ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(6): 1050-1054
doi: 10.25083/rbl/24.6/1050.1054

Received for publication, June, 15, 2018
Accepted, October, 15, 2019

Original paper

Study on the influence of freezing on certain chemical compounds in *Rapana venosa* meat

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Abstract *Rapana venosa* is a marine snail, whose meat has a high biological value and currently represents a culinary delicacy for many restaurants with marine specificity. Therefore, in this paper, the chemical composition of the fresh Rapana meat processed manually, without boiling, but also industrially processed, in the aspect of water content, total protein, raw fat, mineral salts was analyzed. The experimental results revealed a decrease in the values of moisture and fat for the samples of Rapana meat boiled before shell removal. The value of the light hydrolysable nitrogen, the trimethyl amine nitrogen and the enzymatic activity of the superoxide dismutase from Rapana meat frozen at -18°C, for one week, two weeks and 4 weeks was also tracked. The results indicated an early process of proteolysis and amino acids deamination of Rapana meat during freezing and a partial inactivation of the superoxide dismutase enzyme, whose activity registered the lowest value after 4 weeks of freezing.

Keywords *Rapana venosa*, shell removal, enzymatic activity, superoxide dismutase.

To cite this article: MĂRCUȚĂ A-G, MARIN M-P, MĂRCUȚĂ L, DINIȚĂ G, DIACONESCU C, MARIUS H. Study on the influence of freezing on certain chemical compounds in *Rapana venosa* meat. *Rom Biotechnol Lett*. 2019; 24(6): 1050-1054. DOI: 10.25083/rbl/24.6/1050.1054

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Introduction

Rapana venosa is a marine snail belonging to the family Muricidae that lives on the sandy and rocky substrata from the shore line up to 30 meters deep. It is a large gastropod with a globular and thick, spiral shell. It reaches the length of 190 mm and the width of 160 mm and it reproduces through eggs, the incubation time being of about 17 days (TEACĂ & al [1]; AYDIN & al [2]).

The species *Rapana venosa* came to the Black Sea from the Sea of Japan and South China, 50 years ago, first at the mouths of the Danube and then towards the southern areas of the coast, becoming, in a short time, a common faunal element on the the Romanian continental shelf. In 2007, the average value of *Rapana venosa* stocks in Agigea – Eforie Nord, Tuzla and Costineşti locations was estimated at approx. 980 tons (TEACĂ & al [1]).

For a long time, its entering the Black Sea, was considered a disaster, because the main food consisted of mussels, but in the recent years the species has become an exploitable resource, thus having a positive effect on the economy and fishing communities. Rapana meat is very tasty and healthy, has a high biological value, and like most marine delicacies, contains a large number of mineral elements in quantities and forms that are easily absorbed by the body (DANILOV & al [3]; LUO & al [4]). For this reason Rapana has become a traditional product with high demands in restaurants with marine specificity. Only 1% of Rapana meat goes to the Romanian market, due to a very high demand for Rapana meat from Korea and Japan (TEACĂ & al [1]).

In view of the above, the present work aimed to analyze the chemical composition of the fresh meat of *Rapana venosa*, and to highlight some chemical changes that occur during its preservation by freezing.

Material and Methods

The used biological material was provided by a processing plant of *Rapana venosa*, respectively BBG ACCES CONSULT SRL, from the town of Cataloi, Tulcea county. To provided Rapana meat was proceeded as follows: a number of 20 pieces of Rapana, after denisipation by repeated washing, had their shell removed by manual grinding of the meat with a fork, eviscerated to completely remove the digestive tract, washed, divided into four lots, of which three were frozen and stored at -18°C, for one week, two weeks and four weeks. One batch was the control batch as a fresh fishery product (NICOLAE & al. [5]).

At the same time, the factory provided us with Rapana meat processed in an industrial style, as follows: the raw material was introduced into a water basin for sand removal, then the Rapanas were introduced into the sieve and lowered into a sterilizer where the boiling took place, at a temperature of at least 100°C, for 7 minutes, and last, the meat was extracted from the shell using a special fork and eviscerated. The body of the Rapana was washed with water jet, then the mucilage and the violet color were cleaned by rubbing with sea salt in a proportion of 30%. The finished product obtained is a clean, white-yellow flesh, which was frozen at -35°C, then packed and stored at -18°C.

