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Determination of fat oxidation and lipid profile of a yellowfin tuna *(Thunnus albacares)* sausage using lactic acid bacteria *(Lactiplantisbacillus plantarum)*

Determinación de la oxidación de grasas y perfil lipídico de un embutido de atún aleta Amarilla *(Thunnus albacares)* utilizando bacteria Láctica *(Lactiplantisbacillus plantarum)*

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Abstract: The objective of this research was to determine the fat oxidation and lipid profile of a Yellowfin (*T. albacares)* sausage using *Lactic* Bacteria (*L. plantarum)*. For this purpose, during the sausage production process, lactic bacteria *L. plantarum* was added at a concentration of $8x10^2 UFC/ml$ at a dilution of 10^{-1} plus a control treatment (without lactic bacteria). During the different days of storage (day 0, day 4, day 8 and day 12), fat oxidation was evaluated using the acidity index method and the lipid profile was determined on the fourth day of storage using the gas chromatography method. With respect to the results of the acidity index, the sample did not show lipid oxidation during the 12 days of storage, unlike the control, which showed lipid oxidation from day 0, suggesting that the lactic acid bacteria incorporated into the sausage could prolong storage and mitigate the effects of rancidity processes. In addition, the lipid profile of the treatment under study complies with the nutritional standards established by Codex regulations, since tuna is an excellent source of omega-3, which represents a contribution of 30% of fatty acids.

Keywords: fatty acids, storage, nutritional, quality.

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Resumen: En el presente trabajo de investigación, tuvo como objetivo determinar la oxidación de grasas y perfil lipídico de un embutido de Aleta Amarilla *(T. albacares)* utilizando Bacteria *Láctica (L. plantarum)*. Para el cual, en el proceso de elaboración del embutido se adicionó bacteria láctica *L. plantarum* con concentración de $8x10^2 UFC/ml$ en dilución de 10^{-1} más un tratamiento control (sin bacteria láctea). Durante los diferentes días de almacenamiento (día 0, día 4, día 8 y día 12), se evalúo la oxidación de grasas mediante el método de índice de acidez y la determinación del perfil lipídico al 4 día de almacenamiento por medio del método de cromatografía de gases. Con respecto a los resultados del índice de acidez la muestra, no presentó oxidación lipídica durante los 12 días de almacenamiento, a diferencia del control, que presentó oxidación lipídica desde el día 0; lo que sugiere que la bacteria láctica incorporada al embutido, podría prolongar la conservación y mitigar los efectos de los procesos de rancidez. Además, el perfil lipídico del tratamiento en estudio, cumplen con los estándares nutricionales establecidas por la normativa del Codex, por ser el atún excelente fuente de omega 3, lo que representa un aporte del 30 % de ácidos grasos.

Palabras clave: ácidos grasos, almacenamiento, nutricional, calidad.

Introduction

The meat food industry has been intensifying its efforts to produce and market products low in saturated fats (Azevedo et al., 2020). Also, one of the most crucial challenges is to find solutions to produce nutritious and healthy products that maintain sensory characteristics and have consumer acceptability.

Meat and meat by-products are healthy choices because they contain high quality protein, vitamins (B6 and B12) and minerals (iron, selenium and zinc) (Saadoun & Cabrera, 2016).. However, the lipid profile of these products, are questioned mainly when it comes to the high content of saturated fatty acids and low content of polyunsaturated fatty acids (Delgado-Ramos, 2022). The consumption of meat products is approximately 3 to 4 times per week, which is related to the increase in the consumption of "ready-to-eat" products (WHO, 2020).

Fish has been part of the diet for many years, and the motivation to know and study its composition and benefits arose several decades ago. Many fish species have a high commercial value and different types of processing (fresh fish, frozen fish, meals, oils, etc.), according to this demand influences the crucial decision on processing levels in the fishing industry (Cifuentes and Rincón, 2022).

Yellowfin tuna (*T. albacare*) is a fishery species of great acceptance by the population, due to its high nutrient content, however, the content of fatty acids is notably different between species depending on several factors such as season or closed season, fishing area, climate, depth of habitat of the species of interest, salinity, environment, maturity, gender; in addition, this tuna is used for the production of sausages (Otero, 2019).

Biopreservation can be defined as shelf-life extension and safe nutrition using the natural microbiota and its antimicrobial compounds. In preservation biology it can be used from food production methods with minimal processing and without additives. The application of safe microorganisms and their metabolites to inhibit the growth of pathogenic microorganisms that deteriorate food, using acid bacteria (Duque 2018).

Currently, it has been demonstrated that lactic acid bacteria (LAB) can form biofilms on biotic and abiotic material for industrial purposes or with antagonistic effects against various foodborne pathogens (Tatsaporn and Kornkanok, 2020; Valencia, 2020). The species of major importance are *L. sakei. L. curvatus, L. plantarum, L. pentosus, L casei, P. pentosaceus and P. acidilactici* (Bañon et al., 2011; Guzman, 2020).

