

Preparation and evaluation of appertized from snail *Helix aspersa* M.

Preparación y evaluación de apertizado elaborado con caracol *Helix aspersa* M.

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Abstract

This study includes the development and evaluation of snails (*Helix aspersa* M.) appertized, collected at a heliciculture breeding center, located in Los Niches sector, Curico, Maule region, South-central of Chile. The test was conducted at the Laboratory of Sciences of the Catholic University of Maule, Nuestra Señora del Carmen Campus, Curico. The main objective of this work was to study the influence of appertized on sensory attributes and commercial durability of snail *Helix aspersa* M. Additionally, some specific objectives were proposed as follow: to provide this mollusc with a commercial alternative for it consume, to evaluate its organoleptic characteristics and guarantee the product from both the microbiological and nutritional points of view. Three media cover were used (T₀: water + NaCl 2%; T₁: Water + NaCl 2% + citric acid 0.5% + kilol and T₂: extra virgin olive oil + spices + tocopherol). The product was assessed at two different times, after 30 and 90 days of storage. Two sensory evaluations were conducted to measure various organoleptic attributes and acceptability of the appertized by 14 trained panelists. Amino acid, vitamins, cholesterol, acidity, heavy metals, phosphorus and organochlorines analysis were performed. The presence of both total and fecal contaminant microorganisms was determined. Attributes such as color, flavor, aroma, texture and overall acceptability were also measured. Preserves made by T₀ and T₁ treatments were equally accepted by the panelists. However, preserve from treatment T₂ was rejected because of the detection in them of a very dark color, odor and mealy texture. Positive results regarding the content of amino acids, vitamin C and low cholesterol, as well as the absence of pathogenic microorganisms were obtained for the three treatments.

Key words: *Helix aspersa* M., appertized, microbiological, sensory and chemical evaluation.

Resumen

En el Laboratorio de Ciencias de la Universidad Católica del Maule, Campus Nuestra Señora del Carmen, Curicó, Chile, se elaboraron y evaluaron apertizados de caracoles (*Helix aspersa* M.), adquiridos en un plantel de helicicultura ubicado en el sector Los Niches. El objetivo general fue evaluar la influencia del apertizado en los atributos sensoriales y durabilidad comercial de los caracoles. Como objetivos específicos se planteó otorgar a este molusco una alternativa comercial de consumo, evaluar sus características organolépticas y garantizar el producto desde el punto de vista microbiológico y nutritivo. Para ello se utilizaron tres medios

de cobertura (T_0 : agua + NaCl al 2%; T_1 : agua + NaCl al 2% + ácido cítrico 0,5% + kilol y T_2 : aceite de oliva extra virgen + tocoferol + especias) y dos tiempos de evaluación, después de 30 y 90 días de almacenamiento. Se realizaron, además, dos evaluaciones sensoriales para medir diferentes atributos organolépticos y la aceptabilidad del antipasto por parte de 14 panelistas entrenados. Se realizaron análisis de aminoácidos, vitaminas, colesterol, acidez, metales pesados, órgano-clorados y fosforados. Desde el punto de vista microbiológico se determinó la presencia de microorganismos totales y fecales contaminantes. En la evaluación sensorial se midieron los atributos de color, sabor, aroma, textura y aceptabilidad general. Los resultados mostraron que conservas elaboradas según los tratamientos T_0 y T_1 fueron igualmente aceptadas por los panelistas, por el contrario, conservas con el tratamiento T_2 fueron rechazadas, ya que se detectaron en ellas color muy oscuro, olor desagradable y textura harinosa. Además, se presentaron resultados positivos en los tres tratamientos con respecto al contenido de aminoácidos, vitamina C y bajo colesterol, así como también la ausencia total de microorganismos patógenos.

Palabras clave: *Helix aspersa* M., apertizados, evaluación química, microbiológica y sensorial.

