

Physical Methods in Chemical and Sensory Evaluation of Fish Quality

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Abstract: The raw material used in developing this research were fish of the species, Pargo (*Lutjanus purpureus*, Poey 1867), Cioba (*Lutjanus analis*, Cuvier), Biquara (*Haemulum plumieri*) e Cangulo (*Balistis vetula*, Linnaeus) caught at State of Ceará. Experiments aimed at determining maximum period of storage of the product when stored in ice as well as its physicochemical and organoleptic characteristics over a 16 days period. Physicochemical and organoleptic analysis demonstrated that both the disembowelled fish and the ones which were kept whole suffered considerable loss of quality over the period in which they were stored in ice.

Key words: organoleptic characteristics, loss of quality, stored in ice.

Métodos Físicos Químicos e Sensoriais na Avaliação da Qualidade do Pescado

Resumo: No presente trabalho utilizaram-se como matéria-prima, as espécies, Pargo (*Lutjanus purpureus*, Poey 1867), Cioba (*Lutjanus analis*, Cuvier), Biquara (*Haemulum plumieri*) e Cangulo (*Balistes vetula*, Linnaeus), capturados na costa de Fortaleza - Ceará. Os experimentos efetuados destinaram-se a determinar o período máximo de armazenagem do pescado conservado em gelo, bem como, suas características físico-químicas, organolépticas durante o período de 16 dias. As análises físico-químicas e organolépticas mostraram uma acentuada redução significativa ($p < 0,05$) da qualidade do pescado durante o período em que estiveram armazenados em gelo, tanto para os peixes eviscerados quanto para os inteiros.

Palavras-chave: características organolépticas, perdas de qualidade, armazenados em gelo.

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Introduction

In the period between catching and processing or commercialization, fish are subject to quality deterioration due to the storage conditions on the boat and the nature of their composition, as the edible portion of the fish contains high levels of nitrogenous compounds with low molecular weight, which, during storage on ice, may undergo alterations which compromise their chemical, physical or micro-biological characteristic (SHEWAM & JONES, 1957).

FABER (1952), makes reference to the loss of freshness and consequently decrease in quality, studied by means of tests of an organoleptic, chemical and micro-biological nature, aiming for improved evaluation of the level of deterioration of fish in storage.

According to GROMEL (1974), changes in quality which occur during storage of fish on ice are considered mainly as resulting from the combined action of autolytic enzymes and micro-organisms contaminating muscle, as deterioration is, in turn, due almost exclusively to bacterial action, appearing to be accompanied by the formation of

compounds with a disagreeable odor and alterations in the color of the product (SALES, & PORTO, 2007). Thus, FIEGER & FRILLOUX (1954), following the process of deterioration of shrimps conserved on ice, divided it into three phases: in the first, there was a loss of the sweet flavor (up to seven days in storage); in the second the shrimp became insipid (8-14 days); and finally, in the third stage, deterioration was accompanied by disagreeable odors and flavors.

However, a lot of research has been conducted in terms of relating the pH alterations to trimethylamine (TMA) content, Total Volatile Bases (AKIBA et al., 1969), free amino acids, indol, hypoxantine, histamine and other chemical compounds, as indicators of quality during storage of fish and other seafood (BAILEY et al., 1956; CHEUK & FINNE, 1981 and COBB & VANDERZANT, 1971). Many times, these products are used in isolation, as indicators of post-mortem degradation, or related to organoleptic tests for quality evaluation, these tests being considered efficient for evaluation of the quality of fresh or processed fish, however, their subjective nature also requires, for greater

safety, parallel use of chemical and biochemical analyses and micro-biological exams.

