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CHEMICAL, PHYSICAL AND MICROBIOLOGICAL CHANGES IN TILAPIA (Oreochromis niloticus) DURING MARINATION

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■ABSTRACT: This is a study of the physical, chemical and microbiological changes promoted by the marinating process in tilapia fillets. The marinating process was performed in 5% acetic acid and 4% sodium chloride for 15 min. The fish:solution ratio was (1:2). The marination was carried out at 4 ± 2 0C until the end point, which was assessed by physical and chemical analysis. After marination the total bacterial viable count was reduced. During the marinating process acetic acid and sodium chloride diffuse into the fish tissue until a balanced concentration is reached. Most changes in the components of the filleted fish occurred in the first 7 min. being called "variable grade period" in contrast with "constant grade period" that occurred at the end of the marinating process.

■KEYWORDS: Fish; marinated tilapia; *Oreochromis niloticus*; quality control; marination.

INTRODUCTION

In Brazil tilapia is generally consumed as fresh. The term "marinated fish" is used to define different kinds of semipreserved fish made by immersion in a solution containing acetic acid plus sodium chloride.[6, 12]

The inhibitory effects of these substances on bacteria and enzymes increase with concentration.[10] The initial quality of the raw fish, in terms of their freshness, microbiological load and physical damage, is an important factor that influences the quality of the end product.[7] Owing to consumer preferences, it is not advisable to increase these additives to levels that suppress potential spoilage. Cold, fried and cooked marinated fish may be obtained, according to the processing treatment. The shelf-life depends largely on storage temperature and also on the type of bacteria associated with the marinated fish. *Micrococcu*s sp. and *Pediococcus* sp. have been found in herring tidbits stored at room temperature while *Lactobacillus* sp. and *Leuconostoc* sp. have been isolated from spoiled herring tidbits. Bacteria not completely inactivated after the marination may grow during storage, according to their ability to adapt to the acid medium.[9, 11]

The manufacturers' strategies to increase the safety of fish and fish products consist of developing new thecniques to reduce the number of microorganisms in fish and new minimally processed products which could represent new hazards for health because of the growth.of pathogens The purpose of marinating fish products is to offer ready-to-eat or better, ready to cook convenience products. Depending on the viscosity of the marinade, more or less of it penetrates into the flesh and therefore leads to a special taste and texture.[19] The aim of the present work was to study the physical, chemical and microbiological changes promoted in tilapia fillets by the marinating process stored under cooling (4 ^{o}C) and frozen (-18 ^{O}C).

MATERIAL AND METHODS

Raw materials

Tilapia (Oreochromis niloticus) were caught and stored under refrigeration at 40^{0}C. The fresh tilapia was filleted. The fillets were divided into 5 kg portions and immersed in the marinating solution in a tumbler, at a fish: solution ratio of 1:2. The marinating bath was composed of 5% acetic acid and 4% sodium chloride. Marinating was carried out at 4 ± 20C for 15 min.

Process

The fish were marinated by the process developed by Yeannes & Casales.[23] The experiment was run in triplicate.

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The process (Figure 1) consisted of the following stages: reception of the raw materials, washing, filleting, washing, marinating for 15 min. (tamble), packing (sacks made of polyethylene) and storing (at 4 and - 18 ^{0}C).

Physical and chemical analysis

The chemical composition of filleted tilapia was determined as crude protein (N x 6.5),[2] crude fat[2], crude ash[2] and moisture.[14] Acidity was determined by titration with sodium hydroxide.[16] Sodium chloride was determined by the AOAC[2] method. The pH was measured as described by Lima dos Santos[14] et al., with a digital pH meter. Analyses were performed on 3 samples in each group.

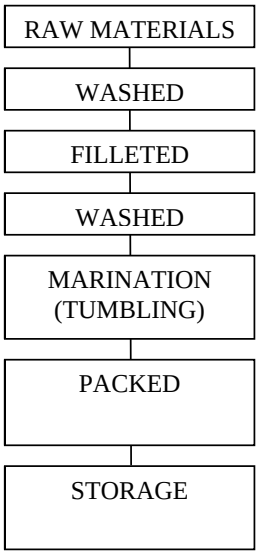

FIGURE 1 - Flow chart of marination process.

Microbiological analysis

For all microbiological counts, 10 g samples were homogenized in 90 mL 0.1% peptone water (Difco, 0118-17-0). From this 10-1 dilution, other decimal dilutions were prepared. Total viable count was determined by the pour-plate method, using Plate Count Agar (Difco, 0479-17) as the medium. Plates were incubated at 37^{0}C for 24 h. Staphylococcus (coagulase-positive) was enumerated by spread-plating on to Baird-Parker Medium (Oxoid CM 275), total coliforms in Brilliant Green Bile Broth and coliforms at 45^{0}C in EC Broth. Presence of Salmonella sp. was assessed following the procedure described in APHA1: after enrichment in 0.1% peptone water overnight and in Rappaport-Vassiliadis Broth (Oxoid CM 669) at 370C for 24 h, samples were streaked on Brilliant Green Agar (Oxoid CM 263) and plates were incubated at 37^{0}C for 24h. Analysis were made in 3 samples in each group. The results of all counts were given as the mean of 3 samples.

