


Iceland  
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# Blue Project eBook

Promotor:



Parceiros:



TINTEX



Operador do programa:





**Title:**

Blue Project eBook

**Promoter:**

Guimarpeixe - Comércio de Produtos Alimentares, S.A.

**Partners:**

TINTEX Textiles, S.A.

Universidade do Minho

Instituto Politécnico de Viana do Castelo

Município de Esposende

Associação Empresarial de Portugal, Câmara de Comércio e Indústria

VISUAL THINKING - Digital Organization, Lda

Matís

**Programme:**

Blue Growth

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# For a Blue Economy of Excellence

Guimarpeixe was born within the industrial culture of Guimarães, founded on strong foundations and dedicated to the industry and commercialization of deep-frozen products, among which the most varied species of fish stand out. It meets the highest market demands, both in terms of installations and equipment, with the best industrial units in the country in this field. Taking into account the quality of its products and the ability to respond to its customers, it was natural that Guimarpeixe felt the need to expand its area of activity, covering the entire northern region of the country and starting its adventure in the international markets. In this context, it was with great satisfaction that, faced with the challenge launched by the Municipality of Esposende, and recognizing the differentiating potential of the Blue Project project, we joined this consortium. As Guimarpeixe is a dynamic company, with a sense of continuous improvement and aimed at the customer, our strategy includes recognizing and guaranteeing the quality of our products before the customer, which is why we have, over time, through various certification mechanisms, of

which we highlight the certification of its Quality Management System in accordance with the NP EN ISO 9001 standard, the strict food safety requirements, also obtaining certification of its Food Safety Management System, as well as by BRC Global Standards for Food : recognition at the highest level as an entity that complies with the highest food safety standards Recently and in a new chapter for the company in terms of certification with recognition by the IFS Food standard (International Featured Standard). We are an environmentally responsible company that seeks to source fish from sustainable fishing, so faced with the challenge of the Blue Project, based on the principles of sustainability, the circular economy, the blue economy and the promotion of differentiating food technology systems, leveraging In a direct way to transfer scientific knowledge to our industry, we have had high expectations from the outset regarding the final results of this project and how different they could be in current fish consumption models. We are proud of this, for promoting this project and being part of this consortium.

João Ribeiro, Guimarpeixe



## **Chapter 1**

# **The Blue Project in a nutshell**



Based on the principles of **Circular Economy**, the Blue Project aims to increase **fish consumption** in school contexts and transform its surplus into **new textile** and consumer products.

The objectives of this initiative include the creation of **innovative products** resulting from scientific work and the development of more **sustainable** food **conservation processes**.

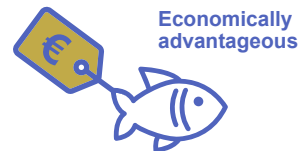
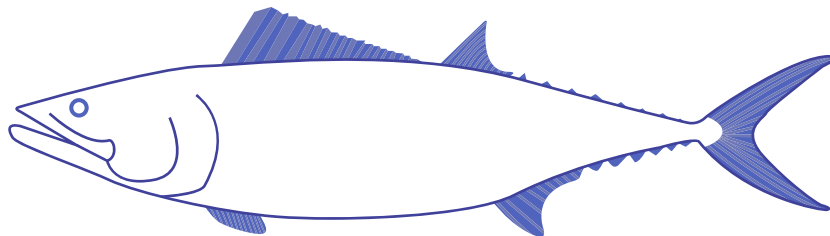
Learn more about the opportunity, goals and SDGs alignment of the Blue Project in this chapter.

# Opportunity

The Blue Project aims to build a strategic line of action, which has as its starting point the promotion of the consumption of **local fish**, ensuring **catch quotas**, promoting the **reuse of surplus** from the Portuguese coast and reducing **food waste**.

The **Atlantic bonito** (*Sarda sarda*) is one of the species that needs to be better known and consumed by the population. This fish exists in abundance, has a flavor similar to tuna and, by comparison, is more economically viable.

## Sarrajão





# Blue Project goals

- 1 Take advantage of the Marine Resources available on the North Atlantic Coast of Portugal
- 2 Create a fresh fish processing unit for the commercialization of the 'Sarrajão' fillets in school canteens
- 3 Use fish leftovers to create textile materials (from the skin) and other food products
- 4 Help municipalities to implement ocean literacy strategies



# SDGs Alignment

The 17 SDGs, unanimously approved by 193 UN member states, meeting in General Assembly, aim to address the needs of people in both developed and developing countries, emphasizing that no one should be left behind. The 2030 Agenda for Sustainable Development is a broad and ambitious agenda that addresses several dimensions of sustainable development (social, economic, environmental) and promotes peace, justice, and strong institutions.

**The Blue Project responds to 6 of these objectives.**



**2** ZERO HUNGER



**The Blue Project is aligned with this goal due to its aim of promoting the consumption of a species of fish unknown to the general public, which allows the diversification of the consumption of fish species through Atlantic Bonito- an economically viable, accessible and extremely nutritious fish species.**

By guaranteeing access to safe and nutritious food, the project embraces food security and promotes sustainable agricultural practices through conscious fishing that maintains healthy marine ecosystems, reducing pressure on them.

**3** GOOD HEALTH AND WELL-BEING



**In terms of health, the Blue Project contributes to the promotion of healthy eating practices through the sustainable consumption of Atlantic Bonito fish. By fostering the health of marine ecosystems, the project not only creates a vital link between human and environmental health, but also stimulates the overall well-being of the ecosystem.**

This species of fish has high nutritional benefits and awares consumers to the possibility of consuming fish species other than the conventional ones.

**4** QUALITY EDUCATION



**The Blue Project plays a crucial role in quality education by connecting the community of the municipality of Esposende to sustainable practices.**

By raising awareness about sustainable fishing, circular economy, healthy and diverse food, the project focuses on communicate to the future generations, in an educational way, the importance of their food quality choices.

As this project is aimed at the younger generations, our goal is to motivate them to learn and take an interest in sustainability.

10 REDUCED  
INEQUALITIES



**The Blue Project aims to reduce inequalities by providing equitable opportunities in the fishing sector. This sector tends to lack interest from communities and is losing importance. As it is an economic activity in which fishermen are small and in a family context, it is necessary to promote better conditions for the sector.**

By fostering social and economic inclusion in the community involved, the project promotes benefits and their fair distribution, in line with the vision of equality proposed by Goal 10. By promoting and making known a species of fish that is less known to the general public, it allows fishermen to explore the marketing of yet another species, resulting in the marketing of a new product.

12 RESPONSIBLE  
CONSUMPTION  
AND PRODUCTION



**The project has led to a reduction in food waste, working from a circular economy and proximity perspective. By transforming surpluses into by-products, the Blue Project has paved the way for more responsible production and more conscious consumption.**

This project highlights the importance of integrating sustainability not just as an isolated objective, but as a fundamental guideline principle at all stages of the food chain and product development. In this way, the Blue Project sets a benchmark in terms of the use of resources.

14 LIFE BELOW  
WATER



**The Blue Project takes an active role in the conservation and sustainable use of marine resources. Guimarpeixe and Tintex comply with meticulous criteria at their factories, showing their focus on responsible production, in line with the principles of Goal 14.**

The project adopts and promotes fishing practices that respect the limits of marine ecosystems, avoiding overfishing and ensuring the preservation of fish populations.

This project emerges as an inspiring example of how local actions can have a global impact on the preservation of marine resources.



## **Chapter 2** **Our consortium**



# The Blue Project Consortium

The consortium presents a joint governance model with a clear definition of roles and responsibilities from a perspective of complementarities between all partners. The Blue Project is coordinated by Guimarpeixe, a company with long experience in processing and supplying quality sustainable frozen fish. The consortium is made up of enti-

ties with complementary skills for generating scientific and technological knowledge, with the participation of a research and development leader focused on serving the food and biotechnology sectors, a tissue company, a polytechnic, a municipality, a university, a business association and a technology company.

The company was born within the industrial culture of Guimarães, based on strong foundations and dedicated to the industry and commercialization of deep-frozen products, among which the most varied species of fish stand out. It meets the highest market demands, both in terms of installations and equipment, with the best industrial units in the country in this field.

### Main activities in the project

- Project coordination, management and evaluation
- Fish cleaning
- Removal of skin for later use in the textile industry and the remaining remains for other food products
- Filleting, conservation, packaging and storage for subsequent transport of fish fillets to the canteens in Esposende

## **Partners**

### **Município de Esposende**



It is the municipality's municipal body and its mission is to define and execute policies with a view to defending the interests and meeting the needs of the local population. In this sense, it is up to you to promote the development of the municipality in all areas of life, such as health, education, social action and housing, the environment and basic sanitation, spatial planning and urbanism, transport and communications, public supply, sport and culture, consumer protection and civil protection.

#### **Main activities in the project**

- Transport of fish/raw material for the project
- Allocation of human resources
- Intermediation with schools in the municipality

# Partners

## Matís



Located in Iceland, the project's donor country, it is an independent, non-profit government research entity. It is a leading research and development organization focused on topics related to the food and biotechnology sectors.

### Main activities in the project

- Creation of a new food literacy with the school community as the target audience
- Sharing knowledge and experience already acquired with Portuguese stakeholders to better communicate and make children aware, informed and passionate about healthy foods
- Active participation in Blue Project conferences and workshops

Based in Vila Nova de Cerveira, TINTEX uses technology as a means to achieve better quality, results and sustainable objectives. With almost 25 years of experience, it operates in the textile sector manufacturing knitted fabrics for the fashion, sports, underwear, footwear, leather goods and home textiles segments, based on sustainable strategies throughout the entire production process.

### Main activities in the project

- R&D activities
- Application of innovative, natural-based functional finishes with therapeutic properties
- Development and optimization of new products with new fabric coating solutions



## Partners

### IPVC - Instituto Politécnico de Viana do Castelo



Escola Superior  
de Tecnologia e Gestão

It is a public higher education institution made up of six schools focused on human, cultural, scientific, technical and qualified professional training, with strong cooperation with the regional community of Alto-Minho.

#### Main activities in the project

- R&D activities
- Creation of new products from leftover raw materials
- Consumer testing

# Partners

## Universidade do Minho



Universidade do Minho  
Escola de Engenharia

It is a research university focused on the regional, national and international socioeconomic environment, which invests heavily in knowledge and R&D through the management of intellectual property, positioning itself as one of the PT HEIs with the most registered patents.

### Main activities in the project

- List of potential components to be used in product formulations
- Formulation and characterization of new products
- Quality control
- Techniques for processing, preserving and stabilizing products
- Consumer testing

## Partners

### AEP - Associação Empresarial de Portugal, Câmara de Comércio e Indústria



It is a national, multi-sectoral association, based in Porto, run by businesspeople for businesspeople, which aims to increase business potential and contribute to a business culture in which management and innovation processes are an intrinsic part of the business.

#### Main activities in the project

- Transversal support in project management and in the development and optimization of all its phases
- Communication and dissemination: (1) Production of content such as e-books, video, among others (2) Organization of conferences and workshops
- Project evaluation analysis reports and their socioeconomic impact

## Partners

### Visual Thinking



It is a technology company with strong skills in developing business intelligence and analytics solutions.

#### Main activities in the project

- Communication and dissemination
- Creation of the Blue Project website
- Development of a platform for sharing educational content and registering interest from potential stakeholders
- Support in the evaluation analysis of the project and its socioeconomic impact

# **Chapter 3**

## **State of the art**

- **Fish Description**
- **Capture Techniques**
- **Nutritional Information**
- **Capture Data**
- **Innovation Opportunities**





# State of the Art

## Fish Description

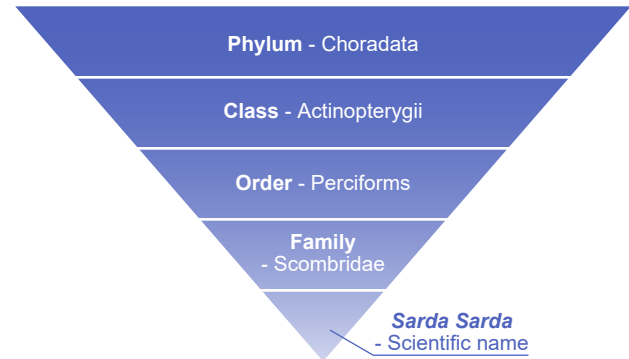
The Sarrajão is a species of Atlantic Bonito, with the scientific name *Sarda sarda*. Pelagic species, it forms large shoals and makes breeding migrations. The body has a hydrodynamic and elongated shape, it is covered with small scales and the tail fin is forked, characteristic of the scombroid family (tuna, mackerel). The dorsal fins are contiguous and the front one is wide. The first dorsal fin has 20 - 23 spines. It has three pairs of fins on the tail peduncle. It presents a blue greenish colour with 5 - 11 dark oblique stripes on the upper part of the body, being vertical in juveniles, and a silver belly.

This species feeds mainly on members of the families Scombridae, Atherinidae, Clupeidae, *Alosa pseudoharengus* and many other species of fish and Cephalopods.

Compared to other common commercial species, it stands out for its favourable features, such as its nutrient-rich lipid/protein composition, high yield, and specific taste.



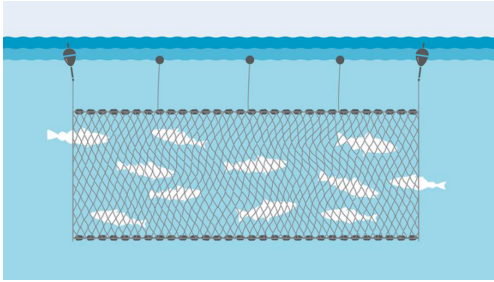
Representation of Sarrajão



Taxonomic representation of the Sarrajão

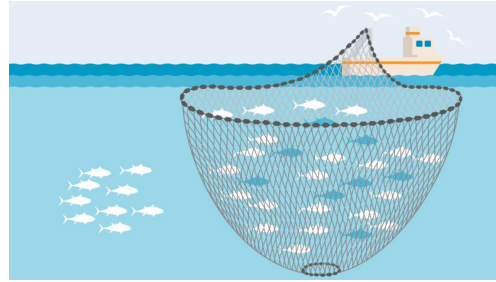
## Capture techniques

Atlantic Bonito is a valuable small tuna species to coastal countries and local communities. The common gears used in fisheries for catching Atlantic bonito are gillnets, purse seines, longlines and trawl fishing.



