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ORIGINAL ARTICLE

Use of Cuban sardines in anchovy production: analysis of physicochemical and sensory factors

Uso de sardinas cubanas en la producción de anchoas: análisis de factores fisicoquímicos y sensoriales

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Abstract The salting-ripening process has traditionally been applied to different pelagic species to obtain a product with typical sensory characteristics distinct from fresh fish. In this regard, the general objective of the research was to evaluate Cuban sardines for the production of anchovy products, aiming for their potential industrial production. The study sought to verify that this species was microbiologically safe for human health and to compare it physically and sensorially with a traditional anchovy product. It was concluded that it was advisable to use this type of sardine for producing anchovy products, as the physicochemical results were quite similar to those found in the consulted literature. Despite having a shorter ripening time (3 months), the product received high consumer acceptance.

Keywords Cuban sardines, anchovy production, salting-ripening process, physicochemical analysis, sensory evaluation, microbiological safety.

Resumen El proceso de salado-madurado se aplicó tradicionalmente a diferentes especies pelágicas con el objetivo de obtener un producto con características sensoriales típicas, distintas a las del pescado fresco. En este sentido, se estableció como objetivo general de la investigación evaluar las sardinas cubanas para la obtención de productos anchoados, con miras a su posible producción industrial. Se buscó comprobar que esta especie fuese microbiológicamente segura para la salud humana y compararla física y sensorialmente con un producto anchoado tradicional. Se concluyó que era recomendable utilizar este tipo de sardina para la obtención de productos anchoizados, dado que los resultados físico-químicos fueron bastante similares a los de la literatura consultada. A pesar de tener un tiempo de maduración menor (3 meses), el producto tuvo una gran aceptación por parte de los consumidores.

Palabras clave sardinas cubanas, producción de anchoas, proceso de salado-maduración, análisis fisicoquímico, evaluación sensorial, seguridad microbiológica.

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PUBLIS EDITORIAL

Introduction

The term "fish" refers to aquatic animals used as food. These can be caught in various bodies of water, such as oceans, seas, rivers, and lakes, or raised through aquaculture techniques. Depending on the type of fish, different varieties of seafood are obtained (Pounds et al., 2022).

In general, all varieties of fish are rich in essential proteins and minerals. Saltwater fish, especially those caught at sea, are particularly abundant in fatty acids, especially unsaturated fatty acids such as omega-3, as well as minerals like iodine, zinc, phosphorus, and selenium. These nutrients help combat the harmful effects of LDL cholesterol, directly benefiting the circulatory system and overall health; they also strengthen the immune system against carcinomas (Eggersdorfer et al., 2022).

Among pelagic species is the sardine, a blue saltwater fish that belongs to the Clupeidae family, within the order Clupeiformes. This group is the most abundant and widely distributed along Cuban coasts and globally, and its fishing is of significant economic and nutritional importance (Claro et al., (2009).

Sardines inhabit relatively warm waters with normal salinity and form large schools of thousands of individuals. They feed on plankton and are commonly found in well-lit surface waters where microscopic forms of phytoplankton abound. During their youth, they migrate toward the coast and shorelines, where small individuals, newly covered by scales and just beginning to acquire their characteristic coloration, frequently group together. Upon reaching adulthood, they move toward oceanic waters and, at certain times, invade deeper waters (Bishop et al., 2017).

Salting is a method of fish preservation used since ancient times, with evidence dating back 3,500 to 4,000 years B.C. In the presence of salt (sodium chloride), the flesh of certain fatty fish can undergo chemical and physicochemical changes, leading to the process known as ripening or "anchovy-making" (Cabrer et al., 2008).

This process requires varying amounts of time, depending on the specific technology applied and the species in question, resulting in a product with distinct sensory characteristics compared to fresh or salted fish. Salted-ripened anchovy is traditionally produced in Southern European countries like Spain, Portugal, France, and Italy, and it is one of the most significant products in the international market. There are references to the trade of "anchovies" dating back to the Middle Ages when salt-ripening was essential to the economy of many Mediterranean towns. In this context, the objective of this research was to evaluate Cuban sardines (S*ardina pilchardus*) for the production of anchovy-like products, with an eye toward their potential industrial production.

Materials and methods

The raw material used in the salting and ripening experiments for this thesis was sardine (*S. pilchardus*) caught off the coast of Havana, Cuba, in February 2015. Coarse salt and olive oil were used for its preparation.

