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Artigo

Shelf life of Yellow Hake: Determinant factors for safe consumption[1]

Vida de Prateleira da Pescada Amarela: Fatores determinantes para o consumo seguro

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Abstract: The quality of fish is affected by various factors such as, source species, freshness, and storage conditions. In free markets, exposure and handling of fish are intense, and often it is not known the origin and date of capture. The aim this work was study physico-chemical, microbiological and sensory parameters to determine the shelf life of Yellow Hake sold in street markets in the city of Santos-SP. Samples were acquired in street markets from different districts of the city and stored in a domestic refrigerator for 72 hours. And it was found that the Yellow Hake acquired on open street markets in the city of Santos-SP should be for immediate consumption. The changes are noticeable deterioration after 24 hours of storage. The results reinforce the importance of the implementation of quality programs in all sectors of sale to ensure food safety for consumers.

Key-words: quality control; shelf life; fresh fish.

Resumo: A qualidade do peixe é afetada por vários fatores, tais como: espécie, frescor e condições de estocagem. Em feiras livres, a exposição e manipulação do pescado são intensas, e muitas vezes não são conhecidas a procedência e a data de captura. O objetivo desse trabalho foi estudar dos parâmetros físico-químicos, microbiológicos e sensoriais para determinar a vida de prateleira da pescada amarela comercializada em feiras livres na cidade de Santos/SP. As amostras de peixe foram adquiridas em triplicata em três feiras em bairros distintos da cidade e armazenadas em refrigerador doméstico por 72 horas. Constatou-se que a pescada amarela adquirida em feira livre na cidade de Santos/SP deve ter consumo imediato. As alterações de deterioração são perceptíveis em 24 horas de armazenamento. Os resultados do trabalho reforçam a importância da implantação dos programas de qualidade em todos os setores de venda visando garantir a segurança dos alimentos e a saúde do consumidor.

Palavras-chaves: controle de qualidade; vida de prateleira; peixe fresco.

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Introduction

Fish has important nutritional value, it is source of high quality protein and rapid digestibility, and it contains low cholesterol and polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid. These fatty acids have cardioprotective effect, reducing the risk of coronary heart disease (AFONSO et al., 2013).

The consumption of fish is recommended at least twice per week by the American Heart Association (2014). The Yellow Hake (*Cynoscionacoupa,* Lacépède, 1801) is one of the species with higher production of extractive fishing in Brazil. It is estimated that approximately 65 tons of Yellow Hake were captured and sold in Brazil between 2009 and 2011 (Ministério da Pesca e Aquicultura, 2011). Fish is highly perishable when compared with other groups of *in natura* foodstuffs (PRENTICE AND SAINZ, 2005). Such perishability can be explained due to the action of autolytic enzymes, pH in the range of neutrality, and it has high water activity, favoring microbial growth (ÖZOGUL et al., 2006). Fish may already have inadequate conditions at the time of purchase, mainly due the inappropriate practices of fish handlers (COSTA et al., 2012; ZANIN et al., 2015). The time between catching and marketing fish, in some cases, reaches long periods and the only way to slow the process of deterioration, is keeping it at low temperatures (GONÇALVES, 2011). Besides the risk associated with outbreaks involving pathogenic microorganisms in the fish sold in Brazil, the fish in the process of microbial deterioration presents formation of histamines that can cause poisoning to the consumer (MORROW et al., 1991; DA SILVA et al., 2010).

The marketing of fish in free markets is common in Santos/SP-Brazil for being located on the coast of Brazil. The points of sale of fresh fish direct to the consumer are characterized by the exposure of the product. The fish of wild origin suffers intense manipulation, and it is often not known the date of its capture. It is up to the consumer the decision to choose the product that suits him/her. Overall, the consumer considers the fish of wild origin as fresher and healthier than the fish in captivity, favoring the purchase at these sites (HALL and AMBERG, 2013; ARAÚJO, et al., 2015).

The aim of this study was to determine the shelf life of Yellow Hake through physical-chemical, microbiological and sensory analysis, commercialized in free street markets in the city of Santos/SP establishing the determinants for the safe consumption of this fish.

Materials and Methods

The samples of Yellow Hake were taken in triplicate at three street markets that occurred on the same day in the city of Santos-SP, Brazil. The fishes were transported in insulated boxes to the Microbiology Laboratory of the Federal University of São Paulo - UNIFESP, Baixada Santista Campus.