WATANABE (1972), studying some aspects of the deterioration of rocket fish (*Macrodon ancylodon*) stored on ice, concluded that sensorial analysis and the findings of the N-TVB and TMA held, demonstrated that the limit of acceptability of fish examined would be around the 15th day after death. The same author, partially confirmed the previously studied results when studying frozen sardines, *Sardinella aurita*, (-18°C) and treated with anti-oxidants, finding that, due to lipid oxidation, the relationship between deterioration, organoleptic properties and nitrogenous bases is complex. The same author found evidence of accentuated variations in the production of N-TVB in the species Tilapia (*Tilapia mossambica*), Mudsucker (*Fabeo congoro*), Tiger Fish (*Hydrocimus vittatus*) and Barbel (*Clarias mossabicus*) frozen due to variation in seasonality and place of catching, and concluded a significant increase in these attributes. They also found that in addition to organoleptic variations due to species, there was also an increase in N-TVB content in fish caught at the end of the year.

COLLINS et al. (1960), making an evaluation of the quality and freshness of shrimps conserved in refrigerated sea

water, proposed the determining of TMA and volatile acid content as methods of indication of bacterial decomposition and determination of nitrogenous non-protein substances as an index of enzyme action.

SAITO & AKAI (1958) found no significant differences between surface and pelagic fish, which live at different temperatures. The results mentioned however, leave no doubt about the relationship between production of TMA and bacterial contamination which, in turn, is a function of the type of handling and storage to which the fish are subjected, this being finally reflected in the quality of the end product (ITO et al., 1969).

HUGHES (1959), found that the herring (*Clupea hargenus*) showed variations in N-TVB, TMA and NH₃ content, resulting from variations such as: season, post-mortem time, maturity of the fish as well as the result of bacterial action.

CASTELL et al. (1970) inoculated pure bacteria cultures in sterilized cod muscles (*Gadus morhua*), finding that those responsible for the production of the fishy smell during decomposition were those of the following types: *Pseudomonas*, *Proteus*, *Achromobacter* and *Serratia*. LAYLOCK & REGIER (1971) and CASTELL (1948; 1949) were able to isolate psychrophiles present in eglefin (*Melanogrammus aeflifinus*) stored at -3°C, in impermeable polythene bags, in

a vacuum, finding that the largest quantities of TMA were produced by the metabolic action of *Pseudomonas putrefaciens*.

CASTELL et al. (1968) studied the production of TMA in cod fillets (*Gadus morhua*) stored between -3° and 26°C showed a small increase in TMA, plus an increase in the speed of bacterial reproduction, this increase being highly significant. The main groups of bacteria responsible for the transformations described, belong to the types *Pseudomonas* and *Achromobacter*, also with involvement of *Flavobacterium*, *Micrococcus*, *Corinebacterium* and *Vibrio* (CASTELL et al., 1948).

SHEWAN & JONES, 1957; FABER, 1952; HILLIG et al. (1958, 59, 60 and 61), performed a broad and systematic study on the formation of several substances as possible indicators of quality in different types of fish, such as polaca (*Pollachius vireus*), small fry, jurel and anchovies stored on ice, wherein they state that the chemical indices (N-TVB, TMA and pH) compared with organoleptic tests, confirm a good correlation with organoleptic characteristics and the final quality of the fish.

The formation of volatile amines in fish muscle occurs more through the action of bacteria which develop in the muscle than through the action of enzymes from

the tissue itself (HILTZ et al., 1969; SALES et al., 2002). Amongst these volatile amines, trimethylamine (TMA), the main product from reduction of Trimethylamine oxide (TMAO), is the most highly studied, as it is associated with the deterioration of fish and consequent protein putrefaction (RONALD et al., 1974).

CASTELL et al. (1970 and 1971), studied the variation in several species of fish, such as: eglefin (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*), polaca (*Polacchius vireus*), cush (*Brosme brosme*), merluza (*Merluccius bilinearis*), red fish (*Sebastes marinus*), catfish (*Anarhichas lupus*), wrapped in polythene bags and frozen at -40°C for 15 hours, concluding that variation in DMA and TMA content between species was very small.

FABER & CEDER QUIST (1953), looking for chemical thresholds which enabled separation of acceptable from unacceptable products discussed a possible analytical use of N-TVB and volatile reducing substances (SVR), (FABER et al., 1956) and FABER (1963), in several species of fish.

