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# Reducing the Bitterness of Tuna (*Euthynnus pelamis*) Dark Meat with *Lactobacillus casei* subsp. *casei* ATCC 393

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**Summary**

During the process of canning tuna fish, considerable amounts of dark tuna meat are left over because of its bitterness, which are then used in the production of animal food. Fermentation with *Lactobacillus casei* subsp. *casei* ATCC 393 was used as an alternative to reduce this bitter taste. Samples of meat were prepared, vacuum packed and then stored at –18 °C. The frozen dark meat was used immediately after defrosting and the experiment was carried out with 2 and 4 % of NaCl with the addition of 2 and 4 % of glucose, respectively. The dark tuna meat was inoculated with lactic acid bacteria (LAB) and fermented at 10 °C for 30 days. The fermentation process was monitored through bacteriological and chemical analyses, when an increase of acidity and the corresponding decrease of pH were observed due to the prevalence of LAB. Sensorial analysis, using a test of multiple comparison, was carried out with pastes of fermented dark tuna meat and presented a significant difference when compared to the paste control, indicating the reduction of bitter taste.

*Key words*: fermented fish, lactic acid bacteria, *Lactobacillus casei*, tuna

## Introduction

About 60 % of the original weight of fish is wasted. When producing a can of tuna, around 18 % of the waste consists of dark meat because of its bitterness, which is then used for animal feed (*1*).

The presence of peptides composed of amino acids with a hydrophobic structure and the oxidation of the lipids (*2*) are responsible for bitterness. Hydrophobic amino acid side chains provoke bitterness of peptides, which is independent from the amino acid sequence. Matoba and Hata explained in their studies how bitterness is developed by hydrolysing non-bitter protein molecules (*3*).

Lactic acid bacteria (LAB) are widely used in the food industry and the simplicity of these microorganisms allows their use in scientific as well as in technological areas (*4*). A wide variety of LAB strains is routinely employed as starter cultures when manufacturing dairy, meat, vegetable and baked products. They are known to change the flavour, texture and appearance of foods in order to delay spoilage and reduce contamination (*5*). One of their important features is the fact that they extend the shelf life of the fermented products compared to the raw substrate (*6*). Metabolic products of LAB have been shown to inhibit the growth of many

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pathogens due to the competition for nutrients and the presence of starter-derivative inhibitors such as lactic acid, hydrogen peroxide and bacteriocins (*7*).

Fox and Wallace (*8*) studied the formation of flavour in cheese and showed that the proteolytic enzymes from lactic acid bacteria play an important role in the degradation of casein and peptides, leading to the production of free amino acids. Microbial peptidases can also reduce bitterness by hydrolysing bitter peptides formed in the cheese. The amino acids that are produced contribute directly to the basic taste of the cheese since they are precursors of other catabolic reactions, which produce volatile aroma compounds.

Arora and Lee (*9*) studied the analysis of variance and revealed significant differences among strains with respect to their specific activities. Overall profiles showed that *Lactobacillus casei* subspecies contained high amino and dipeptidase with relatively weak tripeptidase activities. *Lactobacillus casei* subsp. *casei* strains LLG and ATCC 393 had the highest di- and tripeptidase activities, respectively.

The aim of this work was to study and find out how to reduce the bitterness of tuna fish *(Euthynnus pelamis)* dark meat by lactic acid fermentation, using the *Lactobacillus casei* subsp. *casei* ATCC 393 as a starter.