The heading and partial gutting were done manually. To facilitate handling, the fish was coated with fine salt. Parallel forces were applied to the body and head while keeping the junction between both parts fixed. Once the partial tearing of the flesh and viscera was achieved, a slight rotational movement was made to separate the head from the body. This gutting was partial, as part of the pyloric sac remained in the fish.

After heading and gutting, the fish was filleted. The tail, fins, and belly were cut with scissors, and scalpels were used to obtain the fillets, due to the small size of the fish, which required careful handling.

The *S. pilchardus* fillets were arranged in pans for ripening, forming layers as thick as a fist. After placing a layer of fish, fine salt was added, and the process was repeated. Upon completion, a wooden lid and a 24 kg concrete block were placed to press the fish, facilitating the expulsion of water and fat, as well as the removal of air. As the fish lost water, the pressure was reduced to half its initial value. The fish was covered by the brine formed by the salt and the expelled water, which was renewed periodically.

During the ripening process, which lasted approximately three months at 5°C, physico-chemical and enzymatic transformations occurred, resulting in a product with desired sensory characteristics. The ripening conditions varied between facilities, from climate-controlled environments to those without specific temperature control.

Microbiological analyses were conducted at the Microbiology Laboratory of the Fisheries Research Center (CIP), accredited for microbiological techniques. Five product replicas were evaluated using standardized methods to determine total microorganism count, coliforms, *Escherichia coli, Staphylococcus coagulase-positive,* and *Salmonella* spp., among others. The analyses were performed according to NC 585 (2015).

Physico-chemical analyses were carried out at the end of the ripening process in various laboratories, where total lipids, proteins, ash, pH, NaCl, and water activity were evaluated using standardized methods.

An acceptance and rejection test was conducted to assess the degree of ripening of traditional anchovy, with the participation of 80 consumers. The samples were taken at the end of the ripening process, briefly submerged in brine to remove excess salt, and then olive oil was added to simulate their usual presentation.

Results and discussion

Once captured and dead, fish begin a process of deterioration. In the initial stage, this deterioration is caused by enzymes present in the fish muscle, followed by enzymes produced by microorganisms that enter the fish. However, through the application of appropriate preservation methods and good handling practices, it is possible to keep these indicators within reference limits (Tavares et al., 2021). Table 1 presents the results of the sanitary quality evaluation of the fresh fillets (raw material).

Table 1. Sanitary quality of fresh fillets (raw material)

Microorganism	Reference index			Result
	m	n	c	
Salmonella spp.	0	5	0	Absence
V. parahaemolyticus	0	5	0	Absence
V. cholerae	0	5	0	Absence
Total microorganism count at 30 ºC (CFU/g)	10^5-10^6	5	2	$4x10^2$
				$4.7x10^2$
				$5.5x10^2$
				$4.0x10^2$
				$5.0x10^2$
S. coagulase positive (CFU/g)	10^2-10^3	5	2	$< 10^2$
Thermotolerant coliforms (MPN/g)	0.3 -2.1	5	2	0.36
				0.36
				1.4
				0.3
				< 0.3
E. coli (MPN/g)	< 0.3	5	0	0.36
				0.36
				1.4
				0.3
				< 0.3

The pathogens *S. coagulase*-positive, *Salmonella* spp., *Vibrio cholerae*, and *Vibrio parahaemolyticus* were absent. According to NC 585 (2015) microorganisms that cause foodborne diseases (FBD) must not be present in food and beverages, as their presence poses a risk to the health or life of consumers, which would lead to the food being discarded.

S. coagulase-positive is a gram-positive, facultative anaerobic bacterium widely distributed worldwide. It is estimated that one in three people is colonized, although not infected, by it. It can cause a variety of diseases ranging from relatively benign skin and mucosal infections to affecting the gastrointestinal system, either by the presence of S. aureus or by ingestion of the staphylococcal enterotoxin it secretes (Tavares et al., 2021).

Salmonella spp. is responsible for salmonellosis, a disease that causes various gastrointestinal conditions. In these cases, the bacteria do not invade the bloodstream but focus on the digestive system, causing enterocolitis with severe diarrhea and inflammation in the colon and small intestine, which can result in moderate fever and abdominal pain (Popa & Papa, 2021).

The presence of these pathogens in fish products is associated with fecal contamination, which can result from contamination of the natural aquatic environment, where these microorganisms can survive for months at room temperature, or from improper handling during processing (Sheng & Wang, 2021).