To evaluate the physical and chemical quality of the fish, the following analyzes were performed: pH, presence of ammonia and sulfide gas. The samples were evaluated daily from the time of purchase, and evaluated each and every 24 hours storage, totalizing four evaluation intervals (T0 = time of purchase; T1 = 24; T2 = 48; T3 = 72 hours). The Yellow Hake was stored in a domestic refrigerator at the Laboratory of Microbiology of the Federal University of São Paulo - UNIFESP, Baixada Santista Campus, with constant temperature from 5°C to 7ºC to simulate the everyday life of the consumer. For the determination of pH, it was weighed 10g of sample in a Becker, followed by homogenization with 100mL of water and measured in pH meter (Gehaka - PG 1800) following the recommendations of ADOLFO LUTZ INSTITUTE (2008).

The proof of Eber to release sulfide gas was performed by transferring 10g of homogenized sample to an Erlenmeyer flask (125mL), and closed with two overlapping discs of filter paper with the aid of elastic. Using a pipette, the surface of the paper was soaked with a solution of lead acetate. The flask was placed in the Dubnoff bath so that the bottom of the flask stayed at 3cm above the level of boiling water and it was heated for 10 minutes. The appearance of black spot on the filter paper in contact with vapors indicated the presence of hydrogen sulfide. This reaction is shown to assess the state of freshness of the fish, since the decomposition of muscle releases sulfur that in acidic environment is transformed into hydrogen sulfide (H_2S) (ADOLFO LUTZ INSTITUTE, 2008).

Eber reaction to ammonia, for reaction, 5mL of the reagent Eber was transferred into a Becker, and a portion of the sample was attached to the end of forceps that was inserted in the Becker without touching its walls and the surface of the reagent. The appearance of white and thick smoke indicates that the product is in the beginning of decomposition (ADOLFO LUTZ INSTITUTE, 2008). The evaluation of the appearance of white smoke was standardized and measured at four frequencies: absence, very little presence, little presence and presence.

Analysis of coliforms at 45°C and *E. coli*was performed by rapid methodology Petrifilm (3M) for coliforms at 45°C and *E. coli*, following the manufacturer's recommendations.

For the sensory analysis of Yellow Hake characteristics were evaluated according to the Ministério da Agricultura e Abastecimento (1952):

- Skin: shiny, moist, free of blemishes, bruises, incisions or breaks;
- Color: standard for the species;
- Scales: bright, well adherent to the skin and fins showing some resistance to any movement caused;
- Eyes: clear, bright and prominent, fully occupying the orbits;
- Operculum (membrane covering the gills): rigid, offering resistance to opening;
- Gills: pink or red, moist and bright with natural, proper and mild odor;
- Abdomen: firm, leaving no lasting impression to finger pressure;
- Flesh: firm and elastic consistency;
- Odor: specific, reminding marine plants.

The features were scored as adequate (no point) and inadequate (1 point) for all fish samples. It was made the sum of the points assigned to each feature. The sum ranged from 0 to 7, defining 0 for fish of excellent quality and 7 for fish of poor quality. Sensory analyzes were performed by three researchers after the standardization of the evaluation criteria.

To study the behavior of the variables of interest in each evaluation, it was used the model of analysis of variance (Anova) in blocks and the method of multiple comparison of Bonferroni. The R Development Core Team software (2011) was used to analyze data. In all tests, $p < 0.05$ was considered significant.

Results and Discussion

The pH of the fish indicates that the values were in discordance with the legal advice at the time of acquisition (T0). In the samples collected at the first street market, the average obtained from measuring the pH was 7.19 (0.39).

At the second the average pH obtained was 7.18 (0.20) and at the third the average pH obtained was 7.34 (0.12) all in T0. It is possible to observe a significant increase in pH between T0 and T1, maintenance between T1 and T2, and again increase in T3 (Figure 1). It became evident the pH increase of all the street markets during the evaluation during the 72 hours, reaching values of 7.8. The criteria established by legislation to fresh fish is that the pH should be less than or equal to 6.8 (Ministério da Agricultura e Abastecimento, 1952).

The results of the analyzes of samples collected from these three street markets show the beginning of the decay process already in to and it can be explained by a possible lack of good practice in the handling of the fish during the production chain until the moment of exposure to consumer. Several factors can influence the

quality of the fish, such as species, method of capture, manipulation, lack of temperature control and storage technique.