### Gillnets

A fishing method that uses a rectangular-shaped net with one, two, or three panels held vertically by float lines and ballast lines used alone or in hunts. Generally, it has low environmental impacts with minimal seabed interaction. The size of fish caught can be determined by the mesh size, helping to avoid catching juvenile fish.

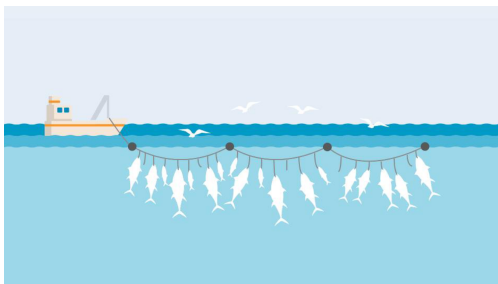


### Purse seines

This technique represents a fishing method that uses a long and high net, which is dropped to surround the prey and reduce its ability to escape. This capture process consists of wrapping the fish by the sides and underneath, preventing its escape through the bottom of the net.

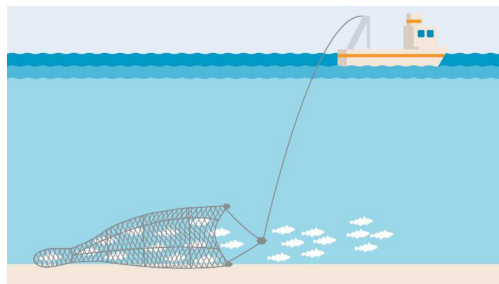
## Capture techniques

Atlantic Bonito is a valuable small tuna species to coastal countries and local communities. The common gears used in fisheries for catching Atlantic bonito are gillnets, purse seines, longlines and trawl fishing.



### Longline

This technique consists of using baited hooks, which are attached to the nets at intervals to attract the target species.



### Trawl fishing

This fishing gear consists of a trawl carried out by a boat, in which the net moves over the bottom and drags the shoals.

# Nutritional and Capture Data Information

## Nutritional Information

The table shows the results of the nutritional characterisation of Atlantic Bonito or Sarrajão (Sarda sarda).

Energy	138 kcal / 577 kJ
Fat	6g
Carbohydrate	0g
Fibre	0g
Protein	21g
Water	73g

Nutritional information of Bonito (per 100 g of edible portion) (Source: Moreiras et al., 1998).

# Nutritional and Capture Data Information

## Capture data

Through information provided by the Portuguese company DOCAPESCA1, in the year 2021 about 237,305.30 kg of Sarrajão was caught, which translates into a total of 722,533.61 €, and the auctions with the highest catch volume are reported on the following table:

Seaports	Catch amounts (kg)
Quarteira	182,728.20
Olhão	26,962.70
Peniche	12,232.10
Sines	3.194.80
Setúbal	1,926.70
Viana do Castelo	1.113.30

Data on catch amount of Sarrajão per seaport during the year 2021.

In the year 2022, around 77,110.00 kg of Sarrajão was caught up to October, which translates into a total of 296,483.93 €, the auctions with the highest volume of catches are reported on Table 2:

Seaports	Catch amounts (kg)
Quarteira	41,918.70
Peniche	10,151.40
Sines	8,538.40
Olhão	5,543.20
Sesimbra	3.256.60
Aveiro	1,728.50

Data on catch quantities of Sarrajão by seaport for the year 2022, until October.

# Innovation opportunities

Due to its high nutritional quality, Sarrajão could be used to develop innovative food products such as meatballs, pates, hamburgers, and fillets. All these fish products are ground-breaking because 1) there are no Sarrajão-based products of this type available on the market and 2) they can be fortified/supplemented with bioactive components and other raw materials with high nutritional value for example, from plant-based (e.g. pulse-crop flours, brassica flours and seaweed) and also animal-based (e.g. whey protein) sources.

In addition to the development of new Sarrajão-based products, different conservation techniques can also be used for these products to promote quality as well as to extend their shelf life. For instance, refrigeration, freezing and others techniques considered promising in the scientific literature such as modified atmosphere packaging (MAP), vacuum packaging and bio-based packaging (i.e., bio-based coatings) could be applied to Sarrajão processed products.



## **Chapter 4**

# **Main scientific results**

### **- Raw materials characterization**

- **Characterization of Fresh Sarrajão**
- **Seasonality Characterization of Sarrajão – Spring / Autumn**
- **Seasonality characterization of Sarrajão over storage time 8 days at 4°C**
- **Overall market trends**
- **Product trends**
- **Target consumer groups**
- **Challenges and opportunities**
- **New seafood product development proposal**



# Characterization of fresh Sarrajão

## Nutritional/chemical characterization

This table summarises the chemical and nutritional parameters obtained for Sarrajão (*Sarda sarda*) marine fish captured in March (Spring) and October (Autumn). The table shows that the fish captured in March and October obtained similar pH values with no significant differences ( $p > 0.05$ ).

Concerning the water activity there are no significant differences for Sarrajão fillets capture in March and October. Results for moisture content showed that the marine fish fillets of March obtained higher values than October fillets ( $p < 0.05$ ).

Regarding protein content it was found that fillets from March were slightly higher ( $p < 0.05$ ) than October fillets ( $25.4 \pm 0.32$  % and  $22.9 \pm 0.35$  %, respectively). The same behaviour was found for ash content, fillets from March were slightly higher ( $p < 0.05$ ) than October fillets ( $1.41 \pm 0.02$  % and  $0.96 \pm 0.02$  %, respectively).

In relation to chloride and fibre content there were no significant differences ( $p > 0.05$ ) between Spring and Autumn marine fish fillets. Both the Spring and Autumn samples had absorbance readings below the detection limit for the

carbohydrate content, meaning that this marine fish species, has no carbohydrate content.

Lipid content results showed that fish fillets from Autumn were 6.32-fold higher than Spring fish fillets ( $p < 0.05$ ).

Parameters	Spring	Autumn
Lipids	$1.21 \pm 0.32^b$	$7.55 \pm 0.29^a$
Carbohydrate	$< 0.002^*$	$< 0.002^*$
Fibre	$1.52 \pm 0.14$	$1.47 \pm 0.11$
Protein	$25.4 \pm 0.32^a$	$22.9 \pm 0.35^b$
Chlorides	$0.27 \pm 0.09$	$0.3 \pm 0.04$
Moisture	$72.64 \pm 0.32^a$	$68.45 \pm 0.39^b$
Ash	$1.41 \pm 0.02^a$	$0.96 \pm 0.02^b$
pH	$5.85 \pm 0.03$	$5.95 \pm 0.01$
$a_w$	0.97	0.97

\* Values under the method's limit of detection.

**Results of chemical and nutritional analyses of fresh Sarrajão fillets, obtained in Spring and Autumn. Mean values + standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .**



## Microbiological characterization

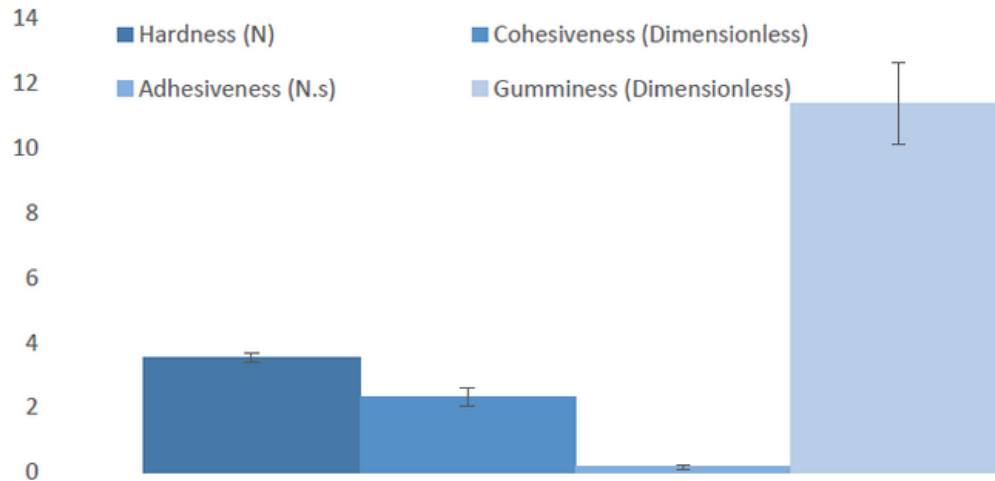
According to the values shown the microbiological analyses demonstrate that were lower than <10 UFC/ml, <100 UFC/ml, <10 UFC/ml, <10UFC/ml and <100 UFC/ml for Escherichia Coli, Moulds and Yeasts, Enterobacteriaceae, Staphylococcus coagulase (+) and Pseudomonas, respectively. Listeria monocytogenes and Salmonella spp. were not detected in 25g of product. According to the applicable legislation, Regulation 2073/2005 and the guidelines defined by the Health Protection Agency (HPA) with these results it can be concluded that fresh Sarrajão is safe for consumption.

Microorganisms at 30°C	Escherichia Coli	Moulds and Yeasts	Enterobacteriaceae	Staphylococcus coagulase (+)	Listeria monocytogenes	Salmonella spp.	Pseudomonas
1x10 <sup>2</sup> UFC/ml	<10 UFC/ml	<100 UFC/ml	<10 UFC/ml	<10 UFC/ml	Not detected in 25g	Not detected in 25g	<100 UFC/ml

Information on microbiological analysis data on fresh Sarrajão

## Texture characterization

Concerning texture results, the Sarrajão fish has a hardness of 3.55 N, a cohesiveness value of 2.33, an adhesiveness value of 0.17 N.s was recorded, and finally, a gumminess value of 11.38.



Texture analysis of hardness, cohesiveness, and adhesiveness of fresh Sarrajão fillets.

## Colour characterization

Concerning colour analysis, the Sarrajão fillets presents a value of 44.79 for the L coordinate, for the  $a^*$  coordinate the value is 3.73 and for the  $b^*$  coordinate the value is 7.53.

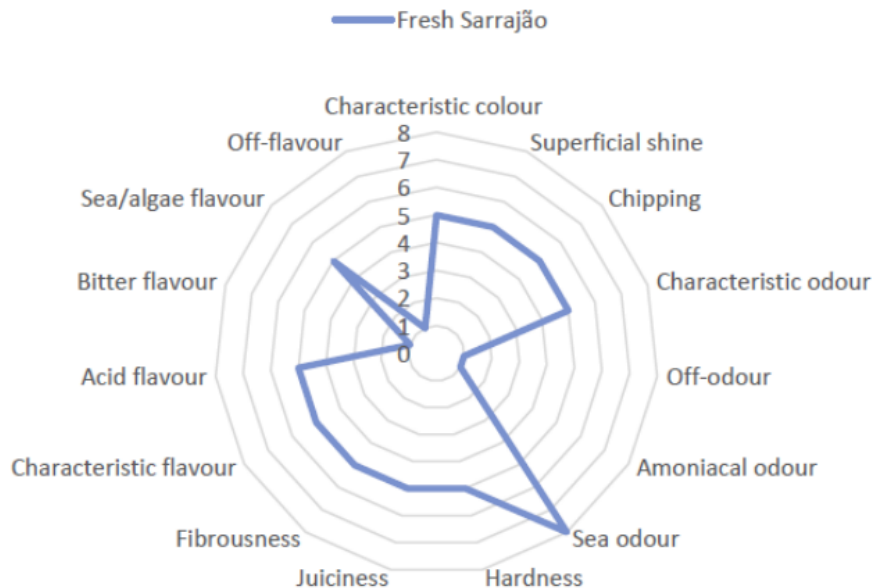


Analysis of the colour of fresh Sarrajão fillets.

## Sensory characterization

For sensory analysis, a quantitative descriptive analysis (QDA®) was carried out with six semi-trained panellists, according to ISO 6658:2005. The attributes evaluated by the panellists were: colour, superficial shine, chipping, characteristic odour, off-odour, ammoniacal and sea odour, hardness, juiciness, fibrousness, characteristic flavour, acid, bitter and sea/algae taste, and off-flavour. These attributes were evaluated on an intensity scale of 10 points (1 - lowest intensity, 10 - higher intensity).

Results show that the panellists scored Sarrajão fresh fillets with a high sea odour, and for all the other positive parameters were given favourable scores.



Sensory analysis of fresh Sarrajão fillets.

# Seasonality characterization of Sarrajão – Spring/Autumn

## Nutritional/chemical characterization

The table summarises the chemical and nutritional parameters obtained for Sarrajão (*Sarda sarda*) marine fish captured in March (Spring) and October (Autumn). The table shows that the fish captured in March and October obtained similar pH values,  $5.85 \pm 0.03$  and  $5.95 \pm 0.01$ , respectively, with no significant differences ( $p < 0.05$ ).

Concerning the water activity there are no significant differences for both marine fish fillets capture in March and October. Results for moisture content showed that the marine fish fillets of March obtained higher values ( $p < 0.05$ ) than October fillets ( $72.54 \pm 0.32$  % and  $68.45 \pm 0.39$  %, respectively), higher than the obtained in an experiment by Altan et al. (2022), which reported 58.05 % of moisture in fish. Regarding protein content it was found that fillets from March were slightly higher ( $p < 0.05$ ) than October fillets ( $25.4 \pm 0.32$  % and  $22.9 \pm 0.35$  %, respectively). Özden et al., (2010) found similar results and reported protein and fat content of Bonito to be 17.78–24.56 % and 1.13–17.37 %, respectively.

Also, Öksüz et al. (2008) reported a protein value of 24.1%, and Altan et al. (2016) 24.43 %, results within the obtained in this study.

Parameters	Spring	Autumn
Lipids	$1.21 \pm 0.32^b$	$7.55 \pm 0.29^a$
Carbohydrate	$< 0.002^*$	$< 0.002^*$
Fibre	$1.52 \pm 0.14$	$1.47 \pm 0.11$
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$a_w$	0.97	0.97

\* Values under the method's limit of detection.