## Materials and Methods

To make the fermentation process viable, the dark tuna meat (frozen grated tuna – *Euthynnus pelamis*) was divided into portions of 400 g and inserted in sterilised flasks. The experiment was performed with the following combinations of NaCl and glucose: 2 % of glucose and 2 % of NaCl, 2 % of glucose and 4 % of NaCl, 4 % of glucose and 2 % of NaCl and 4 % of glucose and 4 % of NaCl. Treatments were developed in an independent way, changing only one of the parameters (NaCl or glucose). The process was developed in aqueous solution with a proportion of 1:1, which means 400 g of fish meat and 400 mL of aqueous solution prepared in agreement with the final concentrations of glucose and NaCl, related above. *Lactobacillus casei* subsp. *casei* ATCC 393 was obtained from the Tropical Culture Collection, from Andre Tosello Foundation (Campinas, SP, BR). The system was inoculated with a quantity of $1.9 \cdot 10^8$ CFU/g (CFU, colony-forming units) and fermented at 10 °C for 30 days. Samples were taken for analysis at predetermined intervals, whilst the lactic fermentation was monitored by the rate of pH values, the lactic acid bacteria count and the total bacterial count. The LAB and total bacterial count were expressed as the log ratio among the counts on De Man, Rogosa, Sharpe agar (MRS agar) and Plate Count Agar (PCA) medium. The slowest growth of LAB on PCA is easily excluded from the total bacterial count.

For microbiological analysis, 10 g were homogenised aseptically for 1 min with 90 mL of 0.1 % peptone saline in a Stomacher 400 (Seward Lab, UAC House, UK). It was serially diluted 10-fold in the same diluent and plated onto appropriate plates. Aerobic mesophilic counts were determined on PCA (Oxoid CM 463, Basingstoke, UK) after incubation at 37 °C for 48 h (*10*). The psychrotrophic bacteria counting was done according to the plating surface on PCA (Oxoid CM 463, Basingstoke, UK) with incubation at 7 °C for 10 days (*10*). LAB counts were determined by plating on MRS agar (Merck, Darmstadt, Germany) with incubation at 30 °C for 48 h (*10*).

In the chemical analysis, pH values were determined by weighing 10 g of each sample blended with 90 mL of distilled water. Total titratable acidity (TTA) expressed as % lactic acid was determined by using the same sample prepared for the determination of pH, the TTA was measured by titrating against 0.1 M NaOH to a final value of pH=8 (*11*). The pH was measured with a pH meter (Corning pH meter 240, Corning, New York).

For the sensorial analysis, samples of 100 g of dark tuna meat (control) and fermented dark tuna meat for the elaboration of pastes were collected. After elaboration, the pastes were submitted for microbiological analysis, like *S. aureus* count, coliform count, *Salmonella* spp. and sulfite-reducing *Clostridia*, which were analysed by standard methods (*10*).

Considering that the samples showed acceptable quality, sensorial analysis was carried out and applied to multiple comparison tests. During these tests, 25 trained judges received one control sample (paste of dark tuna meat) to compare it with 5 codified paste samples. One of the samples was the control paste itself (non-fermented meat, P) and the other four samples were pastes made from fermented dark tuna meat with different treatments. The test used was developed with the objective of evaluating this specific attribute, the bitter taste of the samples. The judges were then asked to evaluate the bitterness of the samples, according to a scale from 1 to 9 points, where grade 9 means that the sample was extremely superior to P (samples less bitter than P), 5 equal to P (samples as bitter as P) and 1 extremely inferior to P (samples more bitter than P) (*12*).

Statistical evaluation was applied to the sensorial analysis, where variance analysis (Anova) was carried out to show significant differences between the samples. Then the Tukey's test was applied and showed a confidence level of 95 % ($p<0.05$), in order to show the differences between mean values (*13*).

## Results and Discussion

Fish decomposes rapidly, therefore this work had to be performed at low temperatures (10 °C), which prolonged the time necessary to reach the rate of fermentation.

Because there is a decrease of free carbohydrates in dark tuna meat, glucose was added to promote adequate fermentation. The glycogenic value varied from 0.05 to 0.85 % in the muscles, making the fermentation process difficult.

The pH decreased and the acidity value increased in all samples (Fig. 1). The initial pH of the dark meat was 6.0. Twenty-four days after the addition of the starter culture *(Lactobacillus casei* subsp. *casei* ATCC 393) the pH decreased, until it reached values between 4.32 to 4.55.

