

Development of quality index method for eviscerated and non-eviscerated octopus (*Octopus insularis*)¹

Desenvolvimento do método do índice de qualidade para polvo *Octopus insularis* eviscerado e não eviscerado

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ABSTRACT - This work aimed to develop a Quality Index Method (QIM) for octopus species *Octopus insularis* and apply it to samples of eviscerated and non-eviscerated specimens in order to establish stability in cold storage. Initially we carried out the recruitment of judges, who were trained to verify the changes undergone by the octopus during storage under refrigeration. The development of QIM Table took place in three experiments. At first, a QIM Table for *Octopus vulgaris* from literature was presented to judges together with specimens of *O. insularis*, so they could assess whether the parameters were suited to this specie. In the second experiment the objective was to validate the table, verifying if changes were needed in the predefined parameters or if it was also necessary to add new ones. Finally, the QIM Table was applied to determine the shelf life of non-eviscerated (NEV) and eviscerated (EV) octopuses (*O. insularis*) during refrigerated storage (2 ± 2 °C). The sum of demerit points for all parameters was named Quality Index (QI). To determine the value of QI at rejection point, N-TVB and N-TMA contents were determined. The data were submitted to analysis of variance and Tukey test at 5% significance, besides correlation and regression analysis by the GLM routine. The developed Quality Index Method for *Octopus insularis* consists of 8 parameters, totalizing 24 demerit points. The NEV samples were rejected at the tenth day of storage, while the rejection of EV samples occurred only at the end of the experiment.

Key words: Mollusk. Sensorial analysis. Shelf life.

RESUMO - O objetivo deste trabalho foi desenvolver o Método do Índice de Qualidade (QIM) para polvo da espécie *Octopus insularis* e aplicá-lo a amostras evisceradas e não evisceradas de forma a estabelecer sua estabilidade sob armazenamento refrigerado. Inicialmente foi realizado o recrutamento dos julgadores, os quais foram treinados para verificar as alterações sofridas pelo polvo durante o armazenamento sob refrigeração. O desenvolvimento da Tabela QIM para a espécie em estudo ocorreu em três etapas: na primeira foi apresentada aos julgadores a Tabela QIM existente na literatura para a espécie *Octopus vulgaris*. Além da tabela, também foram apresentados exemplares da espécie em estudo para que pudessem avaliar se os parâmetros da tabela existente na literatura se adequavam ao *O. insularis*. Na segunda etapa foi avaliado se os parâmetros estabelecidos na etapa anterior eram realmente adequados para a espécie em estudo. Na última etapa a Tabela QIM desenvolvida foi validada. Para determinar em quantos pontos de demérito as amostras NEV e EV foram rejeitadas, foram determinados os teores de N-TVB e N-TMA. Os dados obtidos foram submetidos à Análise de Variância e teste Tukey a 5% de significância, além das análises de correlação e regressão pela rotina GLM. A rejeição das amostras do presente estudo iniciou no 9º dia de armazenamento sob refrigeração. O QIM desenvolvido consistiu de 8 parâmetros, perfazendo um total de 24 pontos de demérito. A amostra NEV foi rejeitada por volta do décimo dia de armazenamento, enquanto que a rejeição da amostra EV ocorreu somente no final do experimento.

Palavras-chave: Molusco. Análise sensorial. Vida útil.

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INTRODUCTION

In Brazil, two octopus species have commercial interest: *Octopus vulgaris*, in the southern region and *O. insularis* (LEITE *et al.*, 2008) in the Northeast and North regions (LEITE *et al.*, 2009). *O. insularis* is the main target of octopus fisheries in northeastern Brazil, where several hundred tons are fished each year, mainly in Ceará fishing with long line pots (BRAGA *et al.*, 2007).

In these states, the octopus catch of the species *O. insularis* has great prospects for growth due to the vast coastline and constant tourism that demands exotic and healthy dishes. The species *O. insularis* has a high water content and a low lipid content, being considered a very healthy meat (MACHADO, 2011; SILVA, 2009). Along with this growth comes the need to expand the trading of that product, even more by putting it in distant markets from the fishing sites to meet a growing demand from consumers in a convenient and secure way.