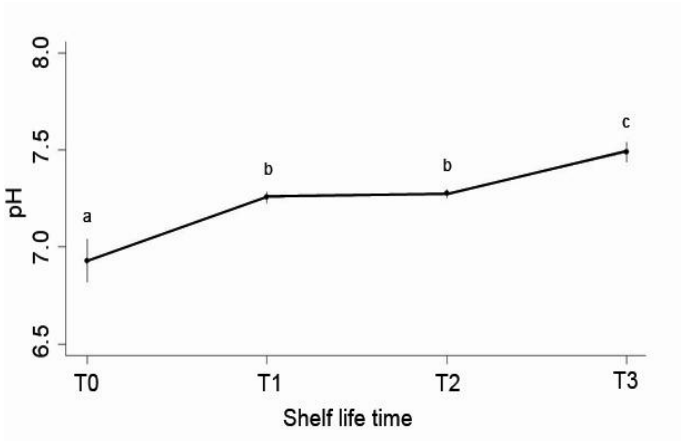

Figure 1 - Measurement of pH of Yellow Hake during four days of storage time in household refrigerator. Storage time in household refrigerator: T0 = the acquisition of the fish; T1 = 24 hours; T2 = 48 hours; T3 = 72 hours. Different letters indicate significant difference between the time intervals ($p < 0.05$).

With the deterioration of the fish, the pH increases due to the decomposition of amino acids, urea and creatinine oxidative deamination, originated by autolysis, and form a conducive environment to enable the bacteria that cause changes in the fish flesh (JACOBSEN et al., 2010). The enzymatic activity and the action of bacteria modify the concentration of free hydrogen ions. As the values go from neutral and alkaline, the product becomes inappropriate for consumption. The pH of fresh fish varies between 6.6 and 6.8 and as the fish deteriorates the pH increases (FONTES et al., 2007). In this regard, the pH values above 7.0 can limit the shelf life of certain fish species (Rey et al., 2012). In a study conducted in Turkey, it was also observed a significant increase in the pH of fresh Haddock with each passing day, rising on average from 6.99 to 7.09 (ALPAS and AKHAN, 2012). Therefore, the determination of acidity can provide an important input in assessing the conservation status of fish and derivatives.

A process of decomposition, by either hydrolysis, oxidation or fermentation, causes changes in pH (ADOLFO LUTZ INSTITUTE, 2008).

The reaction of Eber to hydrogen sulfide is indicated to assess the state of conservation of fresh fish, because the bacterial decomposition in the muscle of the animal releases sulfur, which in acidic environment, turns into hydrogen sulfide (SOARES and GONÇALVES, 2012).

The presence of hydrogen sulfide in fish samples indicates an advanced stage of deterioration (LEHANEA AND OLLEY, 2000). Table 1 shows the results of the

reaction of Eber to hydrogen sulfide of Yellow Hake collected at the street markets 1, 2 and 3. The results obtained were the absence of hydrogen sulfide in samples of street markets 1, 2 and 3 in 0 hour, except for the fish 3 of market 2. From 48 hours of storage, all fish were positive for hydrogen sulfide, except the fish 1 at the street market 3. The confirmation of production of hydrogen sulfide in the samples suggests a limited shelf life of the fish. The data reinforce the need for adequacy and/or implementation of quality programs in establishments selling fish at street markets.

Table 1 – Qualitative analysis of the reaction of Eber to hydrogen sulfide gas of Yellow Hake sold in street markets in Santos/SP, 2014.

	Storage Time (hours)	Fish1	Fish2	Fish3
Street Market 1	0	0	0	0
	24	0	+	+
	48	+	+	+
	72	+	+	+
Street Market 2	0	0	0	+
	24	0	+	+
	48	+	+	+
	72	+	+	+
Street Market 3	0	0	0	0
	24	0	+	+
	48	0	+	+
	72	+	+	+

Legend: *absence (0); presence (+)*

Soareset et al. (1988) analyzed 120 samples of fillet of fish from 10 different species, all maintained at -18°C until the time of analysis.

The highest percentage of positive samples for hydrogen sulfide was detected in 100% of "Pescadinha" (*Cynoscionstriatus*), followed by 88% of Merluza (*Merluciushubbsi)* and Kingklip (*Genypterusblacodes)*, 75% of Sandperch (*Pseudopercisnumida)* and King Weakfish (*Macrodononcylodon*), and 50% of Brazilian Drum (*Umbrina sp.),* Whitemouth Croaker (*Micropogoniasfurnieri*) and Flounder (*Paralichthys sp.).*

Table 2 shows the results obtained by Eber proof for the presence of ammonia in the samples of Yellow Hake. In the street market 1, 2 and 3, the samples showed no white vapor at 0 hour, resulting from the reaction of Eber, except the fish 2 of the street market 2. After 48 hours of refrigerator storage, all samples showed positive results for ammonia. Possible

causes for these results are: inadequate unloading to the point of sale. It is also noted the lack of equipment and techniques for maintaining proper temperature, handling and display of fish. Another factor is the transformation of post-mortem on the transport in step-boat unloading fish, and fish, leading to the deterioration process. Enzymatic reactions cause changes in the composition of muscle; affect the flavor, texture and food safety (CICERO et al., 2014; HAMADA-SATO et al., 2005).