Results of chemical and nutritional analyses of fresh Sarrajão fillets, obtained in Spring and Autumn. Mean values + standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Microbiological characterization

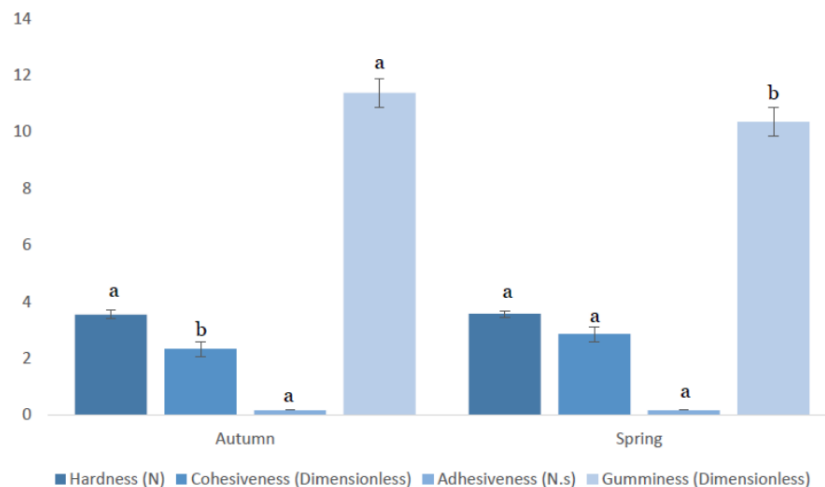
According to the values obtained after the microbiological analyses, it is possible to observe that the values of Microorganisms at 30°C for both Autumn and Spring seasons showed a small difference, as well as the Enterobacteriaceae, Staphylococcus coagulase positive. However, these values are satisfactory according to the legislation, Regulation 2073/2005 and the guidelines defined by the Health Protection Agency (HPA), for this reason the fish is safe for consumption.

Seasons	Microorganisms at 30°C	<i>Escherichia coli</i>	Moulds and Yeasts	<i>Enterobacteriaceae</i>	<i>Staphylococcus coagulase (+)</i>	<i>Listeria monocytogenes</i>	<i>Salmonella</i> spp.	<i>Pseudomonas</i>
Autumn	1x10 <sup>2</sup> UFC/ml	<10 UFC/ml	<100 UFC/ml	<10 UFC/ml	<10 UFC/ml	Not detected in 25g	Not detected in 25g	<100 UFC/ml
Spring	1.15x10 <sup>3</sup> UFC/ml	<10 UFC/ml	<100 UFC/ml	<40 UFC/ml	<20UFC/ml	Not detected in 25g	Not detected in 25g	<100 UFC/ml

Information on microbiological analysis data on fresh Sarrajão from Autumn and Spring.

## Texture characterization

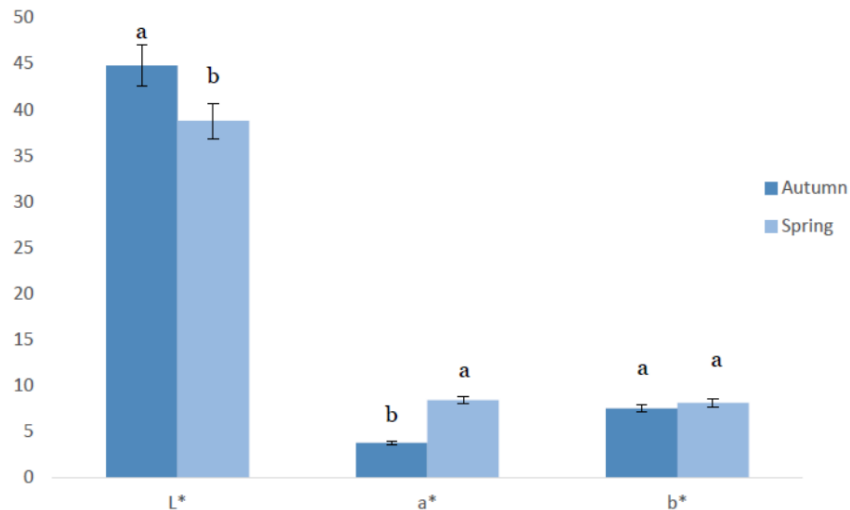
Concerning the texture analysis, it is possible to observe that there were no differences between hardness and adhesiveness of fish fillets captured on March and October. On the contrary significant differences were found on cohesiveness and gumminess of both seasons.



Data concerning hardness, cohesiveness, and adhesiveness of fresh Sarraão fillets, from the Spring and Autumn season.

## Colour characterization

Regarding colour analysis, it is possible to observe that there are significant differences for the luminosity parameter and for the ( $a^*$ ) coordinate between fish fillets captured in March and October. The fish from the Spring season presents lower luminosity values and higher values for the ( $a^*$ ) coordinate, which indicates a redder colour for this sample.



Analysis of the colour of fresh Sarraião fillets, from the Spring and Autumn season.

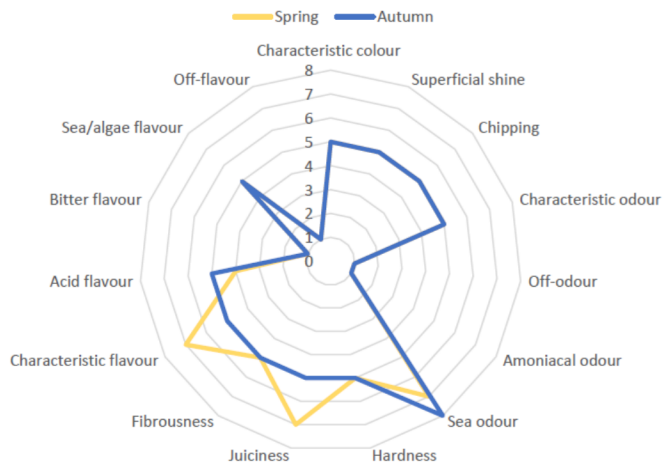


## Sensory characterization

The results of the sensory analysis of for both fish fillets captured in March and October are shown in the figures.

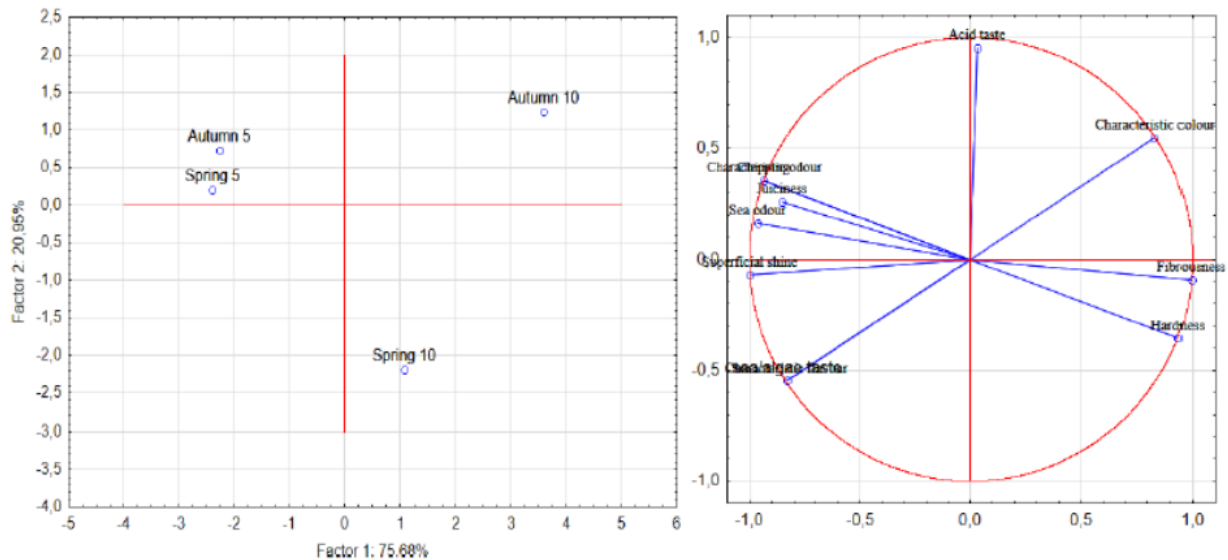
Also, PCA was used to investigate relationships between Spring and Autumn marine fish fillets and the two cooking batch times, 5 min and 10 min. PC1 (Factor 1) and PC2 (Factor 2) summarized almost 76% and 21% of this study information, respectively. A good separation of groups was achieved, with the main differences to be seen in the fillets cooked during 10 min. Fillets cooked for 5 min obtained closer overall scores, regardless of the fish capture seasonality, compared to the other samples. Panellists scored fish samples cooked for 5 min with higher scores for superficial shine, chipping, characteristic and sea odour, juiciness, characteristic flavour and sea/algae flavour compared to fish samples cooked for 10 min. On the other, fillets samples cooked for 10 min were placed further away from these attributes. Instead, fillets samples cooked for 10 min were close to hardness or fibrousness scores, which are characteristics seen as less favourable in fish fillets. However, fillets from the Autumn season cooked for 10 min had higher characteristic colour scores than Spring season cooked for 10 min.

PCA results indicated that cooking time is the most important factor for the overall sensory evaluation: the characteristics considered most valued for fish fillets had higher scores in the cooking time of 5 min, since overcooking (10 min) lead to lower satisfaction scores.



Data from the sensory analysis of fresh Sarrajão fillets from the Spring and Autumn seasons.

## Sensory characterization



Principal component analysis of sensory evaluation-score plot for the mean classification of condition groups (left) and loading plot of different attributes (right), performed on Sarda sarda captured on Spring and Autumn seasons.

# Seasonality characterization of sarrajão over storage time (8 days at 4°C)

## Nutritional/chemical characterization

The results of chemical and nutritional analyses of Sarrajão fillets obtained during storage time until 8 days are shown in the table.

Results show that no significant differences were observed in lipids, carbohydrates, fibre, ash, aw and moisture content during storage time at 4 °C. On the contrary, protein content decreased during storage, chlorides content increased, 2-fold, after 8 days at 4 °C.

Parameters	T0	T8
Lipids	0.83±0.39 <sup>a</sup>	0.97±0.17 <sup>a</sup>
Carbohydrate	<0.002 <sup>a</sup>	<0.002 <sup>a</sup>
Fibre	0.86±0.25 <sup>a</sup>	0.72±0.22 <sup>a</sup>
Protein	24.5±0.05 <sup>a</sup>	23.9±0.17 <sup>b</sup>
Chlorides	0.15±0.01 <sup>b</sup>	0.3±0.02 <sup>a</sup>
Moisture	72.97 <sup>a</sup>	73.25 <sup>a</sup>
Ash	1.7±0.65 <sup>a</sup>	1.32±0.65 <sup>a</sup>
pH	5.97±0.00 <sup>a</sup>	5.95±0.01 <sup>b</sup>
a <sub>w</sub>	0.97 <sup>a</sup>	0.97 <sup>a</sup>

\* Values under the method's limit of detection.

Results of chemical and nutritional analyses of Sarrajão fillets, obtained in time 0d and 8d stored at 4°C. Mean values ± standard deviation (n=3). Means within same column with different superscripts are significantly different at p<0.05.

## Microbiological characterization

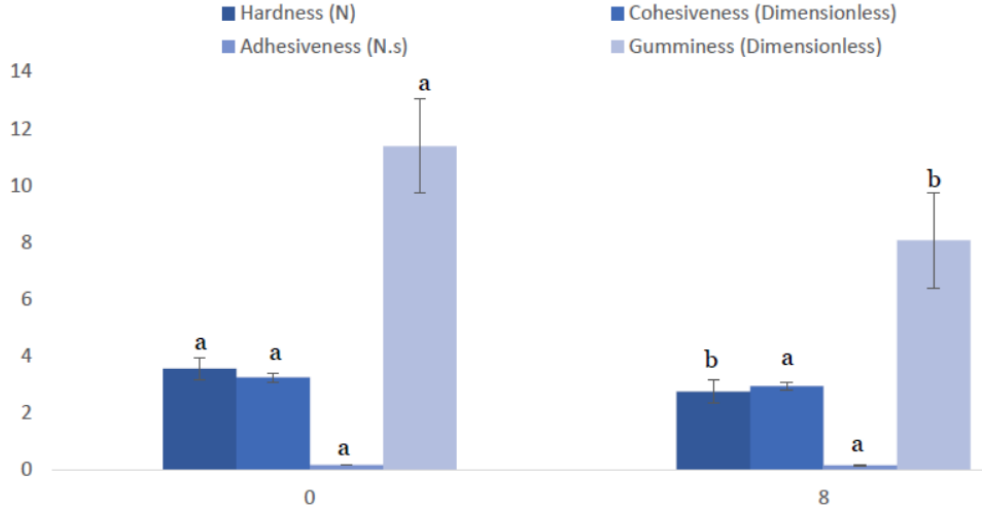
A microbiological analysis was made using Regulation 2073/2005 and the guidelines defined by the Health Protection Agency (HPA). Regarding the microbiological results *Escherichia coli*, Moulds and Yeasts, *Enterobacteriaceae*, *Staphylococcus coagulase (+)*, *Listeria monocytogenes* and *Salmonella* spp. did not show differences during storage time. On the contrary, Microorganisms at 30°C and the *Pseudomonas* UFC/ml increased after 8 days storage at 4°C. However, these values are within the satisfactory values, meaning that the fish is still acceptable for consumption.

Times	Microorganisms at 30°C	<i>Escherichia coli</i>	Moulds and Yeasts	<i>Enterobacteriaceae</i>	<i>Staphylococcus coagulase (+)</i>	<i>Listeria monocytogenes</i>	<i>Salmonella</i> spp.	<i>Pseudomonas</i>
T0	1x10 <sup>2</sup> UFC/ml	<10 UFC/ml	<100 UFC/ml	<10 UFC/ml	<10 UFC/ml	Not detected in 25g	Not detected in 25g	<100 UFC/ml
T8	4x10 <sup>2</sup> UFC/ml	<10 UFC/ml	<100 UFC/ml	<10 UFC/ml	<10 UFC/ml	Not detected in 25g	Not detected in 25g	<400 UFC/ml

Information on microbiological analysis data on Sarrajão, in time 0d and 8d stored at 4°C.