Introduction

Chile has very favorable conditions for snail breeding for instance the climate is mild temperate which facilitates reproduction over a longer period of the year, compared with Europe, where long cold winters cause the animal to stay longer in hibernation, which is the reason behind the production under controlled environments, meaning higher costs of production (Daguzan, 1989). According to Bazán (2005) in recent years there has been a milestone in the history of national heliciculture, as it was completed the first export of live operculated snail, raised under intensive system, whose final destination was Spain. In the field of so-called exotic meats, terrestrial snails represent a large potential market, since their meat is low in fat, its amino acid content is important, its cholesterol level is probably low and mainly because it is a development alternative for the small entrepreneur, who could diversify its products (Martínez and Ballester, 2005). The conservation of this mollusk through an appertized, together its sensory, nutritional and security evaluation, would allow the potential buyer to be sure of what is being bought and to appreciate its organoleptic characteristics after long storage period (Corfo, 1990; Achipia, 2014a). In Chile, the information on physico-chemical, microbiological and organoleptics for the crude product as well as for the appertized, using tin-based containers, is scarce and fragmented

(Schmidt-Hebbel and Pennacchiotti, 1985). Therefore, in 1997 was born the food sanitary regulation in Chile which is also used by appertized products such us of the present research to support the sanitary condition of the final product elaborate (Achipia, 2014 b). This study, hypothesized that: the appertizing process would contribute to optimize the organoleptic characteristics and acceptability of snails *Helix aspersa* M.

The general objective in the present research was to evaluate the influence of appertizing on the sensory attributes and commercial durability of snails (*Helix aspersa* M.) for human consumption. To support the hypothesis and the general objectives it was developed the following specific objectives, to evaluate the organoleptic characteristics and acceptability of the mollusk, through appertizing. Besides to guarantee from the microbiological and nutritional points of view the consumption of appertizing snails.

Materials and methods

Raw material (*Helix aspersa* M.) was provided by a heliciculture breeding center, located on the way to Rauco, in the province of Curico, Maule Region VII (34° 55' 36.66" South Latitude and 71° 17' 04.95" West Longitude), about 20 km west of Curico City, which has about 20,000 mollusks, with September and April the months for breeding them and June the harvest time to supply this investigation (M.O.P., 2007). This cen-

ter has an intensive breeding system with a complete life cycle, protected under greenhouse, with humidity control from 80% to 95%, maintaining temperature between 18°C to 21°C and balanced diet based on wheat flour, chickpea and calcium 95% purity. For the preparation of conservate or appertized, 200 mL glass containers were used with a net weight of 125 g. A completely randomized design in blocks and the experimental unit was the 15 units of snail in a 200 mL bottle. It was applied three treatments with three replication and also for chemical,

microbiology and sensory evaluations were used snails samples from four bottles plus three replication. The results obtained were subjected to analysis of variance (Anova) and where significant differences were detected, a multiple range Tuckey test, with a significance level of 5%, was performed (Little and Hills, 1998) (Table 1). After the conserved product was stabilized, i.e. at 30 and 90 days of storage in the warehouse, chemical, microbiological (presence of *Escherichia coli*) and sensory evaluations were made (Figure 1 and Table 2).

Table 1. Treatments used in the assay.

Treatment	Media coverage
T ₀	(Control) Water + NaCl2%.
T ₁	Water + NaCl 2%+ citric acid, pH 4.5, 80% purity and United States Pharmacopeia (USP) quality + kilol (active ingredient: Pomelo grapefruit extract in doses of 30 ppm).
T ₂	Extra virgin olive oil + antioxidant (active ingredient: tocopherol in doses of 10 ppm).