Statistical analysis

Statistical evaluations of the physical, chemical and bacteriological analyses were done with the computer package, Statistica 6.0.

RESULTS AND DISCUSSION

Physical and chemical analysis

Table 1 presents the results for the humidity content, leached ashes, lipids and proteins and chloride in tilapia fillets (Oreochromis niloticus), cooled or frozen, marinaded or not. In this experiment, the protein content varied between 12.8 and 14.2%, differing from the values found by Biato4, who reported content equivalent to 22.88% for the same species. According to Carbonera[7] et al., the fat content is used as a practical criterion for comparisons between different fish species. Thus, this author considers the fish as fat, when the minimum content of lipids is 10%, semi-fat between 2.5 and 10%, and lean for values below 2.5%. According to the present results, we can classify this fish as lean. With regard to the lipid content of lipids, there was significant variation among the samples ($p < 0.05$). The values for the cooled and frozen samples, irrespective of the marination process, demonstrated a reduction in the lipid content in the later.

Table 1- Physical-chemical composition of the tilapia fillets.

*Tilapia fillets	A	B	C	D	E
Cooled	83.6	1.6	1.4	13.7	0.7
Cooled marinated	81.2	2.9	1.1	13.3	1.4
Frozen	81.7	1.1	1.0	14.2	0.7
Frozen marinated	82.2	1.5	1.0	12.8	4.3

A: moisture; B: ashes; C: lipids; D: proteins; E: chlorides.
** n = 3.*

Leonhardt et al.,[13] worked with two strains of tilapia lineages (Thai and Nilotic) and they found the following lipid contents: 3.0% for the Thai strain (semi-fatty product) and 1.9% for the Nilotic (lean fish). According to Ludorff & Meyer15, in studies carried out with Nile tilapia, the fish showed contents of 1.1% and 78.4% respectively for ashes and moisture. The values found by these authors are close to those obtained in the present study. Beirão3 et al., reporting the chemical composition of the eatable part of fish, crustaceans and mollusks, found moisture contents that varied between 70 and 85%, proteins, between 20 and 25%, ashes, between 1 and 1.5% and lipids, between 1 and 10%. According to the same authors, this composition varies with the species, nutritional state, seasonality, age and gonadal conditions.

As noted by Sales & Sales,[18] quantitative knowledge of the chemical composition of the muscles of the fish is of commercial interest and of great importance for the formulation of an appropriate diet, as well as in the definition of technical procedures for the industries of fish processing industry. It is important to point out that details the chemical composition of the fish can affect the of processing, the flavor, the texture and the stability to fat oxidation, be it by an increase of the unsaturated chains of the fatty acids or the variation of the natural antioxidants, present in the raw material. Regarding the protein content, the value increased from 13.7 to 14.2% during storage of the fillets, due to the loss of water through freezing. During marination there was a reduction in content due to solubilization of proteins in the process. Considering the marination process and subsequent freezing for 15 days, an increase is observed in the concentration of sodium chloride, as a consequence of the diffusion of this salt into the musculature of the fish. Between the cooled and frozen fillets, there was no significant difference ($p > 0.05$) in this content (0.7% NaCl). The sodium chloride is an especially important ingredient of the marinating solution; besides developing the flavor, it contributes to the retention of the moisture. In this study the moisture content in the frozen fillet was 81.7% and in the frozen marinated fillet, 82.2%. The reduction of the pH caused by the presence of the acetic acid approximating the value (pH) of the isoelectric point of the proteins of the fish, restricting the available charges, reducing the protein-water interactions and increasing the protein-protein interactions. During the storage under freezing at -18°C of the marinated fillet, the pH values increased as a function of the time of storage (15 days), but this value was not used to evaluate the deterioration of the fish. This was prove through chemical and sensorial analysis accomplished by other researchers.20 In the marination process a reduction of the pH of 6.4 was observed for 6.0, and an increase of the acidity, of 0.46 for 0.54 in the frozen fillets due to the addition of the acetic acid. The yield of the tilapia fillets processed by marination in tumbler was 88.6%.

Microbiological analysis

The microbiological determinations were performed to complement the evaluation of the hygienic-sanitary conditions, and to assess the procedures related to the processing of the tilapia fillets: cooled, frozen and marinated.

The results of the microbiological tests of the tilapia fillets conserved under refrigeration (4°C) are show in Table 2.