# Texture characterization

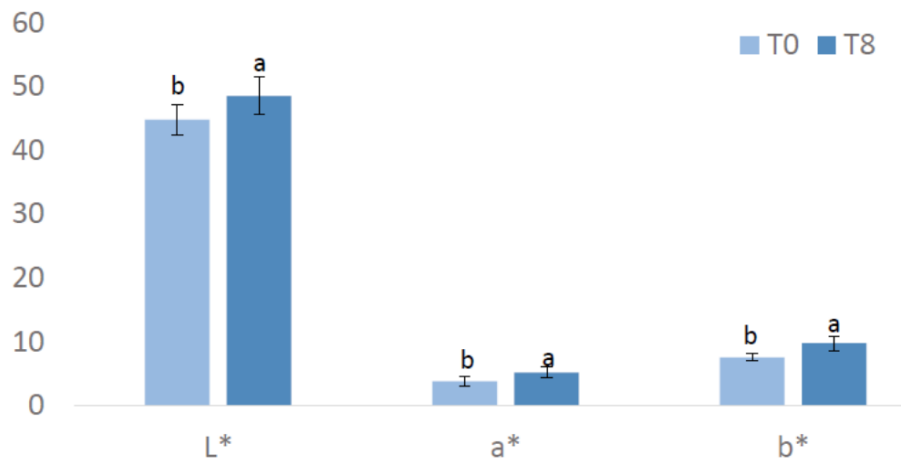
The results of texture parameters of Atlantic Bonito fillets obtained during storage time are shown in the figure. Texture results showed that fillets' hardness and gumminess decreased after 8 days of storage.



Data concerning hardness, cohesiveness, and adhesiveness of Sarrajão fillets, from the Spring and Autumn season.

## Colour characterization

Regarding the colour results, it is possible to observe that there are significant differences for all the analysed parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) during storage time. Fish fillets showed higher values for all the parameters after 8 days of storage, presenting higher brightness, higher reddish colouration, and higher yellowish colouration.

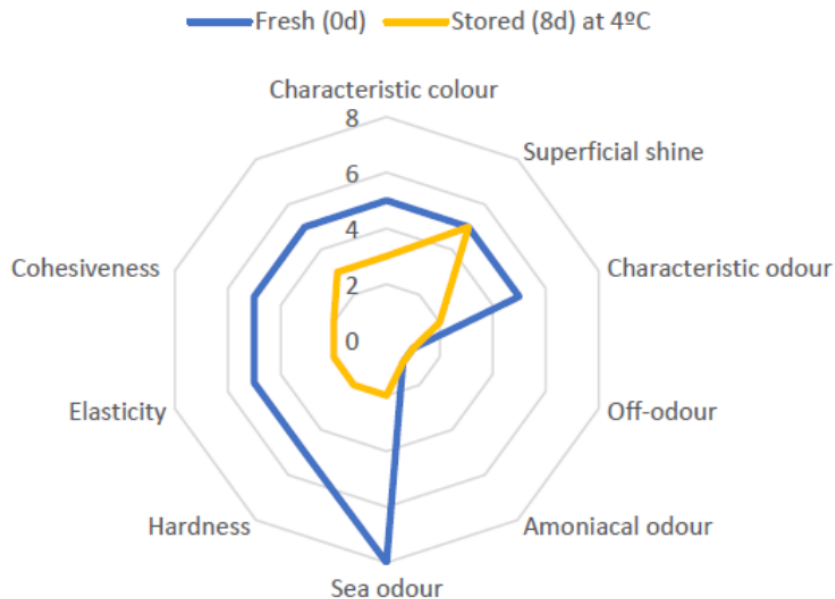


Analysis of the colour of Sarrajão fillets, in time 0d and 8d stored at 4°C.

## Sensory characterization

The results of the sensory analysis of fish fillets during storage time are shown in the figure.

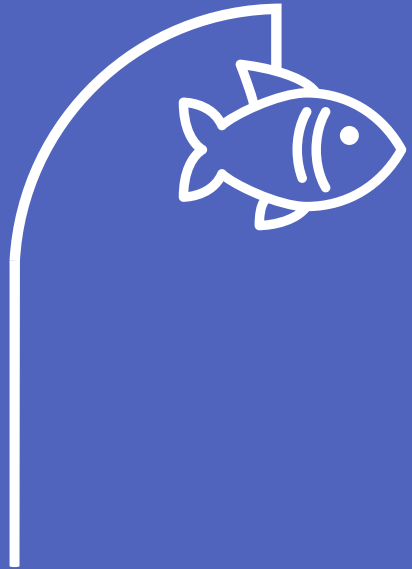
In relation to the sensory analysis, the panelists considered the fish fillet after 8 days of storage as having the lowest score in all attributes. With the exception of the superficial shine which scored the same value in both samples. The panellist did not detect any off-flavour and ammoniacal odour on both samples. With the results obtained it was concluded by the panellists that after 8 days of storage the fish has a satisfactory classification for consumption.



Data from the sensory analysis of Sarrajão fillets, in time 0d and 8d stored at 4°C.

# Products, solutions and market trends for targeted consumer groups

The global seafood market has witnessed significant growth in recent years, driven by increasing consumer awareness of the health benefits of seafood and the rising demand for sustainable and ethically sourced products.





## Overall market trends



**a. Health and Wellness:** Consumers are increasingly seeking healthier food options, and seafood is gaining popularity due to its rich nutritional profile, including omega-3 fatty acids and protein.



**b. Sustainability:** There is a growing emphasis on sustainable and responsible sourcing practices. Consumers are more inclined to choose seafood products that adhere to environmental and social responsibility standards.

## Product trends



**a. Value-added Seafood Products:** Ready-to-cook and pre-marinated seafood products are gaining traction, catering to busy lifestyles.



**b. Alternative Seafood:** Plant-based and lab-grown seafood alternatives are emerging to meet the demand for sustainable and cruelty-free options.



**c. Convenience and Innovation:** Innovative packaging and convenient formats, such as single-serve portions and seafood snacks, are becoming popular.

## Target consumer groups



**a. Health-Conscious Consumers:** Individuals seeking nutritious and low-fat protein sources, including fitness enthusiasts and those with specific dietary requirements.



**b. Environmentally Conscious Consumers:** Consumers who prioritize eco-friendly and sustainable products, often making choices based on certifications and ethical sourcing practices.



**c. Urban Millennials:** The younger demographic values convenience and seeks unique and diverse seafood options, driving demand for innovative products.



**d. Ethnic and Cultural Preferences:** Certain seafood products may appeal more to specific cultural or regional groups, leading to niche markets.

## Challenges and opportunities



**a. Supply Chain Challenges:** The seafood industry faces challenges related to overfishing, climate change, and disruptions in the supply chain. This presents an opportunity for innovation in sustainable practices.



**b. Technology Integration:** Advances in technology, including blockchain and traceability solutions, can address concerns related to transparency and authenticity in the seafood supply chain.

## New seafood product development proposal

In response to the evolving trends in the seafood industry, we propose the development of innovative and diverse seafood products focusing on fish fillets preserved through various processes, sea hamburgers, and sea meatballs. These products aim to cater to the growing demand for healthy, sustainable, and convenient seafood options.

### Product Line:



a. Fish Fillets Under  
Different Preservation  
Processes



b. Sea Hamburgers



c. Sea Meatballs

According to WP4, the purpose of this report is to present the results of the characterisation of the products produced with Sarrajão



## **Chapter 5**

**Main scientific results**

**- New products development**

# Technical specification of components to be used in the Sarrajão products development

## Technical specification of hydrocolloids

### Agar-agar

Name	Agar-agar
Brand	Sosa Ingredients, S.L.
Reference	58050115
Composition	Agar agar (E406)
Description	Texture modifier: Jellying agent. Gelatinases are ingredients of natural origin that have a great capacity to absorb water molecules and in so doing avoid their separation.
Application	The agar-agar must be heated 90°C and used immediately. It can be reheated as many times as necessary. The gelling temperature is 60°C. As this product achieves a soft texture, it is ideal for making false fruit pates without sugar, sheets, or jelly. It can be used with: - Water and all types of liquids with a water content above 80%. - All types of fruit (however, the more acid the fruit, the less it gels).
Organoleptic Properties	<b>Appearance:</b> White-beige fine powder <b>Flavor and odor:</b> Neutral

Physical-chemical properties	pH: 6.0 - 8.0 Particle size: <125µm
Microbiological properties	Mesophilic aerobic microorganisms (cfu/g): <2000 Escherichia coli: Absent in 5g. Moulds and Yeasts (cfu/g): <200 Salmonella: Absent in 10g
Packing	Polypropylene black pot
Storage	Keep in a cool, dry place out of light, in full containers and closed.
Shelf life	Use preferably within 24 months from production date if it has been kept in a sealed packet and in the storage conditions mentioned above.
Allergens	No allergens
GMO	Non GMO

# Alginate

Name	Alginate
Brand	Sosa Ingredients, S.L.
Reference	58050016
Composition	Alginate (E401)
Description	Natural origin ingredients. Sodium alginate is product obtained from kelps, which combined with CLORUR, is used in the process of spherification.
Application	<b>Mix cold.</b> <ul style="list-style-type: none"><li>- Mix the ALGINATE with the liquid that will give the desired flavor.</li><li>- Add drops or larger quantities to the CLORUR mixture.</li><li>- Remove carefully from mixture and leave in water for a few moments.</li><li>- Serve up on the chosen dish.</li></ul>
Organoleptic Properties	<b>Appearance:</b> Creamy-white to light-brown powder <b>Flavour and odour:</b> Neutral

Physical-chemical properties	-
Microbiological properties	Mesophilic aerobic microorganisms (cfu/g): <3000 Enterobacteria: Absent in 1g. Moulds and Yeasts (cfu/g): <50 Salmonella: Absent in 25g
Packing	Polypropylene black pot.
Storage	Store between 5 to 25°C. Keep in a cool, dry place, out of light.
Shelf life	Use preferably within 24 months from production date if it has been kept in sealed packet and in the storage conditions mentioned above.
Allergens	No allergens
GMO	Non GMO



## Carrageenan

<b>Name</b>	Carrageenan
<b>Brand</b>	Danisco Textural Ingredients
<b>Reference</b>	176219
<b>Composition</b>	Processed Eucheuma seaweed (E407 a), xanthan gum (E415), Potassium chloride (E508).
<b>Description</b>	Is a semi-refined carrageenan standardized with xanthan gum, potassium chloride and sugar. It is an off-white powder manufactured from red seaweed.
<b>Application</b>	Processed ham and poultry products in which self-suspending brine is needed.
<b>Organoleptic Properties</b>	<b>Appearance:</b> Off-white powder <b>Flavor and odor:</b> Neutral
<b>Physical-chemical properties</b>	<ul style="list-style-type: none"><li>- <b>pH:</b> 8.0-11.0</li><li>- <b>Energy:</b> 20 kcal/90 kJ</li><li>- <b>Carbohydrate:</b> 6g</li><li>- <b>Fibre:</b> 51g</li><li>- <b>Sodium:</b> &lt;1g</li></ul>

<b>Microbiological properties</b>	<b>Total plate count (cfu/g):</b> <5000 <b>Moulds and Yeasts (cfu/g):</b> <200 <b>Salmonella:</b> Absent in 25g <b>Coliforms:</b> Absent 1g
<b>Packing</b>	Poly-lined bags.
<b>Storage</b>	Store cool and dry. Temperature Max 25°C, with a relative humidity <60%.
<b>Shelf life</b>	-
<b>Allergens</b>	No allergens
<b>GMO</b>	Non GMO

# Xanthan

Name	Xanthan
Brand	Rhodia Food
Reference	11138-66-2
Composition	Xanthan gum (E415)
Description	Its particularly useful for thickening and stabilizing. Its high hydration rate ensures very fast applications after pre-mixing.
Application	Processed ham and poultry products in high self-suspending brine is needed.
Organoleptic Properties	Appearance: creamy-white fine powder Odor: Almost neutral
Physical-chemical properties	<p>pH: 6.0-8.0 Ash: 6.5-16.0 Pyruvic acid: 1.5% max. Total heavy metals (mg/kg): 20 max. Arsenic (mg/kg): 2 Lead (mg/kg): 5 Zinc (mg/kg): 10 Copper (mg/kg): 5</p> <p>Nickel (mg/kg): 2 Manganese (mg/kg): 5 Chromium (mg/kg): 2 Mercury (mg/kg): 1 Cadmium (mg/kg): 1 IPA (mg/kg): 500 Assay: 91.0%-117.0%</p>

Microbiological properties	Total plate count (cfu/g): <2000 Moulds and Yeasts (cfu/g): <100 Staphylococcus aureus: Absent in 1g. Pseudomonas aeruginosa: Absent in 1g. Salmonella: Absent in 25g. Coliforms: Absent 1g. Clostridium perfringens: Absent in 0.1g.
Packing	Net cardboard boxes with inside poly-lining.
Storage	Cool and dry local.
Shelf life	2 years is guaranteed if stored in a correct condition.
Allergens	No allergens
GMO	Non GMO

## Locus Bean Gum

<b>Name</b>	Locus Bean Gum
<b>Brand</b>	Victus Portugal
<b>Composition</b>	Locus Bean Gum (E410)
<b>Description</b>	A flour product obtained after grinding the endosperm of carob seeds. A polysaccharide hydrocolloid composed of combined galactose and mannose units, usually described as galactomannans.
<b>Organoleptic Properties</b>	Appearance: off-white fine powder Odor and flavor: Almost neutral
<b>Physical-chemical properties</b>	<b>pH:</b> 5.0-7.5 <b>Ash:</b> 1.2% max <b>Moisture:</b> 14% max <b>Protein:</b> 7% max <b>Galactomannans:</b> 75% max
<b>Microbiological properties</b>	<b>Total plate count (cfu/g):</b> <5000 <b>Moulds and Yeasts (cfu/g):</b> <500 <b>Salmonella:</b> Absent in 25g. <b>Coliforms:</b> Absent 1g. <b>Escherichia coli:</b> Absent in 1g.
<b>Packing</b>	Multi sheet paper bag, polyethylene interior.
<b>Storage</b>	Cool and dry local.
<b>Shelf life</b>	12 months in original packaging if stored in a correct condition.