Table 2 – Qualitative analysis of the reaction of Eber to ammonia of Yellow Hake sold in street markets in Santos/SP, 2014.

	Storage time (hours)	Fish1	Fish2	Fish3
Street Market 1	0	0	0	0
	24	0	0	+
	48	++	++	++
	72	++	++	++
Street Market 2	0	0	+	0
	24	++	++	+
	48	++	++	+
	72	+++	++	+
Street Market 3	0	0	0	0
	24	+	0	++
	48	++	+	++
	72	+++	+++	+++

Legend:*Absence* (0); *Very Little Presence* (+); *Little presence* (++); *Presence* (+++)

The presence of white smoke indicates that the fish flesh is in the protein degradation process. This enhanced degradation increases the amount of ammonia that, when reacted with hydrochloric acid, form ammonium chloride (NH_4Cl) which is released in the form of vapors (ADOLFO LUTZ INSTITUTE, 2005). The analysis thermo-tolerant coliform of the samples reveals that fecal contamination is present since the acquisition of the fish.

The average coliforms at 45°C obtained in this period was: 2.67, 2.83 and 2.55 (log_{10}/CFU) or $4.7x10^2$, $6.8x10^2$ and $3.6x10^2$ (CFU/g) in the street markets 1, 2 and 3, respectively.

After 24 hours, under simulated domestic refrigeration storage, it was observed that the proliferation of coliforms

at 45°C (Figure 2) differed among collection sites. The street markets 1 and 3 showed increased coliform counts at 45°C, which were 4.0 and 3.8 (log_{10}/CFU) to $10.1x10^4$ and $7.0x10^3$CFU/g, respectively. These data demonstrate that the growth of fecal coliform in seafood is constant, even under refrigeration. After 24 hours of home storage, the proliferation of coliforms at 45° C decreased the growth rate, suggesting the entry into the stationary phase of bacterial growth.

The deterioration of fish is influenced by various factors, from his capture to its processing, marketing and manipulation.

The basic principles of conservation will directly affect the conservation of the product (DEVIDES et al., 2014).

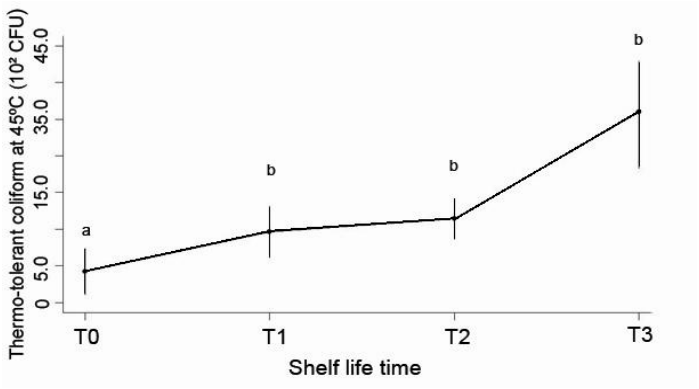

Figure 2 – Means of thermo-tolerant coliforms at 45ºC the Yellow Hake during four storage time in household refrigerator. Storage time in household refrigerator: T0 = the acquisition of the fish; T1 = 24 hours; T2 = 48 hours; T3 = 72 hours. Different letters indicate significant difference between the time intervals ($p < 0.05$).

The manipulation in catching the fish would be the first factor influencing the quality of the product. If capture allows the fish to be in a state of agony, this will exhaust its glycogen reserves, increasing the concentration of lactic acid accelerating *Rigor mortis*, consequently increasing the deterioration process due to pH change.

Soon after capture, it is possible to observe changes in the fish (SOARES AND GONÇALVES, 2012).