SHEWAM & EHREMBERG (1957), demonstrated the existence of a correlation between levels of N-TVB and TMA in fish stored on ice at 0oC and sensory properties, evaluated according to

a point scale established for general appearance and odor of raw fish, and the flavor and odor of cooked fish. Similar results were obtained by ANTONACOPOULOS (1971), who compared the development of N-TVB, TMAO and TMA in frozen fillets with sensory development measured by tasters, observing that chemical and organoleptic transformations were different, thus concluding that it was important to perform organoleptic evaluations in parallel with chemical evaluation, the variation between N-TVB and TMA content being large as a result of many parameters related to fish, or to environmental and catching conditions.

HUGHES (1959) and ITO et al. (1969) determined that the quantity of total volatile bases like NH₃, TMA and DMA, in isolation or together, like TVB, was proposed as an index of contamination or as a degree of deterioration of stored fish. As within one species, the concentration of ATP in live fish is practically constant, thus, the products of degradation, especially inosine and hypoxanthine (JONES, 1965) which accumulate both in initial and final stages of ice-packing of fish, may act as alternatives for evaluation of the degree of freshness (DUGAL (1967).

In Brazil, the literature on the subject is very limited. WATANABE

(1972) studied some aspects of the deterioration of rocket fish (*Macrodon ancylodon*) stored on ice; sensorial and TVB and TMA analyses leading to the conclusion that the limit for acceptability of fish examined was around the fifteenth day following death. ZAMBONI (1963) studied the deterioration of sardines stored in a refrigerator at a temperature of -2°C to 0°C. Upon performing organoleptic analyses side by side with TVB and TMA findings, he concluded that there is a correlation between them in oily fish. WATANABE (1963) partially confirmed the results of ZAMBONI (1963) studying frozen *Sardinella aurita* (-18°C) and treated with anti-oxidants, finding that, due to oxidation of lipids, the relationship between deterioration, organoleptic properties and nitrogenous bases is not simple.

Review of the literature, therefore, led us to face the possibility of, following biochemical changes in the fish, establishing chemical indices for quality evaluation, emphasizing the importance of determining TVB, TMA and pH (SOUZA et al., 2009). The review also showed the lack of research involving Brazilian species, especially in the Northeastern region, research which would be necessary in order to establish national quality standards for species of fish common to

Brazilian waters in current conditions of catching, trading and utilization.

2 - Material and Methods

2.1 Material

The experiment was conducted at the Meat and Fish Technology Laboratory of the Department of Food Technology at the Federal University of Ceará, located in Fortaleza - Ceará during the period between August 1996 and September 1997. The fish studied were: porgy (*Lutjanus purpureus*, Poey 1867), dog snapper (*Lutjanus analis*, Cuvier), common grunt (*Haemulum plumieri*) and trigger fish (*Balistes vetula*, Linnaeus), caught off the coast of Fortaleza - CE, in a total of 100 fish.

Immediately after catching, the fish were placed in thermally insulated crates containing layers of crushed ice and were kept this way until arrival at the laboratory, whereupon they were transferred to the cool room and maintained at 0°C until the end of the experiment.

After twenty-four hours post-catch, the first investigations of Total Volatile Bases (N-TVB), Trimethylamine (TMA) and pH were made, tests which were performed every two days, for a period of 16 days and in double, the fish analyzed being divided into two batches: gutted (G)

and non-gutted (NG), the units being uniform in size and weight.

2.2 Preparation of samples

For the preparation of samples 25g of filleted fish were treated, crushed and homogenized, with 75ml of distilled water, adjusting the pH to 5.2 with a 2N Hydrochloric acid, the homogenate being heated slowly up to 70°C, to be subsequently refrigerated to room temperature and filtered. For the tests, aliquots of 2ml of filtrate were used.

3. Methods

3.1 Determination of bases

In evaluating the total volatile base (TVB) content, free protein extracts were used, prepared from fish muscle treated with trichloroacetic acid solution at 5%, the method proclaimed by HOWGATE (1976). After neutralization with sodium hydroxide solution, the total volatile bases were distilled in a micro-kjedahl distillation device, and titled with standardized acid solution. The concentration of TVB was expressed as mg of N/100g of sample.