According to Amaral and Freitas (2013) food quality and safety are issues of great relevance, especially in the international scenario. In minimalist terms, it can be said that quality refers to the characteristics that make food acceptable to consumers. Sensory evaluation is the most commonly used method for evaluating the quality of fresh fish and has always been considered as the main way to evaluate fish freshness of the fish (BOGDANOVIC *et al.*, 2012).

Several researchers and institutes of the fishing industry have developed methods to evaluate fish freshness based on *post-mortem* changes associated with sensory, physicochemical and microbiological changes (LI; LI; HU, 2013; OCAÑO-HIGUERA *et al.*, 2011). Appearance, odor, taste and texture are the main sensory attributes used to evaluate the fish freshness (ÁLVAREZ *et al.*, 2008; HERNÁNDEZ *et al.*, 2009).

In recent years, great progress has been made in assessing the freshness of fish and consequently in the marketing and quality assurance of fresh fish, mainly at the international level. The scheme known as the Quality Index Method (QIM) is considered a promising method in the evaluation of fish freshness in a fast and objective way (AMARAL; FREITAS, 2013).

Barbosa and Vaz-Pires (2004) established a demerit scoring scheme of 11 items for fresh common octopus (*Octopus vulgaris*) ranging from 0 to 1 or from 0 to 2, depending on the attribute, totaling a maximum of 16 points. However, the QIM should be developed for each species under study, since different species have particular characteristics (ESTEVEZ; ANÍBAL, 2007).

Therefore, the objective of this study was to develop the Quality Index Method (QIM) for octopus of

the species *Octopus insularis* and apply it to samples of eviscerated and non-eviscerated specimens, in order to establish its stability in cold storage.

MATERIAL AND METHODS

Material

A total of 72 specimens of the specie *Octopus insularis* weighing 550 ± 100 g, were obtained from extractive fishing held with long line pots at Itarema municipality, Ceará, during the months of November 2012, and January and April 2013. After capturing, the fishermen slaughtered the octopuses immediately, by immersion in a NaCl 15% solution. They were then immersed in fresh water to remove salt excess and then transported in inboxes with ice until the Meat and Fish Processing Laboratory of Food Engineering Department of the Federal University of Ceará. After weighing and washing, the samples were separated into two groups. In the first group, the octopuses were kept whole, non-eviscerated (NEV) and in the second, they were eviscerated (EV). All fisheries were placed in nylon/polyethylene packages. The samples were stored in a cooler (Electrolux, H500) at 2 ± 2 °C until rejection by physicochemical or sensory indicators.

QIM development

The development of Quality Index Method (QIM) for *O. insularis* species was based on the QIM for octopus *O. vulgaris*, proposed by Barbosa and Vaz-Pires (2004). Judges were recruited by means of questions about age, gender, education level, consumption habits and interest in participating. Three women and two men were selected to compose the sensory team, being 2 individuals over the age of 50 years, 2 in the age group of 30-50 and only 1 with less than 30 years old. All judges had higher education level. Only 2 individuals had the habit of consuming octopus, because this food is not a common dish in Ceará diet. The protocols of sensory tests were previously approved by the Ethics in Research Committee of the State University of Ceará, No.147,279.

Three experiments were conducted independently. The first experiment was carried out to select the parameters that would constitute the QIM Table, while the second was used to validate the Table and the judges' training. The last was the application of QIM Table, with repetition, to determine the shelf life of non-eviscerated and eviscerated *O. insularis* during the refrigerated storage (2 ± 2 °C).