The analyses indicated that the pH decreased with the glucose content (2 %), however, they hinted at the

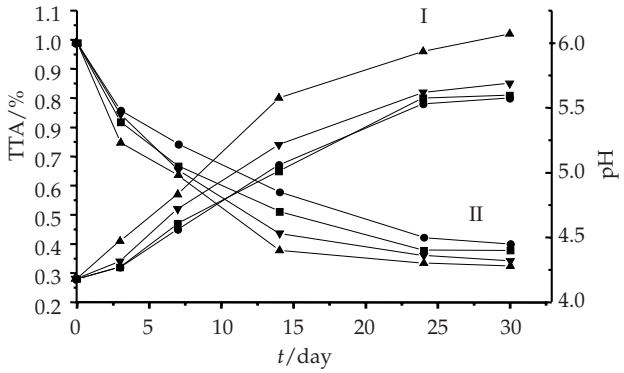


**Fig. 1.** Changes in total titratable acidity (I) and pH values (II) during fermentation of dark tuna meat. Samples were formulated with combinations of various proportions of glucose and NaCl: 2 % glucose and 2 % NaCl (■); 2 % glucose and 4 % NaCl (●); 4 % glucose and 2 % NaCl (▲) and 4 % glucose and 4 % NaCl (▼)

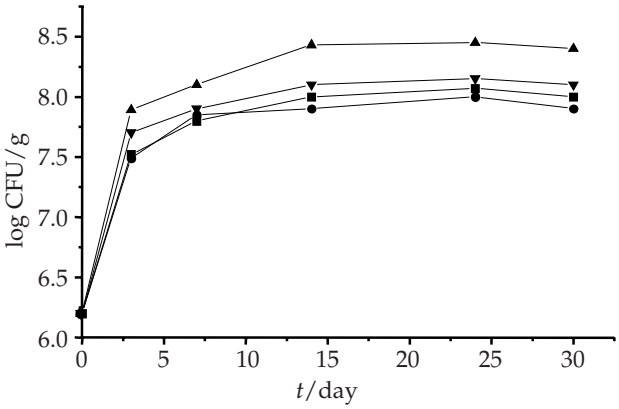


**Fig. 2.** Changes in mesophilic aerobic bacteria counts (log CFU/g) during fermentation of dark tuna meat. Key as in Fig. 1

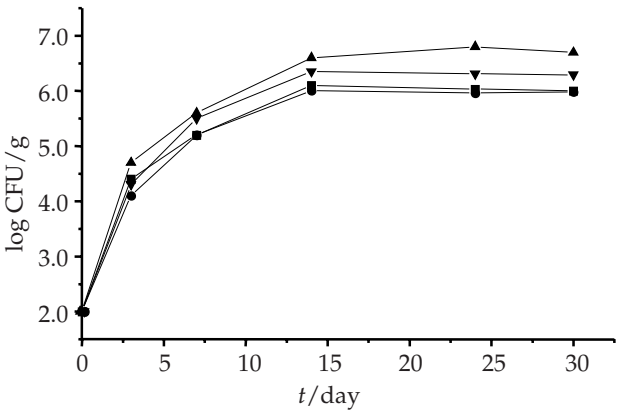


**Fig. 3.** Changes in psychrotrophic bacteria counts (log CFU/g) during fermentation of dark tuna meat. Key as in Fig. 1

fact that increasing the NaCl concentration from 2 to 4 % did not affect the fermentation process. Apparently, when the glucose concentration increased from 2 to 4 %, the pH showed apparent decrease, especially for the treatment using 4 % of glucose with 2 % of NaCl, where the pH rates were 5.23 after 3 days and 4.28 after 30 days of fermentation.

The results are in agreement with the results presented by Morzel *et al.* (*13*). Evaluating the effect of the starter cultures and the decrease of the pH during the fermentation of salmon fillet, it was observed that the highest production of lactic acid occurred with the addition of 5 % of sugar. However, the increase of this concentration did not show an expressive effect and the value of 5 %, conditioning a quantity of sugar that remained in the system after the fermentation, is used as substrate for the growth of pathogenic microorganisms. Nevertheless, the higher increase of NaCl decreases the growth of the LAB and also decreases the pH.