Besides the extrinsic parameters which influence the degradation of fresh fish, intrinsic parameters, such as water activity (about 80%), neutral pH, little connective tissue, facilitate microbial proliferation (SÁNCHES-ALONSO et al., 2012). Colloidal structure of the fish muscle proteins has large quantities of nitrogen free substances (amino acids, and trimethylamine oxide) (Ozden, Inugur, Erkan, 2007) in addition to the amount of acid proteases

(cathepsins), and neutral proteases (calpains) that directly interferes with the process of deterioration speed (AMARAL and FREITAS, 2013).

The values of coliforms at 45°C obtained during the shelf life of Yellow Hake reinforces the importance of hygiene in the handling, the ice cover on the exposed fish and stocking. The law RDC n°. 12 of January 2, 2001 does not provide the coliform analysis for fresh fish. The recommendation of the ICMSF (International Commission of Microbiology Specifications for Food) for the control of microbiological quality of fresh fish, *Escherichia coli* in the question is: Minimum Limit m = 1.1x10 and Maximum Limit M = $5x10^2$ MPN/g (International Commission on Microbiological Specifications for Foods, 1986). In Canada the maximum tolerance of *E. coli* to m=4 MPN/g for M=4x10 MPN/g. Japan has stricter control, not allowing the presence of coliforms (Food Agricultural Organization of United Nations).

Sensory analysis of the Yellow Hake presented acceptable characteristics at the time of acquisition (T0) in the assessed street markets. In 24 hours of storage (T1), the sensory characteristics score points in the evaluation due to the following observations: no protruding eyes, abdomen leaving lasting impression to finger pressure and not firm flesh. The odor of rotten fish, popularly characteristic item for choosing the fish, is present from T2 in all fish. Related attributes of skin, meat, operculum and gills appear as inappropriate from T1. When purchasing fish at street markets there is no record of its validity as it is unknown the origin of that food, as well as the fishing and unloading days. The rapid deterioration of fresh fish can occur due to factors ranging from capture to marketing. According to Lanzarin (2014), even with the maintenance of proper temperature, it occurs the loss of texture of fish flesh, highlighting the importance of achieving the sets of good handling practices. Oliveira et al. (2014) analyzed 29 samples of Arapaima (*Arapaima gigas*, Schinz, 1822) stored in ice (2°C) weekly, during 36 days, to determine the sensory evaluation. The authors found a time limit for the consumption of fish between 26 and 28 days. The most affected features during the first days were on the gills, and the loss of the concavity of the eyes. The fish changes its sensory characteristics, which are fundamental to the buyer and consumer, with the deterioration process, since this simple and affordable method indicates the quality of fresh fish (OCAÑO-HIGUERA et al., 2011). The changes of the fish (releasing odors, mucus formation, abnormal color and changes in texture) occur due to autolysis, oxidation and bacterial activity (Sánchez-Alonso, 2012). The post-mortem changes, which

directly affect the quality and shelf life of the fish, are associated with the degradation of protein and ATP, the pH drop, lipid oxidation, production of undesirable compounds such as trimethylamine (TMA-HIGUERA et al., 2011; TEROVA et al., 2011; CICERO, 2014). According to Oçaño-Higuera (2011), it is essential to apply a preventive process in the chain of fish marketing in order to minimize the risk of occurrence of foodborne outbreak, and to offer the consumer fish meat suitable for consumption. To aim good results, it is necessary to perform a food safety program involving all professionals who come into contact with the fish including: fishermen, salespeople, plant workers that do the fish unloading, stakeholders and retail trade workers. Making a mistake, the handler puts at risk the entire fish production chain and can result in foodborne outbreaks, impairing the health of the consumer. Lanrazin et al. (2011) found that appropriate methods employed in the fish production chain were responsible for a shelf life stable, optimizing costs and increasing the quality of food offered, not endangering the consumer.

Conclusion

This study of the shelf life of Yellow Hake sold in street markets in the city of N) and the volatile bases of low molecular weight (TVB-N) that are produced by bacterial action. Likewise, the muscle undergoes changes in texture, water retention capacity and staining (OCAÑO-SANTOS-SP demonstrated that this type of fish showed signs of deterioration since the time of sale. With the advancement of hours under refrigeration, Yellow Hake showed greater increase in the number of microorganisms and loss of features related to freshness of the fish. Significant changes were observed from 24 hours of storage in this scenario. Initially it is recommended that the period of home storage is as short as possible, avoiding more than 24 hours storage.

To minimize problems associated with foodborne diseases it is necessary to implement quality programs in the establishments of fish marketing, as well as public policies for enforcing the health legislation in the fisheries landing warehouse. Such factors are urgent to guarantee consumer safety, reducing the risk of foodborne diseases.

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