On the first experiment sessions were conducted in groups, when 6 octopus, 3 non-eviscerated (NEV) and 3 eviscerated (EV) were placed in white Teflon

boards and displayed in a stainless steel table, so that all judges could see them. The judges were asked to verify the changes undergone by the octopus *O. insularis* under the refrigeration storage and at the same time establish parameters for the construction of the new Table QIM. It was also asked to observe if the parameters shown in the QIM Table proposed in literature for *Octopus vulgaris* (BARBOSA; VAZ-PIRES, 2004) was suited for the species under study. Parameters that were inadequate for *O. insularis* were discarded or new standards and levels were established, and thus, a new table QIM was proposed. That first step was carried out in about half an hour each session, in which were explained some biological terms of the species, not known by the judges. At that stage, the specimens were evaluated every 2 days, during 20 days, in a total of ten sessions. The octopuses were removed from the refrigerator for sensory session and immediately after the session were returned to the refrigerator to be used at the next session.

The second experiment had another 6 animals, 3 NEV and 3 EV, each animal being one replicate. After this stage, the analysis was performed individually, it means, a judge at a time. The specimens were displayed in white Teflon boards and coded with random numbers with three digits. The QIM Table constructed in the first step was given to the judges to evaluate three pairs of samples, following a balanced design (AB and BA). The objective was to test the table, verifying if changes were needed in the predefined parameters or if it was necessary to add new ones. The same samples were evaluated every 3 days for 18 days in a total of 7 sessions. As in the first experiment, the octopuses were removed from the refrigerator for sensory session and soon after were returned for use in the next session. Suggestions made by the judges were evaluated by the team and when considered necessary, were included in the Table.

In the third experiment were used 30 octopuses in each of two repetitions (EV 15 and 15 NEV), all stored under the same conditions (2 ± 2 °C). In each session we analyzed 3 NEV and 3 EV, which were discarded after evaluation, due to manipulation of the judges of the samples, a possible source of contamination. In the next session, 6 more octopus were evaluated (3 NEV and 3 EV), and so on. It was performed one session every 5 days during 20 days of storage.

Physico-chemical analysis

The determinations were: total volatile basis (TVB-N), following the methodology described by Association of Official Analytical Chemists (2005), and trimethylamine (N-TMA), determined by Conway micro-diffusion technique (BRASIL, 1981).

Statistical analysis

The data were submitted to analysis of variance (ANOVA) and Tukey test to compare means at 5% level of significance. Correlation analysis and linear regression by GLM routine were also performed. All analyses were carried out using the statistical software SAS® “Statistical Analytical Systems” version 9.2 (SAS INSTITUTE, 2009) for Windows.

RESULTS AND DISCUSSION

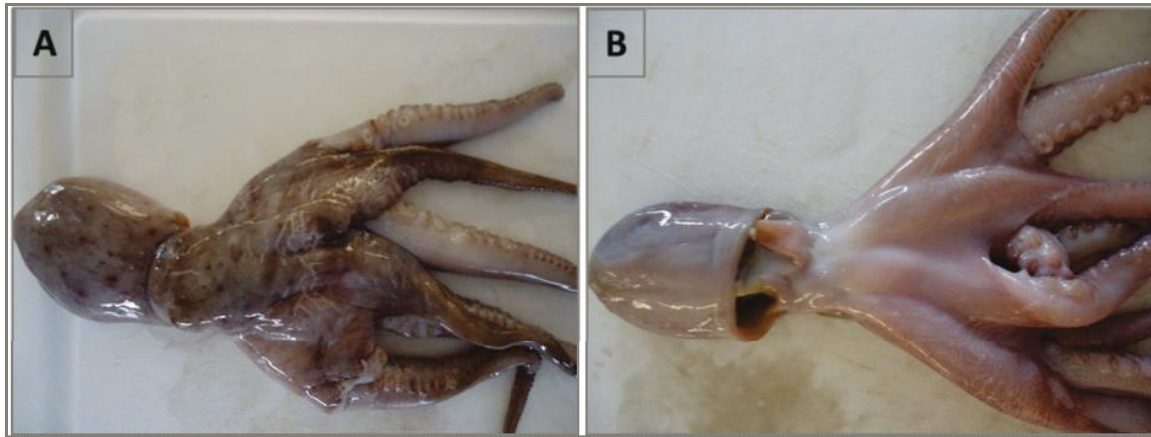
Development of the Quality Index Method (QIM) Table for *Octopus insularis*