Using 2 % of NaCl with 2 % of glucose resulted in acidity 0.22 % after 3 days, and 0.81 % after 30 days. There were apparently almost no changes in acidity with the incorporating of 4 % of NaCl. For the treatment using 4 % of glucose with 2 % of NaCl, the acidity percentage reached 0.31 % after 3 days and 1.02 % after 30 days of fermentation. The results of acidity of the fermentation process correspond to the lactic acid production of the starter *(Lactobacillus casei).*

Figs. 2 and 3 show the growth of mesophilic aerobic and psychrotrophic bacteria counts for all the samples during the fermentation process. Considering the initial counts of $1.0 \cdot 10^2$ CFU/g for psychrotrophic bacteria and $1.6 \cdot 10^6$ CFU/g for mesophilic bacteria, an expressive increase in the count can be observed in the first 3 days of fermentation. Changes in the various samples ranged from 1.2 to $5.0 \cdot 10^4$ CFU/g for psychrotrophic bacteria and from 3.1 to $7.8 \cdot 10^7$ CFU/g for mesophilic bacteria. In the sample of 4 % of glucose with 2 % of NaCl, the growth for psychrotrophic and aerobic mesophilic bacteria ranged from $5.0 \cdot 10^7$ to $2.5 \cdot 10^8$ CFU/g, respectively, after 30 days of fermentation.

The lactic acid bacteria count, as shown in Fig. 4, demonstrated a growth during all processes. The first counts on microbial population varied from 4.8 to $5.4 \cdot 10^6$ CFU/g in different samples. There was a slight growth in all the samples in the first 14 days. After this period, the growth was gradual until the 30th day, when the maximum count varied from 1.0 to $3.6 \cdot 10^8$ CFU/g.

The results showed that the presence of LAB was considerable during the fermentation process. These results showed coherence to other studies. Like in the studies by Adams *et al.* (*15*), there was an increase of the LAB in the fermentation of fish with the addition of glucose and NaCl. Fermentation increases when the glucose value is raised from 1 to 5 %, whilst the increase of NaCl from 1 to 6 % caused a reduction in the fermentation process. Paludan-Müller *et al.* (*16*) studied the fermentation of plaa-som, a typical fermented fish from Thailand with various NaCl concentrations, and reported that LAB growth to levels of $10^8$–$10^9$ CFU/g is required to obtain a sufficient pH decrease in plaa-som, similar to other fermented fish products. However, it was found that the increase of salt concentrations from 6 to 11 % delayed or inhibited the LAB growth and thereby the fermentation process of plaa-som. It was suggested that a maximum of 6 to 7 % was used in plaa-som and other fish products resulting from fermentation in order

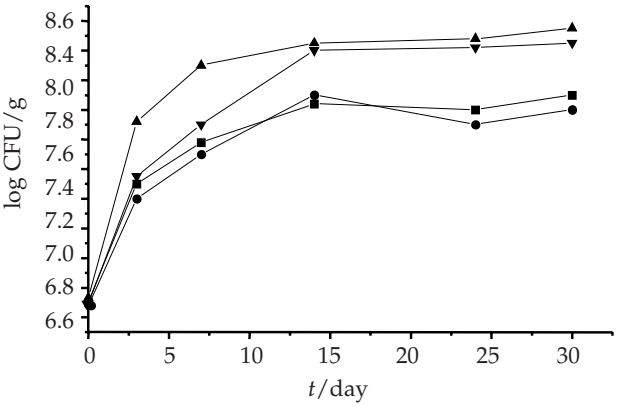


**Fig. 4.** Changes in LAB on MRS agar (log CFU/g) during fermentation of dark tuna meat. Key as in Fig. 1

to facilitate rapid growth of LAB and subsequent decrease in pH below 4.5. In high-salt batches of plaa-som, the growth of LAB was inhibited, allowing the growth of *Staphylococcus* spp. (*17*).