Considering the counts of total coliforms, coliforms at 45°C, *Staphylococcus* coagulase-positive and detection of presence of *Salmonella*, according to the Resolution 12 of the Brazilian Sanitary and Hygiene Inspectorate (ANVISA)[5], the values indicate an acceptable quality for the subsequent marination in the form of frozen fillets.

Table 3 presents the results of the microbiological tests of the frozen fillets (-18°C). In Table 4, the results of the microbiological tests of the samples of the marinated frozen (-18°C) fillets of tilapia are presented.

Table 2 - Microbiological evaluation of cooled tilapia fillets (4 ^{0}C).

	A_1	A_2	A_3
Viable aerobic microorganisms (CFU/g)	40	58	62
Total coliforms (PMN/g)	< 3	< 3	< 3
Coliforms at 45 ^{o}C (PMN/g)	< 3	< 3	< 3
*Staphylococc*us coagulase-positive (CFU/g)	$< 10^2$	$< 10^2$	$< 10^2$
Salmonella sp. (in 25g)	Absence	Absence	Absence

A_1, A_2, A_3: samples

Table 3 - Microbiological evaluation of frozed tilapia fillets (- 18 ^{0}C).

	A_1	A_2	A_3
Viable aerobic microorganisms (CFU/g)	3.2×10^3	1.2×10^3	1.8×10^3
Total coliforms (PMN/g)	< 3	< 3	< 3
Coliforms at 45 ^{o}C (PMN/g)	< 3	< 3	< 3
*Staphylococc*us coagulase-positive (CFU/g)	$< 10^2$	$< 10^2$	$< 10^2$
Salmonella sp. (in 25g)	Absence	Absence	Absence

A_1, A_2, A_3: samples

As can be seen, all the results obtained for total coliforms, coliforms at 45°C, Staphylococcus coagulase-positive and Salmonella sp. are within the acceptance limits for consumption, established by ANVISA (see above).

With respect to the viable aerobic microorganisms, in the Brazilian federal legislation there are no references for the evaluation of fish and its products. However, in the legislation of the state of São Paulo, according to Vieira et al.22, 3 x106 CFU/g is stipulated as the maximum allowed for food. Even though there is no direct implication for public health, an excessive count of these microorganisms would lead to a decrease in the shelf-life of the product. The results obtained for the tilapia fillets are similar to those found by Vieira et al.22 who, in research on fish, found values for viable aerobic microorganisms higher than those found in this work (104 CFU/g).

Table 4 - Microbiological evaluation of marinated frozen (- 18 0C) tilapia fillets.

	A_1	A_2	A_3
Viable aerobic microorganisms (CFU/g)	1.7×10^3	1.1×10^3	1.7×10^3
Total coliforms (PMN/g)	< 3	< 3	< 3
Coliforms at 45 °C (PMN/g)	< 3	< 3	< 3
*Staphylococc*us coagulase-positive (CFU/g)	$< 10^2$	$< 10^2$	$< 10^2$
Salmonella sp. (in 25g)	Absence	Absence	Absence

A_1, A_2, A_3: samples

CONCLUSION

The statistical analysis of the results from the physical and chemical evaluations demonstrate that there were significant differences ($p < 0.05$) among the values of lipids, proteins, moisture, pH and acidity related to the marinating operation. In storage for 15 days, at - 18 °C, only the variations in the values of ashes and chlorides (like NaCl) were not significant ($p > 0.05$). The results obtained in the microbiological evaluation of the frozen and marinated frozen fillets show a reduction in the counts of decomposing microorganisms (viable aerobics) originating from the synergic effect of acetic acid and sodium chloride. The tumbling processing proposed to obtain marinated tilapia fillets, provided the product with a desirable stability during the storage period in the frozen form.

■*RESUMO*: Neste trabalho foram estudadas as variações físicas, químicas e microbiológicas associadas à marinação de filés de tilápia por tambleamento. O processo de marinação foi efetuado com solução de marinação contendo 5% ácido acético e 4% cloreto de sódio. A relação peixe:solução foi 1:2. A marinação foi executada a 4 ± 20C durante 15 minutos. Após o processo de marinação houve uma redução na enumeração das bactérias aeróbias viáveis. Considerando a concentração de ácido acético e cloreto de sódio na solução de marinagem, durante o processo houve um equilíbrio entre os componentes através da difusão nos tecidos (filés) do pescado. O efeito sinérgico induziu à inibição da multiplicação de microrganismos aeróbios viáveis. O rendimento do processo foi 88,6% e isto se deve à desnaturação protéica ocasionada pela perda da água de constituição do pescado. Foi influenciado tanto pelo cloreto de sódio como pelo ácido acético, componentes da solução de marinação.

■ *PALAVRAS-CHAVES*: Peixe; tilápia marinada; Oreochromis niloticus; controle de qualidade; marinação.

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