Lactiplantisbacillus plantarum has a great facility to adapt to environmental niches and the particularity of presenting antibacterial capacity against different pathogenic bacteria (Ruiz et al., 2021). They have been used in sausages as protective cultures inhibiting other undesirable microorganisms. The isolation of LAB that can survive heat treatment of cooked products could be an alternative to improve the shelf life of these products (Miras, 2019).

Several studies indicate that depending on the cooking technique, they suffer losses or increases in nutrients and total lipids (Briones et al., 2020). The variation suffered by the protein matrix from fish when stuffed and added with a live microorganism with probiotic properties is unknown (Martínez, 2016). Therefore, the objective of this research was to determine the fat oxidation and lipid profile of a yellowfin (*T. albacares*) sausage using lactic acid bacteria (*L. plantarum).*

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Materials and methods

Whole, skinless, frozen 3 kg pieces of yellowfin tuna (*Thunnus albacares*), lot 013-A-L-309-22-Z, were obtained from the company Zhongli Importadora Zhongli SA, located in the town of Macarena on the Circunvalación section 3 on the road to Montecristi (Jaramijó). Subsequently, they were used to make sausage.

Obtaining pure strains of *L. plantarum*

For reactivation the pure *L. plantarum* strain (12Lcm) from the DESCALZI laboratory, 1000 µL of the strain was transferred into 100 mL of culture broth and incubated for 24 hours to obtain early stationary phase cells, which will serve as the initial inoculum in the sausage. The bacterial strain was incubated according to the reference for culture conditions at 30°C (Otero, 2019).

Experimental management

The ingredients were weighed and cutterized, obtaining a homogeneous mass, then inoculated with *L. plantarum* ($8X10^8 UFC/ml$) and finally, 300 g were stuffed into synthetic casings and stored refrigerated at 4 °C. Table 1 shows the ingredients and their percentages for the preparation of the fish sausage.

Table 1. *Ingredients used in the production of T. albacares sausage.*

Ingredients	*Percentage*
Fish	91,32
Gelatin	1,14
Milk	5,71
Cumin	0,11
Pepper	0,11
Garlic	0,51
Parsley	0,06
Salt	1
Total	100

Experimental analysis

A one-factorial completely randomized design with a control was used; it is worth mentioning that *L. plantarum was* used for the treatment under study, while a control treatment was prepared without the addition of lactic bacteria.

Experimental measurements

Determination of fat oxidation
It was performed by the acidity index method, 10 g of sample was used in 100 ml of neutralized ethanol (alcohol and ether in similar volumes) (hot), then titrated using a 0.1 M sodium hydroxide solution and 1ml phenolphthalein, shaken vigorously maintaining the elevated temperature of the solution (Villanueva, 2015). Once the procedures were completed, the amount of free fatty acids expressed as oleic acid was calculated.

Lipid profile.
The analysis of saturated and unsaturated fatty acids (FA) was carried out on uncooked sausages by gas chromatography, according to the "AOCS Official Method Ce 1h-05" standard in the laboratory facilities of "LA FABRIL SA".

3. Result

Fish sausage acidity index.
Table 2 shows the acidity values obtained during the different days of storage (day 0, day 4, day 8, day 12), where in using the latic bacteria "*L. plantarum"* it was observed that the sample presented 1.96 mg KHO/g for day 0, with a gradual increase of 3.12 mg KHO/g for day 12, which indicates that the presence of *L. plantarum slowed down lipid oxidation, unlike the control, in which greater lipid oxidation was observed from the initial day, where values close to 4 mg KOH/g were found. plantarum* slowed down lipid oxidation in contrast to the control, which showed higher lipid oxidation from the initial day, where values close to 4 mg KOH/g were found.

The results exposed above agree with Moosavi-Nasab et al (2018), who in their study, showed an increase in the acidity index, with values of 4 mg KOH/g for day 0 and 6.90 on day 3. In addition, Villanueva (2015), mentioned that exposure to open air, temperature variations, light and humidity accelerate lipid oxidation.

According to Codex Alimentarius 329-2017 states that, the acid number in fish oil is ≤ 3 mg KOH/g. It is necessary to emphasize that, lipid oxidation related to high polyunsaturated fatty acid content often contributes to intense seafood odor during prolonged storage (Thanasak

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et al., 2013). Unsaturated acids from marine species are less stable to oxidation, especially in their phosphoglyceride fraction (Granado et al., 2013).