The microbiological analysis carried out on the pastes previously made with fermented meat and non-fermented meat showed acceptable quality, where no growth of *S. aureus*, *Samonella* spp., sulfite-reducing *Clostridia* and coliform was registered. Considering the positive results, the sensorial analysis was carried out and it is presented in Fig. 5.

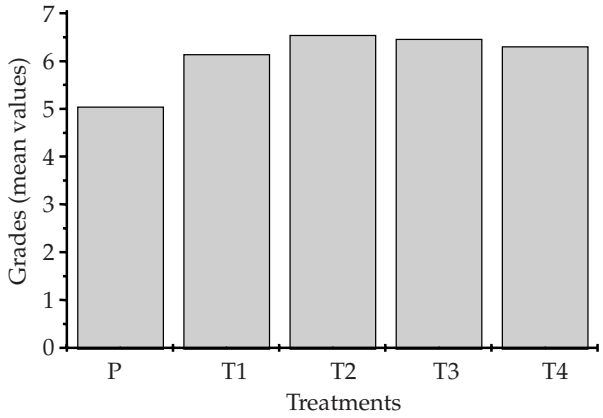


**Fig. 5.** The mean values of grades conceded by the 25 judges to the control (P) and the treatments: 2 % glucose and 2 % NaCl (T1); 2 % glucose and 4 % NaCl (T2); 4 % glucose and 2 % NaCl (T3) and 4 % glucose and 4 % NaCl (T4)

The mean value of the grades of the control sample was 5.04, which indicated that the judges identified correctly the codified control sample among the other samples. The results expressed that all the treatments had grades superior to the control, which indicated that there was a reduction in the bitter taste. However, the results related to the punctuation attributed to the scale used were assessed by the variance analysis and the Tukey´s test for the comparison between the mean values of the samples.

When the variance analysis was applied, significant differences could be observed between the treatments ($p<0.001$). However, using Tukey's test at 95 % of confidence level, it was observed that there were no statistical differences among all the fermented dark tuna meat samples, but they were all different from the control. It could be observed with the results that all treatments obtained superior grades and significant differences in relation to control. The results also indicated a decrease of bitterness with inoculation of *Lactobacillus casei* subsp. *casei* ATCC 393, possibly due to its enzymatic action on amino acids and hydrophobic peptides during fermentation or because the acidity disguised the bitterness of dark meat.

## Conclusions

The pH decreased in all treatments during the whole fermentation period. The performance of fermentation apparently increased when glucose content was raised from 2 to 4 % and it decreased with an increase of NaCl concentration from 2 to 4 %. The inoculation of dark tuna meat with *Lactobacillus casei* subsp. *casei* ATCC 393 significantly decreased the bitter taste.

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# Smanjivanje gorčine tamnoga mesa tune (*Euthynnus pelamis*) korištenjem *Lactobacillus casei* subsp. *casei* ATCC 393

**Sažetak**

Tijekom konzerviranja tune znatne se količine tamnoga mesa odbacuju zbog gorčine, a zatim koriste u proizvodnji hrane za životinje. Da bi se smanjio gorak okus, primijenjena je fermentacija s *Lactobacillus casei* subsp. *casei* ATCC 393. Uzorci su mesa pripremljeni, vakuumirani i zatim spremljeni na –18 °C. Zamrznuto tamno meso korišteno je neposredno nakon odmrzavanja i pokus je proveden s mesom koje je sadržavalo 2 ili 4 % NaCl uz dodatak 2 i 4 % glukoze.

Tamno meso tune inokulirano je s bakterijama mliječne kiseline i fermentirano 30 dana na 10 °C. Proces fermentacije praćen je bakteriološkim i kemijskim analizama kada je povećana kiselost (pad pH) zbog većeg udjela bakterija mliječne kiseline. Paštete fermentiranoga tamnoga mesa tune ispitane su senzorskom analizom, pa se testom višestrukih uspoređivanja ustanovilo da se smanjuje gorčina paštete u usporedbi s kontrolnim uzorkom.