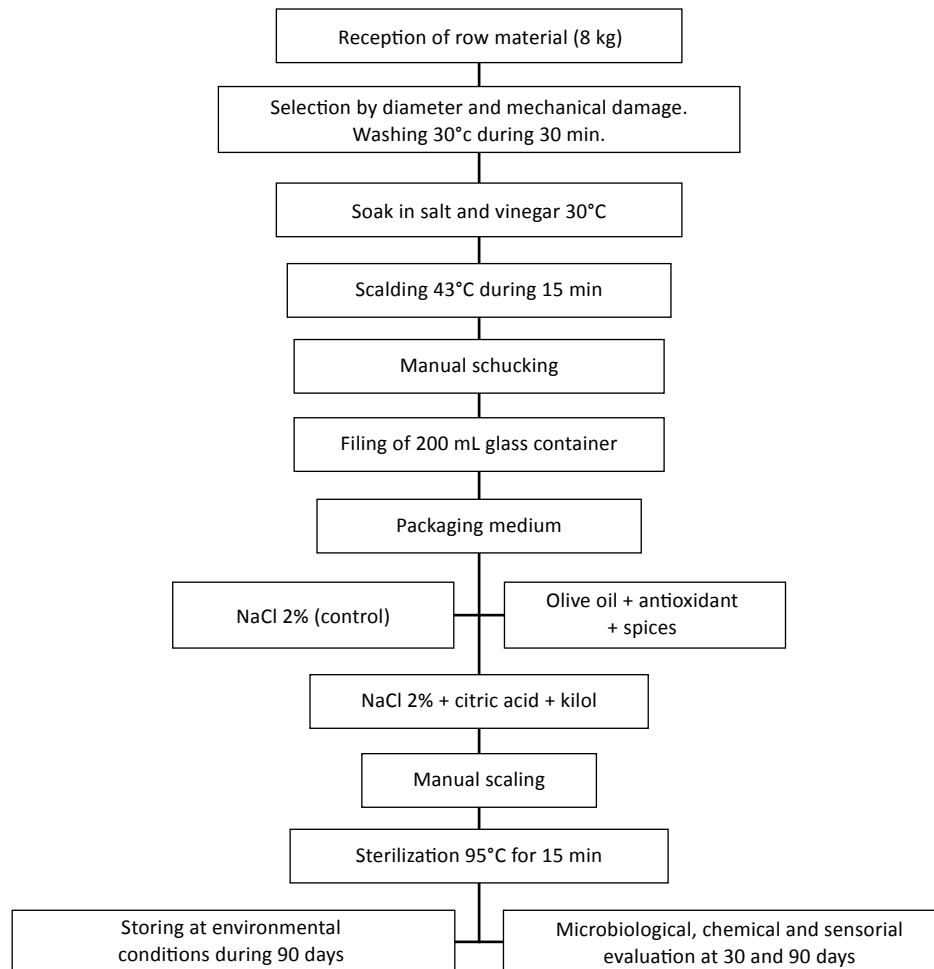


Figure 1. Flow chart for the production of snail (*Helix aspersa*) appertized.

Source: Desrosier, 1994.

Table 2. Chemical and microbiological evaluations.

	Reference
Determination of amino acid content	A.O.A.C. Official method 994.12. 1997
Determination of total cholesterol	A.O.A.C. Official method 2001.11. 1997
Determination of acidity	A.O.A.C. Official method 939.05. 2000
Determination of vitamin C	A.O.A.C. Official method 967.21. 2000
Determination of heavy metals Pb, Cd	A.O.A.C. Official method 999.11. 2000
Determination of heavy metals As, Sb, Sn	A.O.A.C. Official method 985.16. 1990
Determination of heavy metals Hg	A.O.A.C. Official method 988.11. 1992
Determination of organochlorine and organophosphorus	Vives, 2003
Determination of <i>Escherichia coli</i>	A.O.A.C. Official method 966. 24. 2000
Determination of contamination with fecal organisms (Enterobacteriaceae and <i>Clostridium</i> sp.)	A.O.A.C. Official method 991.14; 998.08. 2000

Source: Adapted from Association of Official Analytical Chemistry (A.O.A.C.).

Sensory evaluations were conducted with the participation of 14 panelists, who were properly trained prior to the analysis, according to the assessment guides used (Figure 2 and Figure 3). The panelists abstained from smoking, eating and drinking one hour before tasting. Furthermore, they rinsed their mouth between the tasting of each sample, to avoid confusion later (Stone

and Sidel, 1993). The test was applied by researchers who at random selected samples with different treatments, offering the attributes measured such as; aroma, color, flavor and texture in non-structured records, besides appearance and general acceptability of the snails, using a structured-type record in this case (Witting, 2001).