The initial version of Table QIM, built to evaluate the freshness of octopus species *Octopus insularis*, consisted of seven quality parameters with demerit points ranging from 0 to 3, namely: “external color”, “color of mouth region”, “skin elasticity”, “odor”, “mucus”, “mantle texture” and “eyes”. The sum of points of all parameters was named Quality Index (QI), ranging from 0-21, calculated as an average of the sensory team. At this stage, we perceived that the judges had difficulty in defining the parameter “external region color,” because they were in doubt as to which region it referred: the dorsal or the abdomen (venter). This parameter was then replaced by two other parameters: “color of the dorsal region” (Figure 1A) and “color of the ventral region” (Figure 1B), making a total of eight parameters to be evaluated in the final QIM (Table 1). So, the maximum Quality Index (QI) rose from 21 to 24 demerit points.

In Table 1, fresh octopus was described with the following characteristics: light gray or predominant light brown in the dorsal region, predominantly white on the ventral region, ivory color with light rose spots in the mouth area, elastic skin, sea and seaweed odor, aqueous mucus in small amount, flexible flesh (mantle) to the touch and eyes with translucent cornea and black pupil or dark red, with little turbidity. During the storage, the appearance, odor and texture characteristics suffered changes in different extensions, and, according to the sensory team, reached the maximum conditions of deterioration after 18 days of storage: dorsal region of gray/brown color with strong rose, ventral region of rose color, mouth region of intense rose color, skin without elasticity, intense sour or rotten odor, no mucus, rigid mantle, and opaque cornea and opaque dark red pupil, normally bloodstained.

Determination of octopus *O. insularis* stability stored under refrigeration by the Quality Index Method (QIM)

Most of parameters showed high positive correlation with the storage time, for both non-eviscerated

Figure 1 - Photographs of dorsal region (1A) and ventral region (1B) of octopus *O. insularis***Table 1** - QIM Table developed for *Octopus insularis*

Freshness quality parameter	Definition	Description	Demerit points
Color of dorsal region (eye side)	Predominant color in the animal's body, excepting the mantle, the ventral and the mouth regions	Light gray/light brown	0
		Dark gray/dark brown	1
		Gray/brown with rose spots	2
		Gray/brown with strong rose	3
Color of ventral region (opposite to eye side)	Predominant color of animal's venter	White	0
		White and light rose spots	1
		Slightly rose	2
Color of mouth region	Predominant color of the mouth and the bottom of tentacles	Rose	3
		Ivory with light spots	0
		Ivory with dark spots	1
		Ivory and slightly rose	2
Skin elasticity	Characteristic skin on top of the tentacle (the part nearest the eye) of returning to the initial position after be pulled	Rose intense	3
		Elastic	0
		Moderate elasticity	1
		Low elasticity	2
Odor	Characteristic odor of fresh octopus, perceived all over the animal's body	Without elasticity, sagging	3
		Sea and seaweed	0
		Sea and seaweed less intense, metallic, grass	1
		Sour milk, grass	2
Mucus	Viscous material present all over the animal's body	Intense odor of sour milk or rotten odor	3
		Aqueous (small amount)	0
		Viscous (small amount)	1
		Viscous, cloudy (small amount)	2
		Mucus absence	3

Continuation Table 1

Mantle (flesh) firmness	Texture observed by touching and handle the mantle	Flexible	0
		Little flexible	1
		Little rigid	2
		Rigid	3
Eyes	Appearance of the cornea and pupil	Translucent cornea and black, shining pupil	0
		Slightly opalescent, transparent cornea and black or dark red pupil, slightly muddy	1
		Opalescent cornea and black or dark red pupil, muddy	2
		Opaque cornea and opaque dark red pupil, normally bloodstained	3
QIM score range		0 – 24	

(NEV) and eviscerated octopus (EV) (Tables 2 and 3). Figure 2 illustrates the evolution of demerit points (average of sensory team on both repetitions) of each parameter during the 20 days of cold storage. In Figure 2A is possible to observe that for NEV sample, the score for “color of the dorsal region” increased already on the 5th day, and continued increasing until the 10th day, showing a dark gray /dark brown colored body, level reached by the eviscerated sample only on the 15th. At the end of storage (20th day), both samples showed the same score, around 1,5 demerit points.