Toxigenic *V. cholerae* can survive in aquatic environments for months or even years, and it is associated with zooplankton and other aquatic organisms. Under stress conditions, it assumes a viable but non-culturable form, making it undetectable by conventional bacteriological methods (Salive et al., 2020). Transmission occurs through ingestion of the microorganism from the feces of infected individuals. Transmission mechanisms can be direct (person-to-person) or through cross-contamination via contaminated food, operators' dirty hands, use of contaminated water, or infected fish products (Stein & Chirilã, 2017). In the last 30 years, foodborne cholera outbreaks have been reported, with marine products such as bivalve mollusks, crustaceans, and fish being the most implicated. Cholera cases increased in 2008 compared to 2007, reaching a total of 190,130 cases and 5,143 deaths, representing a 7.6% increase in cases and a 27% increase in deaths (Maponga et al., 2015).

V. parahaemolyticus tolerates common salt and thrives in seawater, being able to grow at a pH of 9 in slightly alkaline media. It is associated with the consumption of shellfish, and in places like Japan, special precautions are required as it can cause gastroenteritis (Ngasotter et al., 2022). Both *V. parahaemolyticus* and *V. cholerae* are halophilic bacteria; therefore, the result obtained was significant for the future use of sardine fillets in anchovy production, as the brining maturation process offers optimal conditions for their growth.

Mesophilic aerobic bacteria were within the reference range for raw products according to the current standard. Although these bacteria are generally considered indicators, they represent a less precise and reliable measure of food poisoning risk compared to other indicators (Hoel et al., 2019).

The presence of mesophilic aerobic microorganisms in food can be directly related to the handling, freshness, or spoilage of the product, and its storage temperature. A relatively low number of these bacteria does not necessarily imply good bacteriological quality, as the food may contain microorganisms that produce enterotoxins or pathogens (Degaga et al., 2022). All known food-associated pathogenic bacteria are mesophilic aerobes, highlighting their importance in food hygiene (Lorenzo et al., 2018).

Thermotolerant coliforms and *E. coli* were found outside acceptable limits, indicating a high degree of fecal contamination. This contamination could be related to water, as well as improper handling from capture, transfer to land by fishermen, and storage. Despite implementing all necessary hygienic-sanitary measures since the acquisition of the sardines, an increase in their contaminant load could not be avoided.

E. coli is an effective indicator of fecal contamination and the presence of pathogenic microorganisms in food and water. It is one of the main pathogenic bacteria in fish products, resulting from contamination from animal or human reservoirs (Yohans et al., 2022). This bacterium invades the intestinal mucosa, causing abundant and constant watery diarrhea, known as “traveler’s diarrhea.”

The presence of gram-negative enteropathogenic microorganisms in fish products is undesirable, and most microbiological specifications require their absence (Lorenzo et al., 2018). However, the anchovy processing technology allowed for addressing the existing microbial load in the raw material (Table 2), as no coliforms were detected in the indicators for anchovy.

Table 2. Sanitary quality of anchovy (final product)

Microorganism	Reference index			Result
	m	n	c	
Salmonella spp	0	5	0	Ausencia
Total microorganism count at 30 ºC (CFU/g)	< $1.1x10^5$	5	2	$1.7x10^4$ $1.5x10^4$ $1.0x10^3$ $1.5x10^3$ $1.6x10^4$
S. coagulase positive (CFU/g)	< 10^2	5	0	< 10^2
Coliforms (CFU/g)	< 10^2	5	0	< 10

The count of aerobic mesophiles increased compared to the results presented in Table 2, although it remained within the reference limit for anchovy (Dambrosio et al., 2023). This increase is related to the growth of moderate and extreme halophilic flora, which is a natural contaminant of salt (Dutta & Bandopadhyay, 2022). These bacteria are transferred to the salted fish, where they find a nutrient-rich substrate that allows them to develop, as they can adapt to environments with a high salt concentration. This phenomenon is favored as the maturation process progresses, increasing the availability of nutrients due to the proteolysis involved in the process. It is important to note that extreme halophiles grow more slowly than Eubacteria (Moran-Reyna et al., 2014), suggesting that they did not significantly contribute to the total number of microorganisms recorded at the beginning.

The absence of *Salmonella* spp. and *S. coagulase*-positive confirms that the product is free of pathogens, thus ensuring its quality and safety. Table 3 shows the chemical composition of anchovy reported in several studies.