Name: _____ Date: _____ Sample: _____

Color |-----|
Light Dark

Flavor |-----|
Tasteless Acid

Aroma |-----|
Tasteless Strong

Texture |-----|
Mealy Cohesive

Indicate presence of flavors/aromas (with an X) Natural to Nuts Sweet Metallic Salty Astringent
 Old-Rancid Bitter Earthy Aquacious Others (please specify).

Figure 2. Guide for sensory evaluation of snails *Helix aspersa* M. appetized.
Source: Adapted from Stone and Sidel, 1993.

Name: _____ Date: _____ Sample: _____

General Acceptance

A. Excellent	9
B. Very good	8
C. Good	7
D. More than regular	6
E. Fair	5
F. Less than regular	4
G. Bad	3
H. Very bad	2
I. Unacceptable	1

Comments

Figure 3. Guide for general acceptance of snails *Helix aspersa* M. appetized.
Source: Adapted from Stone and Sidel, 1993.

Guide for sensory evaluation of snails appertized. You have been selected to participate in the sensory evaluation of snail appertized, product about to hit the market. You will receive a sample of the product and your opinion is sought according to the sensory evaluation sheet attached. You are asked to mark with a vertical cross over 13 cm line in each parameter, depending on the degree of perceived intensity from low to intense, from left to right, respectively.

Guide for general acceptance of snails appertized. You have been selected to participate in the sensory evaluation of snail appertized, product about to hit the market. You will receive a sample of the product and your opinion is sought according to the sensory evaluation sheet attached. Circle the score indicating its acceptability, according to a 1 - 9 scale, corresponding to the indicated grades from unacceptable to excellent, respectively.

Results and discussion

Microbiological evaluations

The absence of total coliforms and fecal contaminants was confirmed for the three treatments of snails appertized, both after 30 days, considered time 0 in storage and at 90 days considered time 1. The absence of thermophilic and mesophilic microorganisms, both the *Clostridium* sp. and coliform *Enterobacteriaceae* family, which includes the species

Escherichia coli, showed that appertized products prepared according to the methodology of this study is commercially sterile and could be marketed without undergoing any alteration of microbial origin (Table 3). These results were according to the Chilean food microbiology regulation (Reglamento Sanitario de los Alimentos, 1996) which must be considered before the consumer may include it in their normal diet. In this same sense the results obtained in this essay were positive related with the issues of the Codex Alimentarius (2003) and FAO and OMS (1983). Present research supports the previously mentioned authors because they are references in food safety related with molluscs and fish as well as well hygienic practices in the food industry.

In 1973, the Food and Drug Administration (FDA) required HACCP controls for conserved foods with low acid to prevent botulism problems in the population, due to *C. botulinum*, concluding on the need for reviewing its current program to regulate food safety, in order to replace it with a preventive HACCP system, as it does with seafood (FAO, 1996). The results obtained in the microbiological evaluation, allowed to verify the effectiveness of the procedures used for cleaning and disinfection during the development of this study, also checked that the temperatures used in the process were appropriate.

Table 3. Microbiological control for the three treatments at 30 and 90 days.

Microorganisms/ Time	Mesophilic Aerobics	Mesophilic Anaerobics	Thermophilic Aerobics	Thermophilic Anaerobics
30 days (t ₀)	Absence	Absence	Absence	Absence
90 days (t ₁)	Absence	Absence	Absence	Absence

Chemical evaluations

Regarding the content of amino acids, it was noted the presence of most of the essential amino acids such as isoleucine, phenylalanine, leucine, lysine, methionine, threonine and tryptophan in the three treatments. Cholesterol content, acidity and vitamin C, is shown in (Tables 4 and 5). According to Bazán (2005) snail meat has low levels of cholesterol and fat, 10 g of meat have 10 mg

of cholesterol. The limit of cholesterol in foods should be between 140 mg/dL and 250 mg/dL (FAO, 1997).