For the “ventral color” (Figure 2B), the samples differ from each other in 10th and 15th day, with the NEV

reaching the highest score. However, by the end of storage time, both samples showed the same score, 2,0 demerit points, corresponding to slightly rose.

In the parameter “color of the mouth region” (Figure 2C) it was observed outlier values for a few judges, which influenced the means of demerit points on the 15th day to NEV sample, but it was still possible to establish a significant linear correlation also for this sample. Therefore, we can infer that, the mouth region of the both samples (NEV and EV) changed to ivory with slightly rose spots (between 1,0 and 2,0 demerit points) at the end of storage. Analyzing these three parameters together, we observe that the octopus non-eviscerated

Table 2 - Mean QIM values (demerit points) of each freshness quality parameter for non-eviscerated (NEV) octopus *O. insularis* stored under refrigeration by 20 days and its correlation with storage time

Parameters	Storage Days					Correlation (R)
	1	5	10	15	20	
Color of dorsal region	0.4	0.8	1.2	1.2	1.6	0.96*
Color of ventral region	0.3	0.9	1.5	1.5	2.1	0.96*
Color of mouth region	0.4	1.0	1.5	1.2	1.9	0.89*
Skin elasticity	0.2	0.6	1.4	1.3	1.8	0.95*
Odor	0.1	0.6	1.3	1.4	1.8	0.97*
Mucus	1.6	1.7	2.1	2.0	2.0	0.79 ^{ns}
Mantle (flesh) firmness	0.3	0.8	0.9	0.9	0.6	0.40 ^{ns}
Eyes	0.7	1.2	1.8	1.7	2.0	0.92*
QIM TOTAL	3.9	7.2	10.4	10.3	12.7	0.99*

*significant ^{ns} non-significant ($\alpha=0.05$)

Table 3 - Mean QIM values (demerit points) of each freshness quality parameter for eviscerated (EV) octopus *O. insularis* stored under refrigeration by 20 days and its correlation with storage time

Parameters	Storage Days					Correlation (R)
	1	5	10	15	20	
Color of dorsal region	0.3	0.4	0.7	1.1	1.4	0.99*
Color of ventral region	0.3	0.7	1.1	1.2	1.9	0.98*
Color of mouth region	0.2	0.5	1.2	1.2	1.5	0.95*
Skin elasticity	0.1	0.3	1.1	1.1	1.5	0.96*
Odor	0.1	0.4	1.1	1.2	1.7	0.98*
Mucus	1.5	1.5	2.3	2.0	1.8	0.50 ^{ns}
Mantle (flesh) firmness	0.3	1.0	0.8	1.2	1.1	0.78 ^{ns}
Eyes	0.6	1.0	1.5	1.5	1.7	0.94*
QIM TOTAL	3.5	5.9	8.1	9.3	12.0	0.95*

*significant ^{ns} non-significant ($\alpha=0.05$)

darkened faster than the eviscerated octopus, in all regions analyzed (dorsal, ventral and mouth).

For the parameter “skin elasticity” (Figure 2D), “odor” (2E) and “eyes” (2H), the rate of evolution of demerit points showed the same pattern for the two samples, increasing faster in the first storage period (10 days) and then more slowly until the 20th day of storage, but never exceeding 2,0 demerit points (on average). So, it was observed a correlation among these parameters, it means, changes in skin elasticity are accompanied by changes in odor and appearance of the animal’s cornea and pupils. The skin loses elasticity and eyes lose transparency, together with development of off odors to the product, all at the same speed. Similar results were observed by Barbosa and Vaz-Pires (2004) in *Octopus vulgaris*, which odor, skin color and skin elasticity, besides the material of the octopus suckers, were the main parameters that determine the rejection of the product, the time that the odor was metallic and grassy, the skin rose and saggy.