## Guar

Name	Guar
Brand	Celeiro
Reference	-
Composition	Guar gum (E412)
Description	Guar is the name of an herbaceous plant of the legume family, grown mainly in India and Pakistan. Guar gum is obtained simply from the dried, ground seeds. Due to its colloidal properties, it is widely used in food and cosmetics.
Application	It is a great thickener and gelling agent. It can be used to thicken sauces, condiments and creams and helps to give consistency and structure to gluten-free pasta.
Organoleptic Properties	<b>Appearance:</b> creamy-white fine powder <b>Odor:</b> Almost neutral
Physical-chemical properties	<b>Energy:</b> 792 kJ/196 kcal <b>Lipids:</b> 1.5g <b>Carbohydrates:</b> 1g <b>Fibre:</b> 75g <b>Proteins:</b> 7g

Microbiological properties	-
Packing	-
Storage	Store in a cool, dry place away from light.
Shelf life	-
Allergens	No allergens
GMO	Non GMO

# Chitosan

Name	Chitosan
Brand	Sigma Aldrich®
Reference	417963
Composition	-
Description	Chitosan is a mucopolysaccharide derived from chitin, extracted from shrimp shells. This polysaccharide presents a wide range of biological activities.
Application	Biocompatible, antibacterial and environmentally friendly polyelectrolyte with a variety of applications including water treatment, chromatography, additives for cosmetics, textile treatment for antimicrobial activity, novel fibers for textiles, photographic papers, biodegradable films, biomedical devices, and microcapsule implants for controlled release in drug delivery. Chitosan, from shrimp shells, practical grade to forms gels with multivalent anions. Gives clear solutions that dry to strong, clear films.

Organoleptic Properties	<b>Appearance:</b> White-beige fine powder or flakes <b>Flavor and odor:</b> Neutral
Physical-chemical properties	<b>Viscosity:</b> >200 cP, 1 wt. % in 1% acetic acid (20 °C, Brookfield) (lit.) <b>Solubility:</b> 1 M acetic acid: 10 mg/mL
Microbiological properties	-
Packing	High density Polyethylene container (white color)
Storage	Store in a cool, dry place away from light.
Shelf life	-
Allergens	No allergens
GMO	Non GMO

# Pectin

Name	Pectin
Brand	Obipektin
Reference	101126
Composition	Pectin gum (E440)
Description	The pectin high viscosity is a pure, high methoxyl pectin. It is standardized to a defined viscosity.
Application	Cloud stabilization and improvement of mouthfeel in beverages. The dosage depends on the composition of the final product.
Organoleptic Properties	<b>Appearance:</b> fine, free-flowing powder, beige <b>Odor:</b> Almost neutral
Physical-chemical properties	<b>pH:</b> 2.8-3.8 <b>Energy:</b> 0kj / 0kcal <b>Carbohydrates:</b> 0g <b>Protein:</b> 0.5g <b>Fat:</b> 0.5g <b>Soluble fibre:</b> 80g

Microbiological properties	<b>Total plate count (cfu/g):</b> <1000 <b>Moulds and Yeasts (cfu/g):</b> <100 <b>Enterobacteriaceae:</b> Absent in 1g. <b>Salmonella:</b> Absent in 25g.
Packing	Packed in a cardboard box with a PE-bag at 25kg.
Storage	If stored in closed packaging, cool and dry.
Shelf life	2 years is guaranteed if stored in a correct condition.
Allergens	No allergens
GMO	Non GMO

# Technical specification of proteins

## Whey Protein

Name	Whey Protein
Brand	NAARMANN
Composition	Produced from fresh skimmed milk, spray dried.
Description	<b>pH:</b> 6.6-6.9 <b>Protein:</b> 48.0-52.0% <b>Fat:</b> 0.1-0.5% <b>Water content of the powder:</b> 3.0-4.5%
Organoleptic Properties	<b>Total plate count (cfu/g):</b> <50.000 <b>Enterobacteriaceae (cfu/g):</b> <10 <b>Moulds and Yeasts (cfu/g):</b> <50 <b>Salmonella:</b> Absent in 25g. <b>Staphylococcus coagulase (+) (cfu/g):</b> <100
Packing	Paper bag with polyethylene-inliner
Storage	Cool and dry
Shelf life	See stamp of package
Allergens	Milk and similar products
GMO	Non GMO

## Pea Protein

Name	Pea Protein isolate
Brand	Cosucra
Composition	Pasteurised skimmed milk
Description	Produced from fresh skimmed milk, spray dried.
Organoleptic Properties	<b>Appearance:</b> yellow coloured powder <b>Flavor and odor:</b> Neutral
Physical-chemical properties	pH: 6.5-7.5
Storage	In dry conditions (max 30°C and 60% relative humidity), closed packing.
Shelf life	See stamp of package
Allergens	No allergens directly incorporated, but possibility of cross-contamination with gluten.



# Milk Protein

Name	Promilk
Brand	Ingredia Functional
Reference	MPI85AFS08
Composition	Milk protein isolate spray dried in powder
Description	Is a dairy ingredient which helps to increase and standardize the protein content of raw materials in many dairy products.
Organoleptic Properties	<b>Appearance:</b> creamy-white free flowing powder <b>Flavor and odor:</b> slightly milk
Physical-chemical properties	<b>pH:</b> 6.5-7.5 <b>Energy:</b> 358kcal /1518kJ <b>Moisture:</b> 5% <b>Protein:</b> 81.5% <b>Fat:</b> 1.5% <b>Carbohydrate:</b> 4.5% <b>Lactose:</b> 4.5% <b>Ash:</b> 7.5%

Microbiological properties	<b>Total plate count (cfu/g):</b> <10000 <b>Enterobacteriaceae:</b> Absent in 1g. <b>Moulds and Yeasts (cfu/g):</b> <30 <b>Salmonella:</b> Absent in 375g. <b>Staphylococcus coagulase (+):</b> Absent in 1g. <b>Escherichia coli:</b> Absent in 1g.
Packing	Polythene lined paper bags
Storage	Cool and dry place.
Shelf life	12 months when stored in a correct condition.
Allergens	Milk and similar products
GMO	Non GMO

## Soy Protein

<b>Name</b>	Soy Protein Isolate
<b>Brand</b>	QingDao Crown Imp. & Exp. Corp. Ltd
<b>Reference</b>	Soypro950IJ
<b>Composition</b>	Milk protein isolate spray dried in powder
<b>Description</b>	Is a soy protein produced from the finest Non GMO raw materials, it has been produced and designed for use within meat, poultry, and fish processing.
<b>Applications</b>	This protein provides strength through water binding in a ratio of 1:5. The product is highly soluble, and it has an excellent dispersion in Ham, it is widely used for injection and can keep Ham fresher.
<b>Organoleptic Properties</b>	<b>Appearance:</b> creamy-white free flowing powder <b>Flavour:</b> Bland
<b>Physical-chemical properties</b>	<b>pH:</b> 7.0-7.5 <b>Moisture:</b> 6% <b>Protein:</b> 90.5% <b>Fat:</b> 0.5% <b>Fibre:</b> 0.5% <b>Ash:</b> 5.5%  <b>Calcium:</b> 0.02% <b>Sodium:</b> 1.2% <b>Phosphorus:</b> 0.7% <b>Potassium:</b> 0.1% <b>Lecithin:</b> 0.2%

<b>Microbiological properties</b>	<b>Total plate count (cfu/g):</b> <20000 <b>Moulds and Yeasts (cfu/g):</b> <100 <b>Salmonella:</b> Absent in 25g. <b>Escherichia coli:</b> Absent in 1g
<b>Packing</b>	Polythene lined paper bags
<b>Storage</b>	Cool and dry place.
<b>Shelf life</b>	12 months when stored in the correct condition
<b>Allergens</b>	Milk and similar products
<b>GMO</b>	Non GMO

# Fish gelatin

Name	Fish gelatin
Brand	Sigma Aldrich®
Reference	G7765
Composition	Mixture of water-soluble proteins of high average molecular masses, present in fish collagen
Description	Gelatin derived from cold water fish skin has low gelling and melting points
Applications	Gelatin from fish skin can be used in the preparation of various gels based on their gelling characteristics, and so be applied in the formulation of edible coatings
Organoleptic Properties	<b>Physical state:</b> solid  <b>Color:</b> light yellow

Physical-chemical properties	pH: 4.0 – 7.0 Viscosity: 7.0 - 10.0 Heavy Metals: < 20 ppm Loss on Drying: < 13.5 % Ash: < 2.0%
Microbiological properties	-
Packing	High density Polyethylene container (white color)
Storage	Stored at room temperature in a dry place
Shelf life	-
Allergens	No allergens
GMO	Non GMO

# Technical specification of aromatic herbal

## Rosemary

Name	Alecrim – <i>Salvia Rosmarinus</i>
Description	Very branched aromatic herb, evergreen, with woody stems, small and thin, opposite, and lanceolate leaves. The upper part of the leaves is green – grayish, while the lower part is almost silver. The whole plant exudes a strong and pleasant aroma. The aromatic herbs are free from pests and diseases.
Parameters	Average weight (pot): 0.2kg Average height: 18cm Color: Dark green
Recommended use	Wash before eating fresh
Packing	Packed in LDPE plastic and packed in a cardboard box
Storage	Ambient temperature, over 12°C.

## Coriander

<b>Name</b>	Coentros – Coriandrum Sativum
<b>Description</b>	A plant with green leaves that are rounder but more like parsley. It is a plant with a characteristic smell and taste and its leaves are soft and delicate. The aromatic herbs are free from pests and diseases.
<b>Parameters</b>	Average weight (pot): 0.2kg Average height: 15cm Colour: Green
<b>Recommended use</b>	Wash before eating fresh
<b>Packing</b>	Packed in LDPE plastic and packed in a cardboard box
<b>Storage</b>	Ambient temperature, over 12°C.

## Oregano

<b>Name</b>	Oregãos – Origanum vulgare
<b>Description</b>	Herbaceous plant with an erect stem, with opposite, oval and pointed dark green leaves. It is characterized by its very aromatic smell and bitter taste. It develops horizontally. The aromatic herbs are free from pests and diseases.
<b>Parameters</b>	Average weight (pot): 0.2kg Average height: 15cm Color: Dark green
<b>Recommended use</b>	Wash before eating fresh
<b>Packing</b>	Packed in LDPE plastic and packed in a cardboard box
<b>Storage</b>	Ambient temperature, over 12°C.

## Basil

<b>Name</b>	Manjerição – Ocimun Basilium L.
<b>Description</b>	Herbaceous plant with green and oval leaves. It is a very aromatic plant with fragrant leaves. The smaller leaves that are present in the center of the plant are more wrinkled. They are cut at least from the 2nd leaf bud ensuring superior freshness. The aromatic herbs are free of any signs of pests or diseases.
<b>Parameters</b>	Average weight (pot): 0.2kg Average height: 16cm Colour: Green
<b>Recommended use</b>	Wash before eating fresh
<b>Packing</b>	Packed in LDPE plastic and packed in a cardboard box
<b>Storage</b>	Ambient temperature, over 12°C.

## Thyme

<b>Name</b>	Tomilho – Thymus vulgaris
<b>Description</b>	A subshrub with full stems, small oval, lanceolate or linear leaves with slightly folded edges, green – grayish in color, opaque on the rafters and whitish on the underside. The aromatic herbs are free from pests and diseases.
<b>Parameters</b>	Average weight (pot): 0.2kg Average height: 15cm Colour: Green
<b>Recommended use</b>	Wash before eating fresh
<b>Packing</b>	Packed in LDPE plastic and packed in a cardboard box
<b>Storage</b>	Ambient temperature, over 12°C.



# Technical specification of micro and macro algae

## Palmaria palmata

Name	<i>Palmaria palmata</i>
Description	Marine macroalgae of the red algae group. It grows on the northern coasts of the Atlantic and Pacific Oceans and reproduces by spreading the male gametes produced in the "leaves" of this species, which in turn fertilize the female gametes.
Parameters	<b>Colour:</b> Dark red <b>Average height:</b> 50cm
Physical-chemical properties	<b>Protein:</b> 9.59% <b>Fat:</b> 1.06% <b>Ash:</b> 39.40% <b>Carbohydrates:</b> 25.94% <b>Total sugars:</b> 14.16% <b>Carotenoids:</b> 8.19 ppm

## Ulva rigida

Name	<i>Ulva rigida</i>
Description	This species of the green algae group has a worldwide distribution in marine environments but can also be found in estuarine environments. It prefers rocky habitats and is easily found in calm waters in the pools between tides. It reproduces by vegetative propagation or by fragmentation.
Parameters	<b>Colour: Green</b> <b>Average height: 20cm</b>
Physical-chemical properties	<b>Protein:</b> 14.59% <b>Lipids:</b> 1.59% <b>Ash:</b> 24.72% <b>Carbohydrates:</b> 27.02% <b>Total sugars:</b> 19.45% <b>Carotenoids:</b> 14.19 ppm

## Porphyra umbilicalis

Name	<i>Porphyra umbilicalis</i>
Description	A marine species of the red algae group that lives mainly in cold, shallow waters around the world. It can reproduce asexually, as all individuals have gametes of both sexes.
Parameters	<b>Color:</b> Dark red to dark brown <b>Average height:</b> 17cm
Physical-chemical properties	<b>Protein:</b> 29.73% <b>Fat:</b> 2.04% <b>Ash:</b> 29.78% <b>Carbohydrates:</b> 26.16% <b>Total sugars:</b> 17.72% <b>Carotenoids:</b> 12.21 ppm

## Gracilaria gracilis

Name	<i>Gracilaria gracilis</i>
Description	It is a genus of red algae with high economic importance to produce agar and for food use by humans and several species of marine animals. Several species are cultivated in the subtropics and tropics. The genus Gracilaria includes moderately sized, free-living, generally erect, linear stemmed, highly branched seaweeds.
Parameters	<b>Colour:</b> Red brown <b>Average height:</b> 40cm
Physical-chemical properties	<b>Protein:</b> 27.93% <b>Lipids:</b> 2.44% <b>Ash:</b> 26.78% <b>Carbohydrates:</b> 26.33% <b>Total sugars:</b> 21.44% <b>Carotenoids:</b> 5.34 ppm

## Spirulina

Name	<i>Spirulina</i>
Description	<p>Spirulina is a microscopic algae that helps to increase energy and vitality thanks to a protein content of more than 65%; it also provides eight essential amino acids in adequate proportions. Because it has a high protein content that is easily absorbed, it is useful for athletes when recovering after physical exertion.</p> <p>It is rich in vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin D and minerals such as magnesium, calcium, and iron.</p>
Applications	Add to tea, juices, smoothies, porridges, soups or as a natural coloring in dishes and desserts.
Parameters	<b>Colour:</b> Green
Physical-chemical properties	<p><b>Energy:</b> 1523kJ / 364kcal <b>Fat:</b> 6.4g <b>Carbohydrates:</b> 4.4g <b>Protein:</b> 69g <b>Fibre:</b> 6.6g <b>Salt:</b> 1.5g <b>Vitamin B3:</b> 14mg <b>Potassium:</b> 1370mg <b>Iron:</b> 43mg <b>Magnesium:</b> 252mg</p>
Storage	Store in a cool, dry place, protected from light.