3.2 Determination of TMA

The extracts for determination of trimethylamine (TMA) were obtained by homogenization of samples with trichloroacetic acid (TCA) at 5%, in the proportion of 1:2, in a Virtis homogenizer for 1 minute. Aliquots of these extracts were used in the test. The content of TMA was evaluated according to the method described by Dyer (1949) and MURRAY & GIBSON (1972), which is based on the fact that amines and basic substances, not fixed in formaldehydes, are released with the TMA concentration in the sample. The quantity of TMA is calculated by comparison with a standard curve obtained through the chemical reaction of known quantities of TMA with picric acid and expressed as mg N-TMA/100g of sample.

3.3 Determination of pH

The pH was measured in disintegrated samples and homogenized with previously boiled distilled water, in the proportion of 1:2 (fish : water), in accordance with the Torry Research Station method, modified by COBB et al. (1971). The pH was found using a combined glass electrode.

3.4 Organoleptic Tests

In the case of Organoleptic tests, evaluation was conducted according to

tests used at the Torry Research Station (Scotland) and described by NORT (1973). These tests enable classification of raw fish according to physical characteristics, in four classes:

Class 1: 1st stage following death, corresponding to fresh fish.

Class 2: 2nd stage following death, corresponding to fish in acceptable condition.

Class 3: 3rd stage following death, corresponding to fish with a strong odor, beginning to deteriorate.

Class 4: 4th stage following death, corresponding to fish in a putrid state.

3.4 Statistical Treatment

The data relating to TVB, TMA and pH were subjected to statistical treatment to establish a relationship between content of TVB, TMA and pH and the in time for comparison between measures, the variance analysis was performed and, when different, from test F, the data were analyzed according to the Tukey test.

4 - Results and Discussion

The TVB, TMA and pH levels obtained every two days, from catch up to the 16th day, are shown in Tables 1, in which TMA and TVB are expressed in mg

N/100g of sample during the evaluation of chemical parameters and in Table 2, the organoleptic characteristics may be seen, based on sensorial evaluations of gutted (G) and non-gutted (NG) fish, stored in ice.

In Table 1, showing analysis of the behavior of TVB, TMA and pH for gutted and non-gutted fish, a difference may be seen between the values for a single day, the average the respective differences being positive, and showing that gutted fish underwent slower deterioration, shown by the lower absolute values of volatile bases (TVB), TMA and pH, values which are significantly higher ($p < 0.05$) than non-gutted fish, or rather, in passing from class 3 to class 4, that is, the 14th day for gutted fish and the 10th day for non-gutted, which confirms the observations made by several authors (BAILEY et al., 1956; CHEUK & FINNE, 1981 and COBB & VANDERZANT, 1971), who stated that the TVB, TMA and pH levels are good indicators of post-mortem deterioration in storage of fish and other seafood products on the 11th day for non-gutted fish.

In terms of physical-chemical properties (Tables 1 and 2), the gutted porgy, dog snapper, common grunt and trigger fish enter the third class on the ninth day, whilst the non-gutted do so only on the seventh day. For gutted fish, it was found that from the 12th day onward, or rather, in passing from the 3rd to the 4th

class, they are significantly higher ($p < 0.05$) than non-gutted fish, similar to the findings of FABER & CEDERQUIST, 1953 and lower than those of WATANABE, 1972, wherein physical-chemical properties changed on the passage from class 4 to 5, that is, on the fifteenth day. This is no surprise as similar findings were observed by other authors (BAILEY et al., 1956; CHEUK & FINNE, 1981 and COBB & VANDERZANT, 1971). Depending on the species, at lower volatile nitrogenous base values, there can be correspondingly worse organoleptic indices or vice-versa. This fact demonstrates that these volatile bases must not be the only ones responsible for the fish odor, despite accompanying the advancement of deterioration and being useful as indicators of quality.

In the case of trigger fish, despite total volatile base values being significantly above those of other fish, there were differences in quality only in the more advanced stages of deterioration, or rather, in passage from classes 3 to 4, that is, on the eleventh day for non-gutted fish and the 13th for gutted (Tables 1 and 2).