The parameters “mucus” and “mantle firmness” did not differ between samples, however, while the “mucus” presented unchanged, viscous, slightly cloudy and in a small amount (1,5 to 2,0 points) from the beginning to the end of the storage (20 days), the “mantle firmness” went from flexible (0,0 point) at the beginning to low flexible (1,0 point) on the 5th day, and has not increased until the end of storage.

Figure 3 shows the average results of Quality Index (total demerit points QIM) adjusted by means of a linear regression analysis, which resulted in R² of 0,92 and 0,99 for the non-eviscerated octopus (NEV) and eviscerated (EV), respectively. Both samples were evaluated by QIM, obtaining 4 demerit points on the

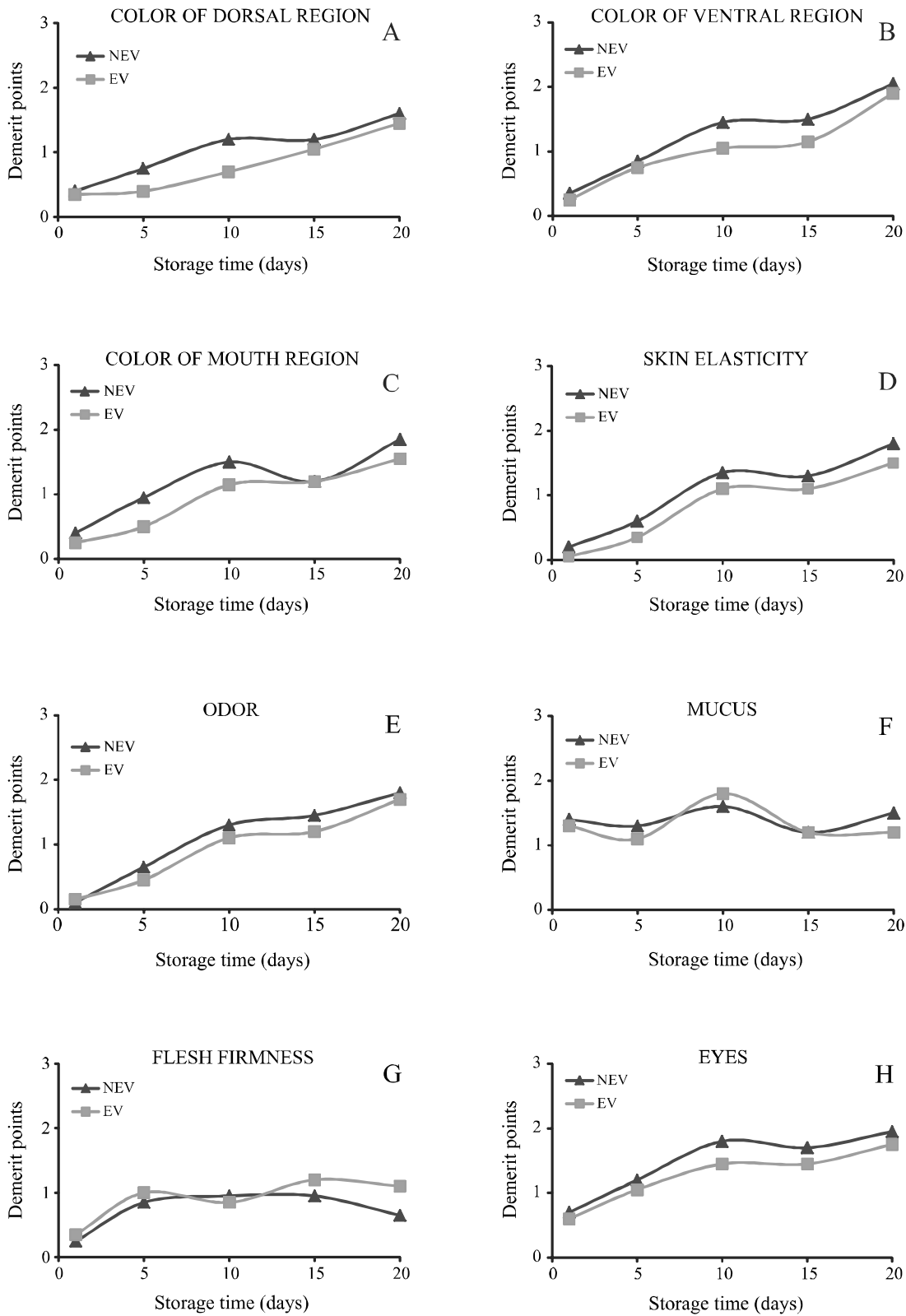
first day of storage due to small, but already noticeable changes in all evaluated parameters. It was observed in this stability experiment that samples reached 12 demerit points at the end of the cold storage time (20 days), unlike the initial experiment of Table QIM development when, after 18 days, under the same conditions, the octopus hit the maximum score (24 demerit points). This happened because in the first experiment, we always performed the evaluations with the same samples that were manipulated by the sensory team and returned to the cold room. Even with the use of gloves, this manipulation was a source of contamination, which caused a more rapid deterioration of the samples. When we decided to dispose of the animals and replace them with others that were in the cold chamber under the same conditions, it was obtained more realistic situation storage, where octopuses are kept without any kind of manipulation.

