone water, an enrichment phase in selective broth, an isolation phase on selective media (XLD e BGA) and biochemicals and serological tests. *Clostridium perfringens* research was performed by a Blood Agar and anaerobic incubation. For *E. coli*, *Pseudomonas* spp. and *Klebsiella* spp. detection a McConkey agar was utilised; blood Agar

for *Streptococcus* spp.; enrichment broth and selective agar incubated at 25 and 30°C was utilised for *Yersinia* spp. and *Listeria* spp. determination. Fungi detection was performed with Agar Sabouraud. Isolates underwent catalase and oxydase tests, Gram coloration, Macromethods (KIA, TSI, Citrate, Ureasi test, Mobility test and Indole test) and Micromethods (API Kit) biochemical tests.

## Results

Series analyses with gastropods matrices were conducted for validation testing of utilised method. Obtained results reported statistically equal dataset ( $P < 0.01$ ) to fish products assessment on repeatability values, mean recovery and robustness of the method. Limits of detection (LOD) were calculated according to the modality described on standard operating procedure (SOP) 18 and 36 for validation parameter calculation. Examined *Helix aspersa maxima* samples revealed a mean concentration of Cd equal to  $0.35 \pm 0.036$  mg/kg. This concentration is considerably higher than the LOD of the method. Pb analysis showed a mean concentration of  $0.05 \pm 0.013$  mg/kg. Hg levels in both species were under the LOD (0.06 mg/kg). *Helix aspersa muller* samples did not reveal a considerable heavy metals levels (Table 1).

Micrbiological results (Table 2) confirm bibliography data (Adegoke *et al.*, 2011; Cirlan *et al.*, 2010; Iovino *et al.*, 2002; Kirkan *et al.*, 2006; Marongiu *et al.*, 1993; Temelli *et al.*, 2006). *Helix aspersa maxima* samples revealed a good hygienic condition. In particular, samples were negative to the most tested microbes except 4 that were positive to *E. coli*, 3 to *Pseudomonas luteola* and 1 to *Klebsiella oxitoca*. *Helix aspersa muller* samples detected 2 positive subjects to *Listeria innocua*, 1 to fungi and 1 to *Streptococcus*.

## Discussion

Obtained results on *Helix aspersa maxima* samples revealed a Cd concentration that cannot be negligible because data are significantly higher than the LOD of the method. These values can be traced to materials for agriculture such as sewage sludge containing high values of Cd that settle in the soil. Terrestrial gastropods living in the soil and vegetation act as bioaccumulator of these substances, thus becoming vectors through their consumption.

Daily uptake of Cd from food and beverages, in the absence of pollution, has been estimat-

**Table 1. Avarage concentration values of heavy metals.**

	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)
<i>Helix aspersa maxima</i>	$0.35 \pm 0.036$	$0.05 \pm 0.013$	<LOD
<i>Helix aspersa muller</i>	<LOD	<LOD	<LOD

Cd, cadmium; Pb, lead; Hg, mercury; LOD, limit of detection.

**Table 2. Dataset of microbiological analysis.**

Microorganisms	<i>Helix aspersa muller</i>	Positive (n)	<i>Helix aspersa maxima</i>	Positive (n)
<i>Salmonella</i> spp.	-	0	-	0
<i>Clostridium</i> spp.	-	0	-	0
<i>Escherichia coli</i>	+	12	+	4
<i>Pseudomonas</i> spp.	-	0	+ ( <i>P. luteola</i> )	3
<i>Klebsiella</i> spp.	+ ( <i>K. oxytoca</i> )	2	+ ( <i>K. oxytoca</i> )	1
<i>Streptococcus</i> spp.	+ ( <i>S. β emolitic</i> )	1	-	0
<i>Yersinia</i> spp.	-	0	-	0
<i>Listeria</i> spp.	+ ( <i>L. innocua</i> )	2	-	0
<i>Fungi</i>	+	1	-	0

ed in the UK to be between 10-30 µg in a 70 kg man (Reilly, 1980; EFSA, 2009). The ingestion of Cd causes symptoms such as nausea, vomiting, abdominal cramp and headache within minute of ingestion. Long-term ingestion of Cd results in serious disease of the kidneys. A recent FAO report established a weekly tolerable dose of cadmium equal to 400-500 mg. This would result in an average concentration in food of 0.04-0.05 mg/kg (EFSA, 2009). A recent EFSA report (2009) recognised a weekly tolerable dose equal to 25 g/kg. Consumption of contaminated foods is one of the most uptake ways for Hg exposition. High levels of Pb results in seious toxic effects in humans. Acute Pb poisoning manifests gastro-intestinal symptoms with dyspepsia, constipation with severe abdominal pain.

These results revealed terrestrial gastropods as heavy metal contamination vehicles for men, (in particular Cd and Pb detected in concentrations higher than LOD), although the EC Reg. 1881/2006 give higher limit values regarding marine molluscs. Microbiological aspects, considering that microbial flora is different between an *epifragmata* (pathogens free) and *corritrice* snail (that even dwells pathogenic saprophytic bacteria), declared a normative lack about the microbiological aspects. This notwithstanding, EC Reg. 2073/2005 establishes the limits for alive gastropods can be used as a valid mean for the consumer protection (Andrews *et al.*, 1975). The presence of microorganisms in foods can be due to food elaboration and treatment. The risk for consumers increases with the consumption of non-termically treated foods.

## Conclusions

A lack of legislative regulation suggests the need of control measures to protect the consumption of this product which is becoming increasingly popular and widespread in the food market. Hence, food business operators must pay attention throughout the production chain and distribution in order to avoid contamination by environmental and human origin bacteria. In general, a good control of spinneret coupled with a particular attention to raw materials quality and manufacturing practices respect, can be good prevention tools for foodborne disease prevention.

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