Rapana meat, fresh or frozen, was shredded in a Porter homogenizer; a part of the homogenate was used as such and chemically evaluated by:

- determination of humidity (drying method in the oven at 103°C ± 2°C, for which 5 g homogenate Rapana meat was taken, and the calculation of the results was done using the classical formula) (DIACONESCU & al [6]);
- determination of total protein (Kjeldhal method for which 2 g homogenized Rapana meat was subjected to mineralization, the nitrogen content was determined and converted into protein equivalent by multiplying with a factor of 6.25) (DIACONESCU & al [6]);
- determination of the gross fat (Soxhlet method: 5 g homogenized Rapana meat was taken into work, from which the fat was extracted until exhausted with organic solvents, and, after removal of the extraction solvent, it was weighed and expressed as a percentage) (DIACONESCU & al [6]);
- determination of the total mineral substances (ash) for which 5 g homogenized Rapana meat was used, calcined in the electric oven at 525 ± 25°C up to the stated mass (BANU [7]);
- determination of the light hydrolysable nitrogen: the method involves the release of ammoniacal nitrogen using a weak base of 10 g fresh Rapana meat, steaming, capturing in an acid solution of known concentration and titration with a 0.1N NaOH solution in the presence of methyl red as indicator (BANU [7]);
- determination of trimethyl amine nitrogen: from 100 g Rapana meat subjected to distillation, the difference between the nitrogen content of volatile bases and the nitrogen content of ammonia and primary amines is trimethyl amine nitrogen (BANU [7]).

Another part of the homogenized frozen Rapana meat was suspended in a saline solution (0.9%), in a ratio of 1:4 (w/v), centrifuged, and the extracts were subjected to the following chemical investigation which consisted in determination of the activity of an antioxidant enzyme present in the cytosol, respectively superoxide dismutase (SOD-Winterbourn spectrophotometric method described by IORDĂCHESCU & al [8]). The method is based on the ability of SOD to inhibit the reduction of tetrazolium salt (NBT^{2+}) by superoxide radicals to a blue formazan. The staining intensity was estimated photocolorimetric at λ = 560 nm. Superoxide radicals are generated in the reaction medium by photochemical reduction of riboflavin. Superoxide dismutase from Rapana meat protein extract decomposes some of the superoxide

radicals, and the amount of formazan formed is smaller. The lower the amount of formazan indicates the more intense the enzyme activity of SOD. One unit of enzyme activity represents the amount of enzyme that produces 50% inhibition under standard conditions:

$$\text{SOD (U/mg)} = \frac{a\%\ inhibition}{50\% \times\ mg\ /\ ml\ protein}$$

Results and Discussions

The values of moisture, protein, raw fat and total mineral substances (ash) in fresh Rapana meat are shown in Table 1. Each value represents the average of five determinations / batch of fresh Rapana meat.

Table 1. Results of chemical analyzes performed on fresh *Rapana Venosa* meat

Species	Water (%)		Protein (%)		Fat (%)		Mineral substance (%)	
	Probes	Average ($x \pm s_x$)	Probes	Average ($x \pm s_x$)	Probes	Average ($x \pm s_x$)	Probes	Average ($x \pm s_x$)
Fresh Rapana meat not boiled*	75.40	**75.10 ± 0.30**	9.60	**9.52 ± 0.13**	1.30	**1.10 ± 0.05**	1.10	**1.03 ± 0.04**
	75.80		9.35		1.10		1.10	
	75.10		9.10		1.05		1.05	
	74.00		9.85		1.00		1.00	
	75.20		9.70		1.07		0.90	
Fresh Rapana meat, boiled*	72.50	**72.82 ± 0.11**	8.90	**9.09 ± 0.09**	0.45	**0.58 ± 0.05**	1.00	**0.95 ± 0.04**
	72.80		9.10		0.65		0.95	
	73.00		9.20		0.47		0.80	
	73.10		8.90		0.65		1.00	
	72.70		9.35		0.70		1,04	

*Fresh Rapana meat, edible, extracted without boiling;**Fresh Rapana meat, edible, extracted in industrial system, with boiling;
*Each value in the table represents the average ± standard error of the mean.

The data in Table 1 showed that for parameters water, total protein, fat and ash determined in uncooked fresh Rapana meat values are found to be relatively close to those reported in the literature, respectively: water-maximum 82%, total protein-10%, fat 1.04-1.86%, mineral substances 0.5-1% (TUDOR [9]).

The free water content is a valuable chemical indicator as it indirectly reflects the proportion of dry matter that concentrates all the nutrient-bearing substances; the percentage of water in fresh Rapana meat is similar to that of lean fish meat, such as the perch for which the literature indicates a value of 78%, and even higher than in the meat of slaughter animals whose water content is on average 74% (PURGE [10]).

The water content in different species of marine animals is variable as it is closely correlated with the fat content, which, in fact, is the only component of the meat with great variability (BANU [7], PURGE [10]); in the case of Rapana meat, the edible part has a very low fat content, respectively on average 1.10% and therefore these mollusks are considered dietary products (TUDOR [9]).

Fresh Rapana meat contains on average 9.52% total protein, but, like fish meat, they are rich in essential amino acids giving biological value to this type of meat (PURGE [10]).

The content of mineral salts in Rapana meat is small, respectively 1.03%, but as provided by the literature, they contain all anions and cations of vital importance for human trophic needs (TUDOR [9]).