It is also noted that the non-eviscerated octopuses showed slightly higher values than the eviscerated ones, at all analyzed times, indicating that its deterioration was more rapid. Barbosa and Vaz-Pires (2004) observed that non-eviscerated *O. vulgaris* specimens reached maximum QIM (16 points) in just 8 days of refrigeration, time considered as the product rejection moment. Already for *Eledone moschata* octopus kept under refrigeration, rejection was detected when the overall sensory score using the European Union (EU) freshness classification system reached 5,5 on the 10th day of storage (LOUGOVOIS *et al.*, 2007).

Physico-chemical analysis

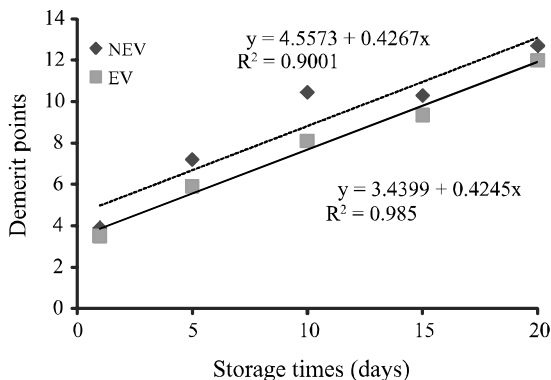
The physico-chemical analyzes were performed to verify if by the time of sensorial rejection, the octopus

Figure 2 - Graphical representation of mean QIM values (demerit points) of each freshness quality parameter for non-eviscerated (NEV) and eviscerated (EV) octopus *O. insularis* stored under refrigeration by 20 days



NEV = non-eviscerated; EV = eviscerated

Figure 3 - Quality Index evolution of octopus non-eviscerated (NEV) and eviscerated (EV) *O. insularis* during refrigerated storage, determined by the QIM method with 24 demerit points