The results obtained for the three treatments of the present study showed a content below the limit, being even more pronounced in treatments T₀ and T₁. With respect the presence of vitamin C in snail meat, the recommended daily allowance is 60 mg to maintain a total body of a gram

and a half. When food is packaged or conserved, it often contains residual oxygen which can react with the food and cause rancidity, loss of color, among others, but the natural or added vitamin C, is responsible for fixing or removing this oxygen (INTA,

1993; Festy, 2007; Challem y Block, 2008). Results from the determination of heavy metals, lead (Pb), arsenic (As), antimony (Sb) and tin (Sn), in addition, organochlorine and organophosphorus are shown in Table 6.

Table 4. Cholesterol content, acidity and vitamin C for each treatment during the time 0 (30 days of storage).

Analysis/Treatment	Cholesterol (mg/dL)	Acidity (I.A.)	Vitamin C (mg/mL)
T ₀	31.45	3.83	0.083
T ₁	1.86	9.33	0.080
T ₂	138.99	7.60	0.080

Table 5. Cholesterol content, acidity and vitamin C for each treatment during the time 1 (90 days storage).

Analysis/Treatment	Cholesterol (mg/dL)	Acidity (I.A.)	Vitamin C (mg/mL)
T ₀	21.58	1.35	0.075
T ₁	1.58	3.12	0.079
T ₂	112.63	3.01	0.085

Table 6. Determination of heavy metals, organochlorines and organophosphorus for each treatment during time 1 (90 days of storage).

Analysis/Treatment	Pb (mg/kg)	As (mg/kg)	Sb (mg/kg)	Sn (mg/kg)	Organochlorines (mg/kg)	Organophosphorus (mg/kg)
T ₀	< 0.01	< 0.03	< 0.05	< 0.05	Undetected	Undetected
T ₁	< 0.01	< 0.03	< 0.05	< 0.05	Undetected	Undetected
T ₂	< 0.01	< 0.03	< 0.05	< 0.05	Undetected	Undetected

Appertized under the three treatments showed a low content of heavy metals and it was not detected in them the existence of organochlorines and phosphorus, which is of great importance to the future health of consumers, as these compounds can become serious contaminants.

The maximum lead content in food is regulated by the Regulation 466/2001 of FAO Council (1997) and the limits are set for products such as milk 0.2 mg/day, meat supply 0.1 mg/day, fats and oils 0.1 mg/day, bivalves molluscs 1.5 mg/day and between 0.05 mg/day and 0.2 mg/day for vegetables (FAO, 1997). Regarding arsenic, this element may be carcinogenic in humans and under current legislation, the maximum level of ar-

senic in the manufacture of animal food is 2 mg/kg to 4 mg/kg and in the case of feed is 1 mg/kg to 4 mg/kg (FAO, 1997).

According to Gallo (2002) studies involving the soft body of the snail for the determination of oligoelements in the specie *H. aspersa*, showed traces content in relation to elements lead and tin. This coincides with the results obtained in the present study in which the amount of lead was less than 0.01 mg/kg and tin less than 0.05 mg/kg in appertized under the three treatments T₀, T₁ and T₂.

In general, all the results from chemical evaluations conducted in each of the treatments, allowed to corroborate the results from other studies that positioned the terrestrial snail meat among the best food in terms

of quality and nutrition. According to Bazán (2005) the features found in the flesh of these mollusks is mediated by the transformation of plant proteins into animal protein of high both biological and gastronomical quality.

Sensory evaluations

Results at time 0, equivalent to 30 days of storage and time 1, at 90 days of storage, are presented in Tables 7 and 8, respectively.

Color. The results of analysis of variance calculated for the attribute color, showed that in both storage times there were statistically significant differences between treatments T_0 , T_1 and T_2 , and Tukey test determined that the T_1 treatment differed from the rest. The statistical difference of T_1 , is provoked by a higher score given by the panelists in the sensory evaluation sheet with respect to the perceived intensity of this attribute. The panelists felt that snails under T_1 treatment showed a lighter color which contrasted with darker color for T_0 and T_2 .