Regarding the batch of Rapana industrially processed, by boiling, we note that the process influenced the water content that reached 72.82%. It is known that during boiling, foods with a high water content will undergo a reduction in their volume by partially eliminating it (PURGE [10]). Chipping or boiling in superheated steam is a method used especially for boiling meat products.

A significant decrease appears in terms of the gross fat content, from 1.10%, to 0.58%, knowing that under the influence of the high temperature, the fat is liquefied (BANU [11]). From the point of view of protein and mineral content, the differences are insignificant, respectively 9.59% compared to 9.09% for proteins and 1.03% compared to 0.95% for mineral salts, which justifies us to consider that the process of short-term boiling (respectively 4 minutes at 100°C), rather affects some vitamins and enzymes (PURGE [10]).

Rapana meat was stored by freezing (-18°C), for 1, 2 and 4 weeks, thawed and an analyzed. The analysis focused on the light hydrolysable nitrogen content, trimethyl amine nitrogen and the activity of an antioxidant enzyme present in the cytosol, respectively superoxide dismutase (SOD). The values of these parameters can be found in Table 2.

Table 2. Results of chemical analyzes performed on frozen *Rapana Venosa* meat

Species	Preservation type	Determined chemical parameter		
		Lightly hydrolysable nitrogen (mg NH_3 /100 g) ($x \pm s_x$)	**Nitrogen fromTMA (mg/100 g) ($x \pm s_x$)**	**SOD activity (U/mg) ($x \pm s_x$)**
Rapana meat, edible part	Fresh meat, manually eviscerated	12.9±0.12	0.08±0.02	3.05±0.03
	Frozen for a week	20.5±0.10	1.06±0.01	2.55±0.09
	Frozen for two weeks	26.1±0.11	1.38±0.01	2.40±0.02
	Frozen for four weeks	29.05 ±0.09	2.11±0.05	1.45±0,08

*Each value in the table represents the average of five determinations ± standard deviation ± standard error of the mean

The data in Table 2 showed that the light hydrolysable nitrogen from Rapana meat registers significant quantitative growth during the storage in a frozen state, from 12.9 ± 0.12% in fresh meat, to 20.5 ± 0.10 % after one week of freezing, up to 29.5 ± 0.09% after 4 weeks of freezing. This indicates an early process of proteolysis and deamination of Rapana amino acids even during freezing (BANU [7]).

The literature states that up to a value of 30 mg NH_3/100 g, lobsters, lobsters and other frozen mollusks are considered good for consumption (BANU [7]; DUYFF [12]).

Similar results are registered in the case of trimethyl amine nitrogen (TMA), which from the value of 0.8 ± 0.01 mg nitrogen from TMA / 100 g determined in fresh Rapana meat, has increased slightly, after one week and 2 weeks of freezing, so that at the end of the fourth week of freezing it reached the value of 2.11 ± 0.05 mg nitrogen from TMA / 100 g. It is known that trimethyl amine (TMA) is produced by reducing trimethylamine oxide under the action of enzymes secreted by the altering bacteria typical of the marine environment, so the freezing-thawing process, even for a long time, does not completely cancel the bacterial attack (BANU [11]).

Determination of SOD activity revealed significant differences between fresh and frozen Rapana meat hydrolysate, respectively 3.05 ± 0.03U / mg compared to 1.45 ± 0.08U/mg after 4 weeks of freezing, due to partial inactivation of the enzyme by freezing. The decrease in SOD activity in extracts obtained from Rapana frozen meat may be due to changes produced by the reactive oxygen species within the amino acid chain, at the level of the hydrophobic core radicals of the enzyme molecule: the literature specifies that reactive oxygen species affect these positions with the formation of carbonyl derivatives, accompanied by the total or partial inactivation of the enzyme (PAPUC et al. [13]; PAPUC et al [14]). Moreover, Blanchard and Mantle (1996) suggested that species of free radicals in oxygen influence the conditioning of fish meat at refrigeration temperatures (BLANCHARD & al [15]). SOD activity was minimal in the case of frozen Rapana meat extract for 4 weeks.

Conclusions

Summarizing the results, we can conclude the following:

- the industrial processing of Rapana meat, which required the boiling operation, significantly influenced the fat content, for which values of 0.58% were registered, compared to 1.10% in the case the meat extracted without boiling;
- the short-term boiling process, respectively 4 minutes at 100°C, does not significantly affect the protein and mineral content of the processed Rapana meat;
- the long-term freezing of the Rapana meat, respectively 4 weeks, determined an early process of proteolysis and amino acid deamination, as well as decreased activity of the superoxide dismutase enzyme;
- the freezing of Rapana meat, at a temperature of -18°C, triggers processes that affect proteins and enzymes, processes that are proportional to the storage period.

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