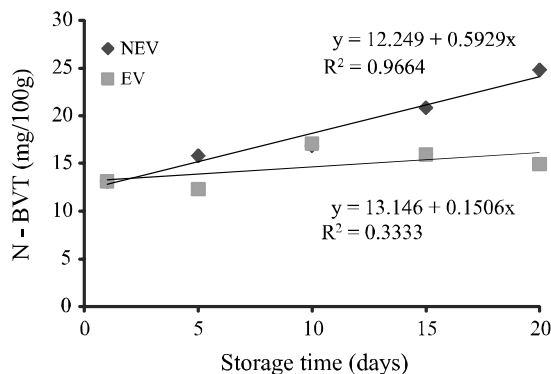
NEV = non-eviscerated; EV = eviscerated

was out of the physico-chemical standards required by law. In Brazil, there are no maximum limits for volatile basis values (N-TVb) and trimethylamine (N-TMA) for cephalopods, but the Agriculture Defense Secretary of the Ministry of Agriculture, Livestock and Supply (BRASIL, 1997) establishes the value of 30 mg/100 g as a maximum value of N-TVb and 4 mg/100 g of N-TMA as limit for fishery, except elasmobranchs. Thus, these values were adopted as limit values to indicate the state of freshness of the octopus in this study.

The initial values of N-TVb were around 13 mg N-TVb/100 g, indicating that the samples were in good quality at the beginning of the experiment, according to Ogawa and Maia (1999). By the twentieth day, any of the samples reached the maximum limit of 30 mg/100 g, even though there was a tendency to increasing values, as shown in Figure 4.

According to Vaz-Pires *et al.* (2008), the N-TVb analysis has been useful as an indicator of deterioration,

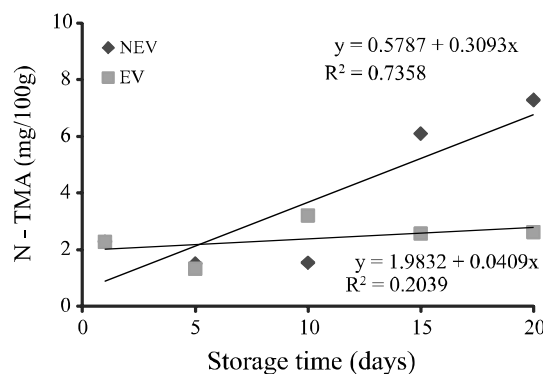
Figure 4 - Evolution of N-TVb contents during storage of octopus *O. insularis*



having low use as a freshness index. This justifies the fact that the samples from the present study did not reach the established maximum limit, but were rejected sensorially.

As far as N-TMA, on the first day of analysis it was recorded values close to 2,30 mg N-TMA/100 g for both samples, which showed similar behavior until the 10th day of the experiment. The two samples diverged from the 15th day of storage, the N-TMA contents to EV octopus remained unchanged until the end of the experiment, while the NEV octopus reached 7,70 mg N-TMA/100 g on the 15th day, exceeding the limit set by law, and continued to increase until the end of the experiment (Figure 5).

Figure 5 - Evolution of N-TMA contents during the storage of octopus *O. insularis*



The initial N-TMA content in *O. vulgaris* octopus samples stored under refrigeration, reported by Atrea *et al.* (2009) was 1,3 mg N / 100 g, being this value lower than those found in this study. However, on the 9th day of storage, the samples of these authors reached an N-TMA content of 30 mg N / 100 g.

It was possible to establish a significant correlation between the demerit points QIM with N-TVb values ($\alpha = 0,05$) and N-TMA ($\alpha = 0,15$) for non-eviscerated octopus, but there wasn't significant correlation between the QIM and the physico-chemical parameters for EV samples. It means that while the sensory quality was already compromised, chemical indicators didn't accuse deterioration to the eviscerated octopus.

CONCLUSIONS

The QIM developed for octopus non-eviscerated and eviscerated *Octopus insularis* consists of 8 parameters with scores from 0 to 3, making a total of 24 demerit points. The QIM Table was successfully applied

to assess the stability of non-eviscerated and eviscerated specimens stored under refrigeration. The rejection of non-eviscerated samples occurred around the fifteenth day of storage when they reached around 10 demerit points. For the eviscerated sample rejection occurred on the twentieth day of storage, where the samples reached about 11 demerit points, with physico-chemical parameters still within the laws' limits.

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