Color results presented by T_1 is justified by the presence of ingredients as kilol and citric acid in this treatment (Table 7). Kilol, which possesses antioxidant activity, helps maintain the organoleptic characteristics, conservation and/or stability of the food, does not alter the food nutritional value, protects from protein degradation, prevents oxidation of lipids, among others. This product is used in the food industry, since it is a seed extract (80%) and pulp (20%) of orange and composed of other natural elements, ascorbic acid (vitamin C), glycerides, tocopherols, aminoacids and other non-toxic, being currently the most widely used preservative in the food industry for salmon in Chile (ISP, 1998). Meanwhile citric acid in packed pork meat, stabilizes the color; additionally, for the case of bacon and cured meats, it promotes the appearance of color and prevents black spots on shrimp (ISP, 1998).

Taste. Scores given by the panelists with respect the intensity perception for this attribute was similar for each treatment. They considered that for snails from T_0 , T_1 and T_2 the trend showed bland flavor. The ingredients used in the manufacture of each conserved food or treatment had no significant impact on the flavor of the snails (Table 8). Only some

panelists, in the comments and observations of the evaluation sheet, showed that those with T_2 treatment presented a flavor similar to olives. This last, because one of the ingredients of T_2 was extra virgin olive oil. The extra virgin olive oil (oleic acid does not exceed 1 g/100g) containing mainly glycerides composed by a mixture of saturated and unsaturated fatty acids, the unsaturation is mainly due to oleic acid content between 67% and 83%, and only about 2% is fully unsaturated (Desrosier, 1994).

Aroma. According to the evaluation of the panelists, preserves under T_2 treatment had a higher score. They perceived, in this treatment, a sensory attribute intensity as highly strong smell and unpleasant. Different was the assessment for preserves with T_0 and T_1 , which were evaluated with a bland and pleasant aroma, similar to freshly prepared snail. The big difference in the content of T_2 relative to T_0 and T_1 was the use of extra virgin olive oil as a medium of cover, also the antioxidant tocopherol and some spices were added. Perhaps the negative result in the attribute aroma, was as a result of deterioration or decomposition of olive oil, after being subjected to high temperatures for sterilizing the appertized. When oxidation is the main problem in the deterioration of the oil, the amount of oil that undergoes these changes may be small, but resulting odors are strong, very sharp and out of proportion to the volume of oil damaged (Desrosier, 1994).

Texture. According to the evaluation given to the three treatments by the panelists, preserves under T_2 treatment received the lowest score regarding the texture attribute. According to the panelists, snails texture for T_2 treatment was described as mealy. Different evaluation received treatments T_0 and T_1 in which the texture achieved higher values, being more cohesive and described as similar to the original texture of the mollusk (Table 8). The negative results for T_2 for this sensory attribute, are due to oxidation and decomposition of the olive oil. According Desrosier (1994) oxidation can influence the texture resulting in product viscosity. In relation to the generally accepted treatment, preserves made as T_0 and T_1 , were similarly accepted by the panelists. Preserves from

treatment T_2 were rejected, as very dark color, odor and mealy texture were detected on them. The general acceptance of snails with treatments, T_0 and T_1 by the panelists,

is justified by the positive results of these two treatments presented in the evaluation of sensory attributes, flavor, aroma and texture and the color, especially in T_1 .

Table 7. Parameters evaluated for each treatment at time 0.

Parameter	T_0	T_1	T_2
Color	3.24 b*	6.74 a	3.92 b
Flavor (Taste)	5.06 a	4.99 a	4.63 a
Aroma	3.41 b	2.46 b	11.99 a
Texture	9.27 a	8.69 a	3.69 b

* Averages followed by the same letter have not significantly differences at 5%, according to Tukey test.

Table 8. Parameters evaluated for each treatment at time 1.

Parameter	T_0	T_1	T_2
Color	2.62 b*	7.48 a	2.09 b
Flavor	4.16 a	4.54 a	4.12 a
Aroma	3.66 b	2.72 b	12.41 a
Texture	9.06 a	7.98 a	3.12 b

* Averages followed by the same letter have not significantly differences at 5%, according to Tukey test.