## Chlorella

<b>Name</b>	<i>Chlorella sp.</i>
<b>Description</b>	Chlorella is an alga that grows in fresh water. It is a microscopic alga with a unicellular structure and is characterized by containing the highest concentration of chlorophyll found in any other plant. Chlorophyll has the quality of attracting heavy metals and other harmful substances to itself and then eliminating them from the body. It is also a natural source of vitamins, minerals, fiber, nucleic acids, amino acids, and enzymes.
<b>Parameters</b>	Colour: Green
<b>Physical-chemical properties</b>	-
<b>Storage</b>	Store in a cool, dry place away from sunlight.

# Technical specification of liquid smoke

## RudinSmoke Euro 100

<b>Name</b>	RudinSmoke Euro 100
<b>Brand</b>	Ruitenberg
<b>Reference</b>	RD0962/RD0960/RD0959
<b>Composition</b>	Hardwoodmix (contains oak, hickory)
<b>Description</b>	Partially neutralized smoke condensate with high browning capacity and medium flavor.
<b>Applications</b>	<p>In co extrusion systems as a crosslink agent.</p> <p>In the brine bath, injector, and tumbler as a browning and flavoring agent for sausage, meat, and fish.</p> <p>In dip and showering applications.</p>
<b>Organoleptic Properties</b>	<p><b>Appearance:</b> clear liquid</p> <p><b>Colour:</b> brown</p> <p><b>Flavour:</b> smoke</p> <p><b>Taste:</b> mellow smoke flavor</p>

<b>Physical-chemical properties</b>	<p>pH: 4.5-5.0</p> <p><b>Moisture:</b> 85% max.</p> <p><b>Carbohydrates:</b> 9%</p> <p><b>Fibres:</b> 2%</p> <p><b>Sodium content:</b> 980 mg/100 g</p> <p><b>Salt:</b> 2450 mg/100 g</p> <p><b>Ash:</b> 2% max.</p> <p><b>Nutritional value:</b> 36kcal /152kJ</p> <p><b>Acidity (% HAC):</b> 2-5</p> <p><b>Carbonyls (g/L):</b> 80-100</p> <p><b>Phenols (g/L):</b> 7.0-10.5</p> <p><b>Staining index:</b> 90/110</p> <p><b>Benzopyrene:</b> 10 ppb max.</p> <p><b>Benzo-a-anthracene:</b> 20 ppb max.</p>
<b>Microbiological properties</b>	<p><b>Total plate count (cfu/g):</b> negative</p> <p><b>Moulds and Yeasts (cfu/g):</b> negative</p> <p><b>Enterobacteriaceae (cfu/g):</b> negative</p>
<b>Packing</b>	Polypropylene black pot.
<b>Storage</b>	Cool (6-21°C) in closed original packaging.
<b>Shelf life</b>	At least 9 months under recommended storage conditions.
<b>Allergens</b>	No allergens
<b>GMO</b>	Non GMO

# Technical specification of leguminous plants

## Lupine

Name	Lupine flour 250g
Brand	Celeiro
Reference	-
Composition	100% Lupine flour
Description	Lupin flour obtained from the milling of skinned white lupin.
Applications	Given its characteristics it is used for nutritional enrichment and to favor the thickness of your various recipes. Add to other flours in the production of bread, biscuits, and desserts in the proportion of 10-20%. Also use it to thicken and enrich soups and sauces.
Organoleptic Properties	Colour: Yellowish
Physical-chemical properties	Energy: 1493kj / 358kcal Lipids: 12g Carbohydrates: 9g Fibres: 27g Protein: 40g Salt: 0.06g
Microbiological properties	-
Packing	-
Storage	Store in a cool and dry place.
Shelf life	-
Allergens	May contain traces
GMO	Non GMO



## Chickpeas

Name	Chickpeas flour
Brand	-
Reference	-
Composition	Chickpea flour
Description	Chickpea flour is a type of flour with low carbohydrate content and great nutritional power, besides not containing gluten, which makes it a great ally in the kitchen. It has a great nutritional contribution, being very rich in vitamins of group A, B, C, E. It is also rich in omega 6, iron and can triple that provided by meat and fiber, as it helps improve intestinal transit and regulate our body. In addition, it contains slow absorption carbohydrates, which facilitate digestion and allow diabetics to control glucose imbalances in the body, giving them the same energy as sugar
Applications	Cooking your favorite bakery, pastry, or confectionery recipes.

Organoleptic Properties	Colour: creamy Odor: Almost neutral
Physical-chemical properties	Energy: 1483 kj / 353 kcal Fat: 6g Carbohydrates: 44.5g Fibre: 21.7g Salt: 0.08g Phosphorus: 366 mg Iron: 6.2mg
Microbiological properties	-
Packing	-
Storage	Store in a cool, dry place off the ground.
Shelf life	-
Allergens	May contain traces of nuts, soya, and sesame.
GMO	Non GMO

## Beans

Name	Bean flour
Brand	-
Reference	-
Composition	Bean flour
Description	Bean Flour is obtained from the processing of dehulled broad beans in mills.
Applications	Cooking your favorite bakery, pastry, or confectionery recipes.
Organoleptic Properties	<b>Colour:</b> off-white <b>Odor:</b> Almost neutral
Physical-chemical properties	<b>Energy:</b> 359 kcal <b>Fat:</b> 2.6 g <b>Carbohydrates:</b> 71.3 g <b>Salt:</b> 0.006 g <b>Protein:</b> 12.6 g
Microbiological properties	-
Packing	-
Storage	Store in a cool and a dry place.
Shelf life	-
Allergens	May contain traces of nuts, soya and sesame.
GMO	Non GMO

# Lentils

Name	Lentils flour
Brand	-
Reference	-
Composition	Lentils flour
Description	Flour from organic farming that provides a high quantity of proteins, iron, and vitamins B1 and B2.
Applications	This flour can be used to bake rustic breads and various rustic pastries.
Organoleptic Properties	Color: creamy Odor: Almost neutral
Physical-chemical properties	Energy: 1399 kj / 331 kcal Fat: 1.2g Carbohydrates: 50.4g Fibre: 11.2g Salt: 0.061g Proteins: 24g
Microbiological properties	-
Packing	-
Storage	Store in a cool and dry place.
Shelf life	-
Allergens	May contain traces of nuts, soya and sesame.
GMO	Non GMO

# Peas

Name	Pea flour
Brand	-
Reference	-
Composition	Pea Flour
Description	Source of vegetable protein and numerous minerals such as calcium, sodium, iron, zinc, selenium, potassium, and phosphorus. The low-calorie intake and an almost non-existent amount of fats make it a more than advisable food for weight loss. The main properties of this flour are a high protein content, being a legume flour and low fat, making it a very healthy product to have in your kitchen and include it in your recipes.
Applications	It is recommended to use for all types of recipes, sweet and savory. On the packaging, you can enjoy a suggested recipe.
Organoleptic Properties	Color: brown Odor: Almost neutral
Physical-chemical properties	Fat: 1.8g Carbohydrates: 42.6g Fibre: 19.8g Salt: 0.002g
Microbiological properties	-
Packing	-
Storage	Store in a cool, dry place away from light.
Shelf life	-
Allergens	May contain traces of nuts, soya and sesame.
GMO	Non GMO

# Quinoa

Name	Quinoa Flour
Brand	-
Reference	-
Composition	Quinoa Flour
Description	Quinoa flour retains many of the natural properties of quinoa, thanks to the relatively simple production process. Once the seed coat is removed and the seeds are sufficiently dry, they are ground into flour. A simple and honest product, which is sufficient.
Applications	The bold flavor with nutty notes of quinoa flour can add a surprising new dimension to all kinds of products such as cakes, pies, biscuits, waffles, and pancakes.
Organoleptic Properties	<b>Color:</b> brown <b>Odor:</b> Almost neutral
Physical-chemical properties	<b>Energy:</b> 1510kJ / 361kcal <b>Fat:</b> 6.5g <b>Carbohydrates:</b> 58g <b>Fibre:</b> 11g <b>Protein:</b> 12g
Microbiological properties	-
Packing	-
Storage	Store in a cool, dry place off the ground.
Shelf life	-
Allergens	No allergens
GMO	Non GMO

# Buckwheat

Name	Buckwheat flour
Brand	-
Reference	-
Composition	Buckwheat flour
Description	High fiber content, promotes and regulates intestinal transit, helping to combat constipation. The low glycaemic index of its carbohydrates means that sugar levels remain stable for longer.
Applications	It is recommended to use as a substitute for traditional flour in all types of recipes, sweet and savory.
Organoleptic Properties	Colour: brown Odor: Almost neutral
Physical-chemical properties	Energy: 346kcal Fat: 3.4g Carbohydrates: 70g Fibre: 3.7g Salt: 0.01g Protein: 13g
Microbiological properties	-
Packing	-
Storage	Keep in a cool, dry place.
Shelf life	-
Allergens	May contain traces of cereals with gluten, peanuts, nuts, and sesame seeds.
GMO	Non GMO

## Locust Bean

Name	Locust Bean Flour
Brand	-
Reference	-
Composition	Locust Bean Flour
Description	Is an energizing food with multiple benefits thanks to being rich in mucilage that helps treat respiratory problems, throat discomforts and digestive problems such as diarrhea, indigestion, stomach and intestinal discomforts.
Applications	It can be used in the same way as any other flour. In addition, due to its natural sweet taste, it can be used as a cocoa substitute in the preparation of smoothies, desserts and pastries in general.
Organoleptic Properties	<b>Color:</b> Creamy <b>Odor:</b> Almost neutral
Physical-chemical properties	<b>Energy:</b> 286kcal <b>Fat:</b> 0.48g <b>Carbohydrates:</b> 44g <b>Fibre:</b> 43.8g <b>Salt:</b> 0.3g <b>Protein:</b> 4.61g
Microbiological properties	-
Packing	-
Storage	-Store in a cool, dry place.
Shelf life	-
Allergens	May contain traces of nuts, soya and sesame.
GMO	Non GMO

# Technical specification of fish oil

## Fish oil

Name	Fish oil
Brand	Naturitas
Reference	-
Composition	Mainly triglycerides with abt. 0.1 – 0.5% moisture and abt. 0.1% impurities
Description	-
Applications	-
Organoleptic Properties	<b>Physical state:</b> liquid <b>Color:</b> Transparent, light Brown <b>Odor:</b> Faint, fish
Physical-chemical properties	<b>pH:</b> 5.0 -7.0 <b>Boiling point:</b> > 200°C <b>Solidification point:</b> app. -10°C – 0°C <b>Flash point:</b> > 160 °C
Microbiological properties	-
Packing	Gelatine capsules
Storage	Stored in a dark, fresh and dry place
Shelf life	-
Allergens	No allergens
GMO	Non GMO



# Technical specification of other coating components

## Tween 80

Name	Tween® 80
Brand	BioChemica
Reference	A1390
Composition	Polyethylene sorbitol ester, sorbitol and oleic acid.
Description	Tween 80 is a polyethylene sorbitol ester, with a calculated molecular weight of 1,310 daltons, assuming 20 ethylene oxide units, 1 sorbitol, and 1 oleic acid as the primary fatty acid.4 Fatty acid constituents of this product are determined by transesterification to yield fatty acid methyl esters, which are identified by gas chromatography. Typically, the fatty acid composition is approximately 70% oleic acid with several other fatty acids such as palmitic acid indicated.
Applications	Tween 80 has been widely used in biochemical applications including: solubilizing proteins, isolating nuclei from cells in culture, growing of tubercle bacilli, and emulsifying and dispersing substances in medicinal and food products

Organoleptic Properties	Appearance (liquid): yellowish-brownish, clear, oily
Physical-chemical properties	<b>Density (d 25°C/25°C):</b> 1.06 - 1.09 <b>Hydroxyl-No.:</b> app. 75 <b>Saponification No.:</b> 45 – 55 <b>Specific gravity:</b> 1.07 (25 °C) <b>HLB (hydrophile-lipophile balance) value:</b> 15.00 <b>Critical Micellar Concentration (CMC):</b> 13-15 mg/liter <b>Brookfield Viscosity:</b> 400-620 cps (25 °C, neat) <b>Micelle molecular weight:</b> 76 kDa3
Packing	High density Polyethylene container (transparent)
Storage	Room Temperature Keep container sealed
Shelf life	-
Allergens	No allergens
GMO	Non GMO

# Glycerol

<b>Name</b>	Glycerol
<b>Brand</b>	Sigma Aldrich®
<b>Reference</b>	G9012
<b>Composition</b>	1,2,3-Propanetriol Glycerin
<b>Description</b>	Glycerol is odorless, colorless, viscous in nature, and exists as a sweet tasting liquid. It can be derived naturally as well as from petrochemical feedstock.
<b>Applications</b>	Glycerol has a wide variety of applications, and is one of the most valuable and versatile chemical substances in nature. It can be used as an emollient, solvent, sweetening agent, in pharmaceutical formulations, cosmetics, foodstuffs and toiletries. It is very stable and can be easily stored under normal temperature; also, it is non-irritating and has no adverse impact to the environment. Glycerol is used both in sample preparation and gel formation for polyacrylamide gel electrophoresis. Glycerol (5-10%) increases the density of a sample so that the sample will layer at the bottom of a gel's sample well. Glycerol is also used to aid in casting gradient gels and as a protein stabilizer and storage buffer component.