In Tables 2 and 3, it was seen that the organoleptic properties, for raw fish, measured according to NORT (1973) develop in the same way as TVB, TMA and pH, it being impossible, however, to

establish more precisely, the corresponding function. It was also found that amongst the different species, there is no apparent

fixed relationship between percentile values, whether of TVB or TMA, with organoleptic classes.

Table 1 – Average values of total volateis bases (BVT), trimetilamina (TMA) and pH em fish muscle of Pargo, Cioba, Biquara and Cangulo stored in ice during 16 days.

Fish	Days	Eviscerados (E)			Não Eviscerados (NE)		
		BVT	TMA	pH	BVT	TMA	pH
Pargo	2	3,10 ^b	0,15 ^b	6,90 ^a	4,50 ^b	0,17 ^a	6,95 ^a
	4	7,15 ^a	0,48 ^a	7,20 ^a	8,68 ^b	0,53 ^a	7,50 ^a
	6	7,20 ^a	0,78 ^a	7,45 ^a	9,70 ^b	0,92 ^a	7,90 ^a
	8	8,80 ^b	4,05 ^{ab}	7,75 ^a	10,30 ^b	5,08 ^b	8,01 ^a
	10	13,20 ^b	5,75 ^{ab}	7,90 ^a	17,20 ^b	7,85 ^b	8,02 ^a
	12	18,60 ^b	8,70 ^b	8,01 ^a	26,70 ^b	13,40 ^b	8,06 ^a
	14	25,30 ^b	10,35 ^b	9,75 ^b	35,45 ^b	15,65 ^b	10,98 ^b
	16	32,70 ^b	13,25 ^b	10,05 ^b	42,70 ^b	17,70 ^b	12,09 ^b
Cioba	2	4,20 ^b	0,17 ^b	6,85 ^a	4,30 ^b	0,22 ^a	6,85 ^a
	4	6,35 ^a	0,55 ^a	6,93 ^a	6,95 ^b	0,64 ^a	6,97 ^a
	6	7,26 ^a	0,86 ^a	7,28 ^a	11,72 ^b	0,93 ^a	7,29 ^a
	8	8,01 ^{ab}	4,09 ^b	7,36 ^a	15,45 ^b	5,45 ^b	7,45 ^a
	10	8,08 ^a	6,75 ^b	8,01 ^{ab}	17,57 ^b	8,76 ^b	8,02 ^a
	12	12,10 ^b	9,60 ^b	8,05 ^a	25,40 ^b	10,25 ^b	8,09 ^a
	14	18,35 ^b	11,25 ^b	10,25 ^b	32,54 ^b	12,36 ^b	11,02 ^b
	16	26,50 ^b	14,05 ^b	11,75 ^b	36,64 ^b	17,42 ^b	13,04 ^b
Biquara	2	4,08 ^b	0,18 ^a	6,90 ^a	4,50 ^b	0,35 ^a	6,90 ^a
	4	6,09 ^b	0,62 ^a	7,01 ^a	6,80 ^b	0,70 ^a	6,99 ^a
	6	8,20 ^b	0,92 ^a	7,05 ^a	11,75 ^b	0,92 ^a	7,50 ^a
	8	8,60 ^{ab}	5,00 ^b	7,09 ^a	12,25 ^b	6,25 ^a	8,03 ^a
	10	10,35 ^b	7,35 ^b	8,05 ^a	17,65 ^b	9,26 ^b	8,45 ^a
	12	19,60 ^b	10,46 ^b	8,09 ^a	24,25 ^b	11,28 ^b	8,75 ^a
	14	25,45 ^b	12,26 ^b	10,98 ^b	33,45 ^b	13,35 ^b	10,76 ^b
	16	36,50 ^b	15,72 ^b	12,76 ^b	36,65 ^b	18,45 ^b	12,98 ^b
Cangulo	2	5,20 ^b	0,19 ^a	6,82 ^b	5,10 ^b	0,45 ^a	6,87 ^a
	4	7,00 ^{ab}	0,72 ^a	7,03 ^a	6,90 ^b	0,86 ^a	7,35 ^a
	6	8,50 ^a	0,97 ^a	7,25 ^a	11,95 ^b	0,95 ^a	7,68 ^a
	8	10,65 ^b	6,08 ^b	8,45 ^a	16,45 ^b	7,22 ^b	8,25 ^a
	10	19,75 ^b	8,55 ^b	8,65 ^a	18,25 ^b	9,97 ^b	8,65 ^a
	12	26,65 ^b	11,64 ^b	9,08 ^a	25,27 ^b	11,35 ^b	8,92 ^a
	14	33,36 ^b	13,28 ^b	10,85 ^b	34,45 ^b	15,65 ^b	10,75 ^b
	16	37,33 ^b	16,82 ^b	12,25 ^b	37,45 ^b	19,22 ^b	12,45 ^b