Table 2. Percentage of Acidity Index

Acid number (mg KOH/g)				
Treatments	**DAY 0**	**DAY 4**	**DAY 8**	**DAY 12**
T1 (with lactic bacteria)	1,96[a]	2,48[a]	2,32[a]	3,12[b]
CONTROL (without lactic bacteria)	3,27[b]	3,37[b]	3,55[b]	3,72[a]

Determination of the lipid profile by gas chromatography.

Table 3 summarizes the result of the lipid profile in the yellowfin tuna and lactic bacteria sausage, which presented 58.74 % of saturated fatty acids, among acids (C12:0- 2.27%; C14:0-8.47% and C16:0- 27.64%) respectively, 36.03 % of monounsaturated acids comprised between (C16:1n7- 4.34%; C18:1ns9cis- 21.40%), a total of 5.20 % of polyunsaturated fatty acids (C18:2 (n-6) - 2.46 % and C18:3 (n-3/n-6) 1.18 %, as well as, 4.95 % of trans fats and 2.08 % between omega3/omega6.

The variation of lipid composition is given according to the marine species, as well as to factors such as: seasonal variation, which is directly linked to the increase of unsaturations in FA, the age or stage of life of the individual (Cedeño, 2019). On the other hand, Bravo and Pozo (2015) stated that the percentages in sausages with commercial formulations of mortadella possess: 37.34 % of total saturated acids; 40.74 % of monounsaturated, 21.92 % total polyunsaturated 21.92% and 1.76 % of total trans fats. Meanwhile, in sausages, values of 36.42 % total saturated acids; 39.58 % total monounsaturated; 24 % total polyunsaturated and 2.05 % of trans fats were found.

In contrast, Pinto (2015), reported values of total fatty acids in tuna: SFA 112.17 mg/100g; MFA 75.30 mg/100g; PUFA 226.59; n-3 171.77mg/100g; n- 6 48.01 mg/100g; n-3/n-6 0.39 mg/ 100g. in this way, it is mentioned that, according to the criteria established by the Ministry of Health and Social Protection in resolution 333 of 2011-17. For nutritional labeling and labeling in Colombia. Species were classified as excellent or good sources of PUFA, this resolution establishes that a food should be classified as "high" or "excellent source of", When at least 45 % of the acids present that are found in a food come from polyunsaturated fats, which contribute more than 20 % of the energy value of the product (Atehortúa et al., 2017).

Elshehawy & Farag (2019) noted that DHA is usually more abundant in fish lipids than EPA. The study by Peng et al. (2013), the essential amino acid (EPA) ratios for yellowfin and bigeye tuna were 44.95 % - 45.64 %. Yellowfin tuna presented higher concentrations of DHA (20.22 % of total fatty acids), C20:4 (ARA) and C20:5 (EPA), in addition, n-3/n-6 ratios ranged from 3.29 - 4.56 %.

This study shows that these tuna species have a high nutritional value, despite the fact that most marine fish are not able to efficiently synthesize polyunsaturated fatty acids, they acquire them through their phytoplankton diet, they also depend on ecological and biological factors so that the levels of omegas 3 and 6 are high, the different varieties of fish have mandatory nutritional elements in the human diet fundamentally EPA and DHA GAs (Atehortúa et al., 2017).

Table 3. Lipid profile of tuna sausage with *L. plantarum*

Carboxylate Type		**%**
C4:0	Butyric	1,13
C6:0	Caproic	1,23
C10:0	Cupric	1,80
C12:0	Lauric	2,27
C14:0	Myristic	8,47
C15:0		2,47
C16:0	Palmitic	27,64
C17:0	Margaric	1,34
C18:0	Stearic	12,39
Total Saturated		58,74
C14:1		1,19
C16:1 (n-7)	Palmitoleic acid	4,34
C18:1 t	Trans Isomers	4,95
C18:1 (n-9)	Oleic	21,40
C18:1 c	Cis isomers	1,27
C20:1 (n-9/n-11)	Eicosenoic acid	1,15
C22:1 (n-9/n-11)	Erucico	1,73
Total Monounsaturated		36,03

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C18:2 (n-6)	Linoleic	2,46
C18:3 (n-3/n-6)	Linolenic	1,18
C22:2		1,56
EPA-DPA-DHA		----
N.I		----
Total Polyunsaturated		5,20
Trans fats		4,95
Omega3/Omega6 Ratio		2,08

4. Conclusions

During storage at refrigerated temperature (4°C), the raw yellowfin tuna sausage inoculated with lactic acid bacteria (*L. plantarum*) contributed to the prolongation of the autooxidation of the lipids present in the fish until the eighth day of storage. In relation to the lipid profile, the sausage presented a large amount of palmitic acid (27.64%), oleic acid (21.40%), as well as an Omega3/Omega6 ratio of 2.08%. This made it possible to obtain an innovative product, of good quality and with a high nutritional value for the consumer.

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