Figures 4 and 5 show different sensory profiles from the evaluated snails appertized (T_0 , T_1 and T_2). In Figure 4, it can be seen that for both evaluation times, 30 and 90 days of storage, for treatment with snails under T_0 , the perceived quality attribute with a higher degree of intensity by the panelists was the texture. The attributes, flavor, aroma and color were perceived at a lower intensity than texture, but similar between them.

Regarding preserves in treatment T_1 , quality attributes perceived with greater intensity by the panelists were texture and color. The two remaining attributes, flavor and aroma, were perceived at a lower intensity. Mollusks in treatment T_1 were those with the highest widespread acceptance and also the best evaluation of all quality parameters present in the evaluation guide used by the panelists. In studies on preserved snails, it was concluded that the addition of additives such as citric acid 0.03% during the pre-cooking stage provoked good results in texture and appearance of the product (Corfo, 1990). Figure 5 shows the sensory profile of snails with treatment T_2 , showing that in both evaluation times the aroma attribute was perceived

with a greater degree of intensity, but in this case, negatively evaluated by the panelists as, 'strong and unpleasant'. Appertized preparations with this treatment were rejected by the panelists. It can be said, that according to the responses obtained in the two evaluation guides used for the sensory evaluation and analysis of data, only the T_0 and T_1 treatments are recommended, T_2 treatment discarded by the refusal of the panelists. Citric acid was used for T_1 , proving its efficiency when considering this treatment was the one with the highest acceptance by the panelists.

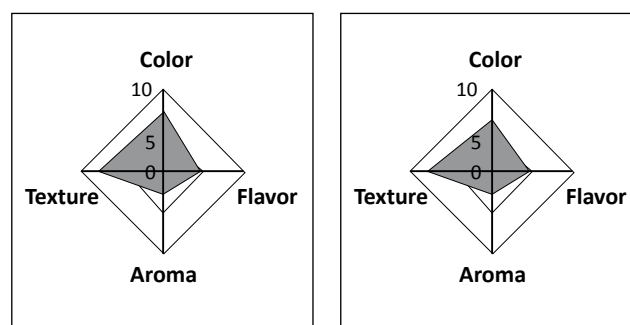


Figure 4. In Left and Right, average profile of the sensory characteristics of snails appertized corresponding to T_0 , at time 0 and 1, respectively.

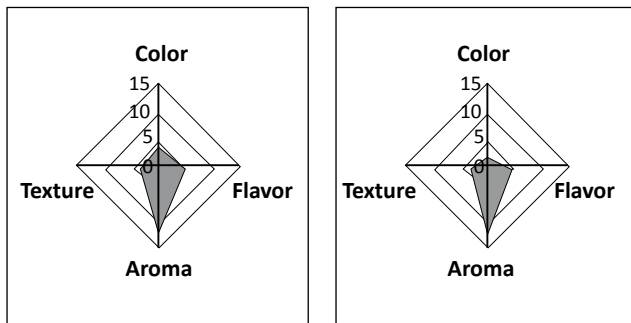


Figure 5. In Left and Right, average profile of the sensory characteristics of snails appertized corresponding to T0, at time 0 and 1, respectively.

Conclusions

- The mollusks subjected to treatments T0 (Control: water + NaCl 2%) and T1 (water + 2% NaCl + citric acid + kilol -active ingredient: pomelo grapefruit extract doses of 30 ppm-) were accepted by panelists with positive results in the organoleptic characteristics, freshly prepared snail-like taste, pleasant aroma and firm texture also in the case of T1 a lighter color.
- Appertized under T2 treatment (extra virgin olive oil + antioxidant -active ingredient: tocopherol in doses of 10 ppm-) was rejected by negative results in three of the parameters evaluated, presenting, dark color, unpleasant flavor and mealy texture.
- The three treatments showed positive results regarding the content of amino acids, vitamin C, low cholesterol and heavy metals as well as the total absence of total microorganisms and fecal contaminants.

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