a b Means in a column by different letters are different (p>0,05).

Table 2 – Average value of organoleptic characteristics of fish Pargo, Cioba, Biquara e Cangulo, storage in ice during 16 days.

Fish	Days	Organoleptic characteristics (Evisceration)				Observações
		Odor das Gueiras Odour of	Cor dos Olhos	Carne e Abas Abdominais	Textura do Músculo	
Pargo	2	9	5	5	5	
	4	8	4	4	4	
	6	7	4	3	3	
	8	6	3	2	2	
	10	5	3	2	2	
	12	4	2	1	1	
	14	3	2	1	1	
	16	1	1	0	0	
Cioba	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	4	
	8	6	3	2	3	
	10	5	2	2	3	
	12	4	2	1	2	
	14	3	1	1	1	
	16	2	0	0	0	
Biquara	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	3	
	8	6	3	2	2	
	10	5	2	2	2	
	12	4	2	1	1	
	14	3	1	1	1	
	16	2	0	0	0	
Cangulo	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	3	
	8	6	3	2	2	
	10	5	2	2	2	
	12	4	2	1	1	
	14	2	1	1	1	
	16	1	1	0	0	

Table 3 – Average value of organoleptic characteristics of fish Pargo, Cioba, Biquara e Cangulo, storage in ice during 16 days.

Fish	Days	Características Organolépticas (Não Eviscerados)				Observações
		Odor das Gueiras	Cor dos Olhos	Carne e Abas Abdominais	Textura do Músculo	
Pargo	2	9	5	5	5	
	4	8	4	4	4	
	6	7	4	3	3	
	8	6	3	2	2	
	10	5	3	2	2	
	12	3	1	1	1	
	14	2	2	1	1	
	16	1	1	0	0	
Cioba	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	4	
	8	6	3	2	3	
	10	5	2	2	3	
	12	3	1	1	1	
	14	2	1	1	1	
	16	1	0	0	0	
Biquara	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	3	
	8	6	3	2	2	
	10	5	2	2	2	
	12	3	1	1	1	
	14	2	1	1	1	
	16	1	0	0	0	
Cangulo	2	9	5	5	5	
	4	8	4	4	4	
	6	7	3	3	3	
	8	6	3	2	2	
	10	5	2	2	2	
	12	3	2	1	1	
	14	2	1	1	1	
	16	1	1	0	0	

5 - Conclusions

In view of the results obtained, it is possible to present the following conclusions:

5.1. Development of N-TVB, although slower than that of TMA, constitutes a safer index for the evaluation referred to in the previous conclusion, due to its better linear behavior.

5.2. Although there is, for raw fish, a positive relationship between organoleptic properties represented in four classes, and the values for nitrogenous bases, N-TVB

and TMA, it was not possible to establish the corresponding function, nor fixed relationships between them.

5.3. It therefore seemed to us that gutting is useful as a way of prolonging the validity of fish to be stored on ice, it being important to remember that gutting of fish was done twenty-four hours after catching and that if it was done immediately following the catch, on board, there would be a strong probability that the operation would be even more effective in prolonging the useful life of the fish.

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