Table 3. Chemical composition of anchovies

Parameter	Anchovy (Aizpún et al., 1979)	Anchovy (Chiodi, 1962)	Anchovy (Czerner, 2011)	Common sardine
Moisture (%)	71.03	74.35	57.7	48.83 (0.15)
Protein (%)	18.35	19.99	19.61	22.05 (1.23)
Lipids (%)	9.83	2.77	4.6	2.7 (0.26)
pH	-	-	5.7	6.4 (0.04)
a_w	-	-	0.781	0.95 (0.003)
NaCl (%)	-	-	17.78	15.18 (0.51)
Ash (%)	2.2	2.97	20.18	3.11 (0.05)

This decrease is related to their role as an energy reserve necessary for cellular development during spawning. According to Aizpún et al. (1979) and Jensen et al. (2007), the differences in composition recorded for the same month in different years are linked to less favorable environmental and feeding conditions, resulting in lower lipid content.

Moisture and pH values are within normal ranges for similar fish, although they present slight variations, possibly due to factors such as species, water salinity, temperature, and climate. The ash value is comparable to that reported by other researchers, indicating an adequate mineral level; however, Czerner (2011) reported significantly higher values. The methodology used in this study is reliable and conforms to established standards.

The product showed high water activity (0.95±0.03) (Maneffa et al., 2017). The NaCl percentage was 15.18±0.51, near the lower limit (14-21%). This product is classified as a preserve since it does not undergo thermal treatment; its preservation depends on reducing water activity and a high salt concentration, which limits microbial development and guarantees commercial stability. To achieve optimal conditions in the anchovy curing process, salt must be added, which would also provide a bacteriostatic effect and reduce the number of present microorganisms (Margiati et al., 2024).

The production of anchovies remains an artisanal and meticulous process, where the quality of the final product depends on the skill and cleanliness of the handlers. High-quality anchovies are characterized by having a flexible yet firm texture, avoiding a leathery consistency; a color that ranges from reddish-brown to a light caramelized tone; and a proper balance of aromas and flavors that should harmonize the oil, salt, and fish.

Sensory evaluations should be performed by trained judges; however, in this study, a preference or rejection test was chosen, conducted with regular anchovy consumers without the need for training (NC 1032-1, 2014). Table 4 presents the values obtained in the sensory evaluation.

Table 4. Anchovy acceptance or rejection and preference test

Consumer	Acceptance or rejection		Preference	
	Acceptance	Rejection	Anchovy 1	Anchovy 2
80	62	18	12	68

The results of the sensory evaluation revealed that out of 80 consumer judges, 62 accepted the product, which translates to a 77.5% acceptance rate. This percentage suggests a favorable response to the product, indicating that most consumers consider it to meet their expectations of quality, taste, and texture. An acceptance level above 75% is generally seen as positive in the market (Ruiz-Capillas & Herrero, 2021), and in this case, it is attributed to the quality of the production process and attention to critical sensory aspects. However, the 22.5% of judges who rejected the product offer opportunities to identify areas for improvement, such as texture, flavor, or salinity, which is crucial for optimizing production.

In comparison, Anchovy 1 had a 15% acceptance rate, while Anchovy 2 reached 85%. This high acceptance level for Anchovy 2 suggests it was received more favorably, likely due to differences in texture, flavor, or freshness, reflecting a more optimal production process. An 85% acceptance not only indicates good quality but also great potential for commercialization. In contrast, the low acceptance of Anchovy 1 highlights the need to investigate the causes of this negative perception, such as excessive salt or inadequate texture, to improve the product.

Conclusions

The presence of sanitary quality indicators such as thermotolerant coliforms and *E. coli* was identified in the sardine fillets; however, pathogens such as *Salmonella* spp., *V. cholerae*, *V. parahaemolyticus*, and *S. coagulase* positive were not detected in the raw material used. The salting-maturation process of sardine fillets (*S. pilchardus*) proved effective in eliminating coliforms at the end of the process. After processing, the chemical composition of the fillets was similar to that reported in previous studies, although the water activity and NaCl percentage of the final product did not reach optimal levels for anchovy production. Despite this, sardine fillets (*S. pilchardus*) with three months of maturation showed good consumer acceptance, suggesting the product's potential in the market.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Iván N. Chaple and Oscar Ros: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, drafting the original manuscript and writing, review, and editing.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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