<b>Organoleptic Properties</b>	<b>Colour:</b> Colourless <b>Appearance:</b> Viscous Liquid <b>Turbidity:</b> Clear
<b>Physical-chemical properties</b>	<b>Vapor density:</b> 3.1 (vs air) <b>Vapor pressure:</b> <1 mmHg (20 °C) <b>Autoignition temperature:</b> 698 °F <b>Refractive index:</b> n20/D 1.474 (lit.)
<b>Microbiological properties</b>	-
<b>Packing</b>	High density Polyethylene container (transparent)
<b>Storage</b>	Room Temperature Keep container sealed

# Lactic acid

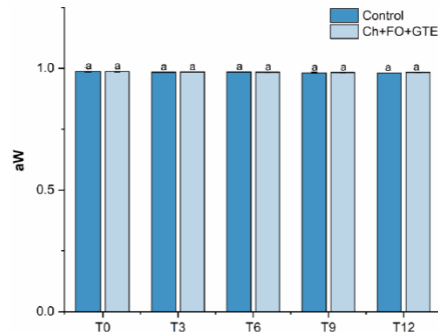
Name	L-(+)-Lactic acid
Brand	Sigma Aldrich ®
Reference	L1750
Composition	L-(+)-Lactic acid
Description	L-(+)-Lactic acid is the only naturally occurring lactic acid in humans and mammals. Commercially, few bacteria like Lactobacillus casei, L. delbrueckii, Streptococcus lactis produces L-Lactic acid by fermentation process. Lactic acid activates hydroxycarboxylic acid receptor, G-protein coupled receptor 81 (GPR81).
Applications	Lactic acid can be used as a component in substrate solution II for lactate dehydrogenase reaction; as an additive in storage solution A and as a supplement in the artificial gastric juice preparation for evaluation of degree of resistance of Lactobacillus to the gastric stresses.
Organoleptic Properties	Colour: White Appearance: Powder Turbidity: Clear

Physical-chemical properties	Initial boiling point and boiling range: 122 °C at 20 hPa 122 °C at 15 hPa Flash Point: 110,00 °C - closed cup Melting point: 53 °C pH:1.2 Density: 1,200 g/cm3 Water solubility: 100 g/l at 20 °C - soluble
Microbiological properties	-
Packing	Glass bottle
Storage	Recommended storage temperature 2 - 8 °C
Shelf life	-
Allergens	No allergens
GMO	Non GMO

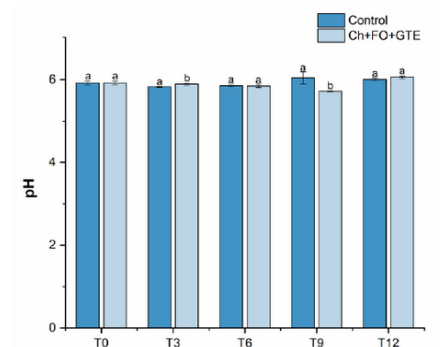
# Development of edible coatings to extend sarrajão shelf-life

## Shelf-life evaluation of coated sarrajao fillets

**pH and water activity:** The figures shows the water activity ( $A_w$ ) and the pH of coated and no coated sample (control). The coating application did not significantly affect the  $a_w$  ( $p < 0.05$ ) and the pH of control and coating samples were similar after 12 days of storage (T12).



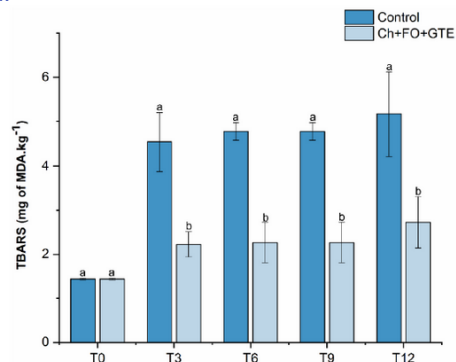
Water activity ( $A_w$ ) of coated and no coated fillets over the storage period. Errors bars represent the standard deviation of  $n=3$  replicates. a-b Different lower-case letters indicate significant difference between samples on the same day of storage ( $p < 0.05$ ).



pH value of coated and no coated fillets over the storage period. Errors bars represent the standard deviation of  $n=3$  replicates. a-b Different lower-case letters indicate significant difference between samples in the same day of storage ( $p < 0.05$ ).

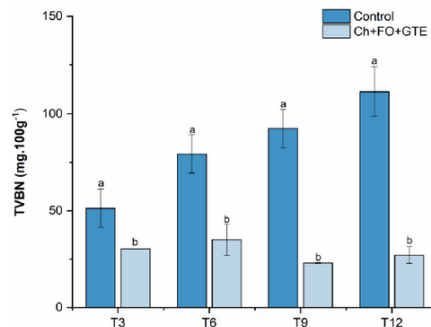
## Shelf-life evaluation of coated sarrajao fillets

**Lipid and protein oxidation:** The figure presents the thiobarbituric acid reactive substances (TBARS) (mg of malonaldehyde (MDA).kg<sup>-1</sup>) of coated and no coated fillets over the storage period, that is an indicative of the lipid oxidation in the fillets. The coating application retarded lipid oxidation during 12 days (TBARS < 3 mg MDA/kg), indicating the potential of the formulation composed by chitosan (Ch), fish oil (FO) and green tea extract (GTE) to delay lipid oxidation.



Thiobarbituric acid reactive substances (TBARS) (mg of malonaldehyde (MDA).kg<sup>-1</sup>) of coated and no coated fillets over the storage period. Error bars represent the standard deviation of n= 3 replicates. a-b Different lower-case letters indicate significant difference between samples on the same day of storage (p < 0.05).

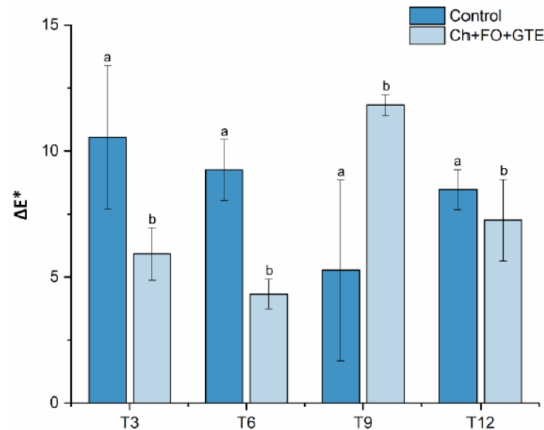
The application of Ch + FO + GTE in Sarrajão fillets also delayed the protein oxidation, as observed in the figure that presents the total volatile base nitrogen (TVBN). TVBN of coated samples did not exceed the limit of acceptability during 12 days, as a consequence of low post-mortem autolysis and bacterial degradation. The TVBN value of coated samples were slow than control sample.



Total Volatile Base Nitrogen (TVBN) (mg.100g<sup>-1</sup>) of coated and no coated fillets over the storage period. Error bars represent the standard deviation of n= 3 replicates. a-b Different lower-case letters indicate significant difference between samples in the same day of storage (p < 0.05).

## Shelf-life evaluation of coated sarrajao fillets

**Colour changes:** The effects of chitosan-based coating application on the color properties ( $\Delta E^*$ ) of Sarrajão fillets during refrigerated storage were assessed and presented in the figure. According to  $\Delta E^*$  results, some color changes were detected between coated and no coated Sarrajão fillets samples over time. At the end of storage (T12) the colour changes were more pronounced in the control sample than the coated sample.



Color properties ( $\Delta E^*$ ) of coated and no coated fillets over the storage period. Error bars represent the standard deviation of  $n=3$  replicates. a-b Different lower-case letters indicate significant difference between samples on the same day of storage ( $p < 0.05$ ).

## Shelf-life evaluation of coated sarrajao fillets

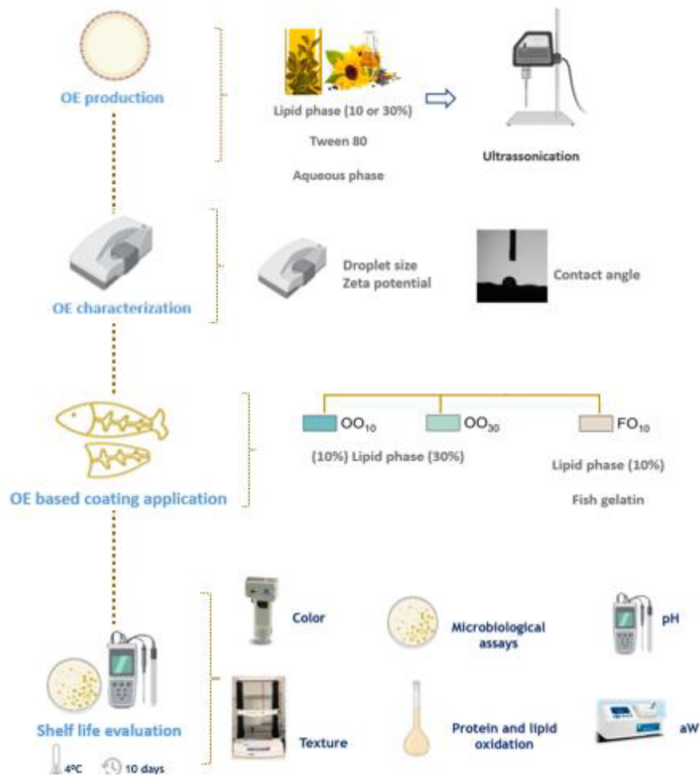
**Texture parameters:** Texture parameters of coated and no coated Sarrajão fillets samples during storage at 4 °C are shown in the table. The coated fish fillets' hardness was maintained during 3 days of storage, indicating that the hardness parameter was less changed in coated fillets over storage time. Gumminess and chewiness parameters of the control was higher than coated fillets during the storage time, indicating that more energy is required to disintegrate the control fish fillets. So, the results indicated that the chitosan-based coating acted as a barrier to water loss.

Texture parameters (hardness, gumminess and chewiness) obtained for the coated and no coated samples during the storage time (T0 to T12).

Time (days)	Sample	Hardness (Kg)	Gumminess (hardness × cohesiveness × springness)	Chewiness (hardness × cohesiveness)
0	Control	5.33 ± 0.37 <sup>a</sup>	1.12 ± 0.32 <sup>a</sup>	0.35 ± 0.03 <sup>a</sup>
3	Coated	5.22 ± 0.68 <sup>a</sup>	1.04 ± 0.32 <sup>a</sup>	0.30 ± 0.06 <sup>a</sup>
	Control	6.72 ± 0.78 <sup>b</sup>	2.29 ± 0.24 <sup>bde</sup>	0.64 ± 0.05 <sup>bc</sup>
6	Coated	9.58 ± 1.10 <sup>c</sup>	1.37 ± 0.05 <sup>a</sup>	0.29 ± 0.03 <sup>a</sup>
	Control	9.91 ± 1.29 <sup>c</sup>	1.49 ± 0.14 <sup>ac</sup>	0.41 ± 0.09 <sup>ad</sup>
9	Coated	11.97 ± 0.53 <sup>d</sup>	1.84 ± 0.24 <sup>bc</sup>	0.44 ± 0.12 <sup>ad</sup>
	Control	12.31 ± 0.29 <sup>d</sup>	2.38 ± 0.07 <sup>d</sup>	0.88 ± 0.22 <sup>c</sup>
12	Coated	12.07 ± 0.06 <sup>d</sup>	1.78 ± 0.16 <sup>b</sup>	0.43 ± 0.01 <sup>d</sup>
	Control	12.96 ± 0.22 <sup>e</sup>	2.07 ± 0.15 <sup>be</sup>	0.91 ± 0.20 <sup>c</sup>

\*\* Different letters show significant differences (p < 0.05) in the same column over the storage period.

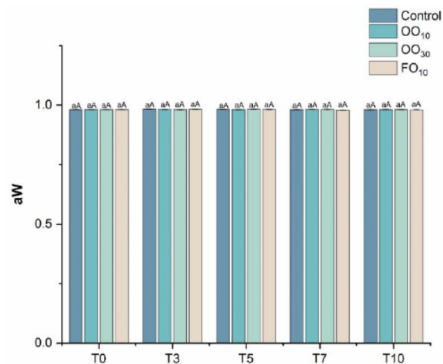
# Oregano oil emulsion based coated fillets





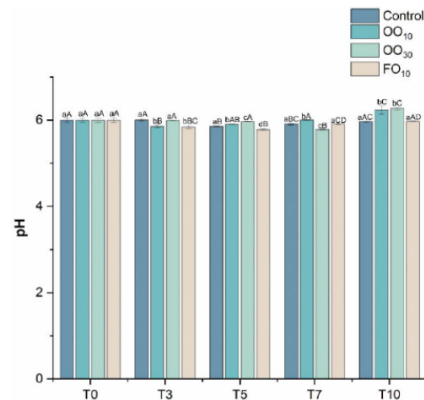
## Oregano oil emulsion based coated fillets

**pH and water activity:** The water activity ( $A_w$ ) of OE-based coated Sarrajão fillets and control sample are shown in the figure. All samples presented similar  $A_w$  ( $p < 0.05$ ) during 10 days of storage, demonstrating that coating application did not affect significantly the  $a_w$  ( $p < 0.05$ ).



Water activity ( $A_w$ ) of coated and no coated fillets over the storage period. Error bars represent the standard deviation of  $n=3$  replicates. A-C Different capital letters indicate significant difference between the same sample during the storage time ( $p < 0.05$ ). a-b Different lower-case letters indicate significant difference between different treatments in the same day of storage ( $p < 0.05$ ).

Regarding the pH value, all samples presented  $pH \leq 6.00$  during 7 days of storage (T7). However, at the end of storage (T12) significant differences ( $p < 0.05$ ) were observed between Sarrajão fillets coated with OE emulsion (OO10 and OO30) and OE emulsion and fish gelatin (FO10) and control sample, as can be observed in the figure.

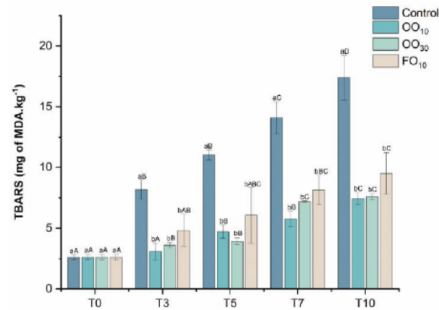


pH value of coated and non coated fillets over the storage period. Errors bars represent the standard deviation of  $n=3$  replicates. A-C Different capital letters indicate significant difference between the same sample during storage time ( $p < 0.05$ ). a-b Different lower-case letters indicate significant difference between different treatments in the same day of storage ( $p < 0.05$ ).

# Oregano oil emulsion based coated fillets

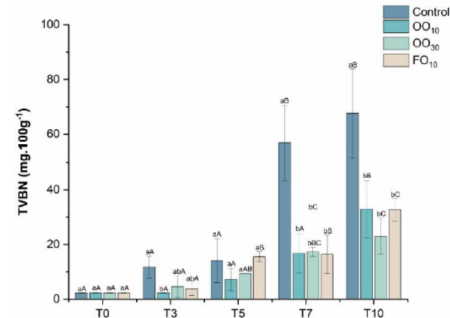
**Lipid and protein oxidation:** OE emulsion-based application in Sarrajão fillets reduced significantly the process of lipid oxidation. All coated Sarrajão fillets presents similar value ( $p < 0.05$ ) of thiobarbituric acid reactive substances, demonstrating the antioxidant potential of oregano oil coating formulations.

The figure presents the total volatile base nitrogen (TVBN) of OE-based coated Sarrajão fillets and control sample. Similar to the lipid oxidation, all coated Sarrajão fillets presents similar value ( $p < 0.05$ ) of TVBN, indicating that the coating application in addition to retard the lipid oxidation, delayed the protein oxidation process.



Thiobarbituric acid reactive substances (TBARS) (mg of malonaldehyde (MDA).kg<sup>-1</sup>) of coated and no coated fillets over the storage period. Error bars represent the standard deviation of n= 3 replicates.

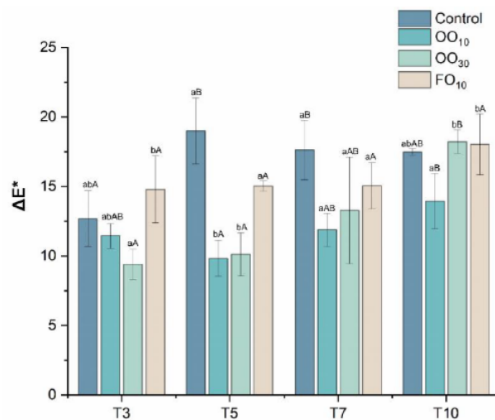
A-C Different capital letters indicate significant difference between the same sample during the storage time ( $p < 0.05$ ). a-b Different lower-case letters indicate significant difference between different treatments in the same day of storage ( $p < 0.05$ ).



Total Volatile Base Nitrogen (TVBN) (mg.100g<sup>-1</sup>) of coated and no coated fillets over the storage period. Errors bars represent the standard deviation of n=3 replicates. A-C Different capital letters indicate significant difference between the same sample during the storage time ( $p < 0.05$ ). a-b Different lower-case letters indicate significant difference between different treatments in the same day of storage ( $p < 0.05$ ).

## Oregano oil emulsion based coated fillets

**Colour changes:** The figure presents the effects of OE based-coating application on the color properties ( $\Delta E^*$ ) of Sarrajão fillets during refrigerated storage. As observed, at the end of storage (T10) all coated samples presented similar ( $p < 0.05$ ) color changes than the control sample.



Color properties ( $\Delta E^*$ ) of coated and no coated fillets over the storage period. Errors bars represent the standard deviation of  $n=3$  replicates. A-C Different capital letters indicate significant difference between the same sample during the storage time ( $p < 0.05$ ). a-b Different lower-case letters indicate significant difference between different treatments in the same day of storage ( $p < 0.05$ ).

# Bluecast: application of by-products from the agri-food industry as natural preservatives for fish-based products

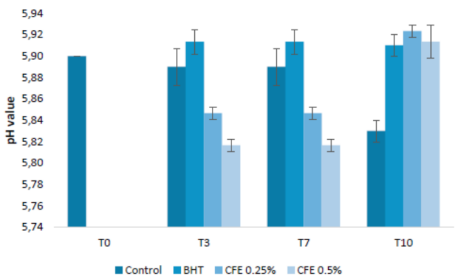
## Shelf-life evaluation

**pH and Aw:** The pH value is a crucial parameter affecting the sensory attributes and microbial stability of fish products. The table summarizes the pH values of different fresh fish burger formulations over the storage period (T0, T3, T7 and T10) at 4 °C. In this study, the initial pH of all formulations was relatively similar, around 5.90. Over the storage period, there were slight fluctuations observed. The control samples exhibited a minor decrease in pH by day 10, reaching 5.83. Interestingly, the addition of BHT at 0.02% and CFE at both 0.25% and 0.5% concentrations maintained pH levels relatively stable throughout the storage period, with no significant differences compared to the initial pH. However, it's worth noting that by day 10, there was a slight increase in pH for the CFE 0.25% and CFE 0.5% groups compared to their initial values. Water activity (aw) is another critical factor influencing microbial growth and chemical reactions in fish products. The initial aw values were quite close among all formulations, ranging from 0.9701 to 0.9724.

Throughout the storage period, there were minor fluctuations observed in aw values for all samples. The control samples exhibited a slight decrease in aw by day 7, followed by a slight increase by day 10, but these changes were not statistically significant ( $p < 0.05$ ). Similarly, the samples with added antioxidants (BHT and CFE) maintained aw values close to their initial levels, with only minor fluctuations observed over the storage period.

pH and water activity (aw) for different fresh fish burger formulations: Control; Butylated hydroxytoluene (BHT) 0.02%; Chestnut Flowers Extract (CFE) 0.25% and 0.5%, stored at 4 °C for 10 days. Values (mean  $\pm$  standard deviation, n =3) followed by different uppercase letter in the same line and lowercase letter in the same column are significantly different ( $D<0.05$ )

Parameter	Sample	Storage (days)			
		0	3	7	10
pH	Control	5.90 $\pm$ 0.00	5.89 $\pm$ 0.02aA	5.89 $\pm$ 0.02aA	5.83 $\pm$ 0.01aB
	BHT		5.91 $\pm$ 0.01aA	5.91 $\pm$ 0.01aA	5.91 $\pm$ 0.01bA
	CFE 0.25%		5.85 $\pm$ 0.01bcA	5.85 $\pm$ 0.01bcA	5.92 $\pm$ 0.01bB
	CFE 0.5%		5.82 $\pm$ 0.01cA	5.82 $\pm$ 0.01cA	5.91 $\pm$ 0.02bB
aw	Control	0.9724 $\pm$ 0.0009	0.9716 $\pm$ 0.0009aA	0.9705 $\pm$ 0.0007aA	0.9716 $\pm$ 0.0007aA
	BHT		0.9701 $\pm$ 0.0006aA	0.9706 $\pm$ 0.0010aA	0.9697 $\pm$ 0.0003bA
	CFE 0.25%		0.9712 $\pm$ 0.0007aA	0.9703 $\pm$ 0.0014aA	0.9704 $\pm$ 0.0004bA
	CFE 0.5%		0.9709 $\pm$ 0.0004aA	0.9700 $\pm$ 0.0009aA	0.9702 $\pm$ 0.0003bA



DH values of fish burger samples over the storage period of 0 (T0), 3 (T3), 7 (T7) and 10 (T10) days at 4°C. Control: Butylated hydroxytoluene (BHT) 0.02%; Chestnut Flowers Extract (CFE) 0.25% and 0.5%. Columns represent the mean (n =31), and the bars represent the standard deviation.

## Shelf-life evaluation

**Lipid and protein oxidation:** Total volatile basic nitrogen (TVB-N) is known as a product of bacterial spoilage and endogenous enzyme action, and its level is often used as an index to evaluate fish quality. The levels of these compounds, which increase with the onset of microbial spoilage, are primarily responsible for the fishy odours, which increase as spoilage proceeds. The table presents the results of TVB-N for different formulations of fresh fish burgers stored at 4°C for 10 days. In terms of TVB-N levels, the control samples show a significant increase ( $p < 0.05$ ) over the storage period, reaching 21.05 mg N/100g by day 10, indicating substantial protein degradation. However, the addition of 0.02% BHT appears to effectively limit this increase, keeping TVB-N levels significantly lower throughout the storage period compared to control samples. Likewise, the addition of 0.25% CFE also shows an attenuating effect on TVB-N accumulation, although not as pronounced as BHT. On the other hand, the addition of CFE at a concentration of 0.5% resulted in lower TVB-N values, keeping the values constant throughout the storage period.

At the end of storage (10 days), the control samples had a higher value of TVB-N. The TVB-N content of all the samples did not exceed the maximum level for acceptability for marine fish (i.e., 35 mg N/100g) [7] at day 10.

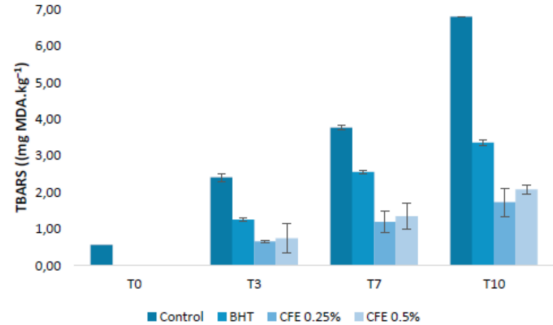
Lipid oxidation is one of the major factors that cause fish products quality loss, off-flavours, and discoloration. Accelerated by factors such as oxygen, iron, light and temperature, the lipid oxidation in meat is commonly evaluated by the TBARS assay, which measures the malonaldehyde (MDA) content (a main secondary product formed in the process). The TBARS values of samples expressed as MDA content (mg.kg<sup>-1</sup>) over the storage period at 4 °C.

The control samples exhibit a sharp increase in TBARS levels over the storage period, reaching 6.80 mg/kg by day 10. However, the addition of antioxidants, particularly BHT (synthetic) and CFE (natural) at both concentrations, effectively limits lipid oxidation, as evidenced by significantly lower TBARS levels compared to the control group throughout the storage period.

These results suggest that both BHT and CFE have potential as antioxidants in preserving the quality of fresh fish burgers during refrigerated storage. However, it's worth noting that while CFE shows effectiveness in limiting both protein degradation and lipid oxidation, BHT appears to be more potent in preserving protein integrity, especially at the lower concentration of 0.02%.

# Shelf-life evaluation

## Lipid and protein oxidation:



Lipid oxidation values of fish burger samples over the storage period of 0 (T0), 3 (T3), 7 (T7) and 10 (T10) days at 4°C. Control; Butylated hydroxytoluene (BHT) 0.02%; Chestnut Flowers Extract (CFE) 0.25% and 0.5%. Columns represent the mean (n = 3), and the bars represent the standard deviation.

Parameter	Sample	Storage time (days)			
		0	3	7	10
TVBN <sup>1</sup>	Control	2.38±0.00	16.38±0.00aA	16.38±0.00aA	21.05±4.04aA
	BHT		2.38±0.00bA	4.71±4.04bcdA	4.71±4.04bA
	CFE 0.25%		7.05±4.04bA	9.38±0.00bcA	9.38±0.00bA
	CFE 0.5%		2.38±0.00bA	2.38±0.00bdA	2.38±0.00bA
TBARS <sup>2</sup>	Control	0.57±0.18	2.42±0.41aC	3.77±0.37aB	6.80±0.13aA
	BHT		1.26±0.03bC	2.56±0.28bB	3.36±0.39bA
	CFE 0.25%		0.66±0.04cC	1.20±0.04cB	1.73±0.08cA
	CFE 0.5%		0.75±0.11bcC	1.35±0.07cB	2.08±0.01cA

<sup>1</sup>TVBN expressed as milligram of Nitrogen per 100 grams of fresh fish burger

<sup>2</sup>TBARS expressed as milligram of Malonaldehyde per kilogram of fresh fish burger

Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric acid reactive substances (TBARS) for different fresh fish burgers formulation: Control; Butylated hydroxytoluene (BHT) 0.02%; Chestnut Flowers Extract (CFE) 0.25% and 0.5%, stored at 4°C for 10 days. Values (mean ± standard deviation, n=3) followed by different uppercase letter in the same line and lowercase letter in the same column are significantly different (p < 0.05).

## Shelf-life evaluation

**Texture parameters:** texture parameters including hardness, resilience, cohesion, springiness, gumminess, and chewiness, were assessed for different fish burger formulations over a storage period of 10 days at 4°C are shown in the table.

Hardness, an important textural attribute, exhibited variations across formulations and storage times. Initially, the control samples showed a hardness of  $28.31 \pm 0.33$  kg, with slight fluctuations over the storage period, reaching  $33.27 \pm 3.14$  kg by day 10. Notably, formulations with BHT and CFE at both concentrations generally displayed comparable or slightly lower hardness values compared to the control samples. It is also possible to notice an increase in this parameter in samples enriched with CFE at both concentrations throughout the storage period.

In this study, the resilience of the fish burger was also evaluated. This parameter is an indicative of the burger's ability to recover its shape after deformation, remained relatively stable across formulations and storage times. The control samples exhibited resilience values ranging from 8.61% to 5.79% over the 10-day storage period. Similarly, formulations with BHT and CFE 0.25% and 0.5% maintained resilience within similar ranges, suggesting minimal impact on the burger's elastic properties. However, there is a tendency for this parameter to reduce over storage time.

Cohesion and springiness, defining the internal strength and elasticity of the burger, respectively, showed moderate variations. While the control samples displayed cohesion values between 0.28 to 0.32 and springiness values between 56.86% to 63.47% over the storage

period, formulations with different preservatives showed slight deviations, especially noticeable in the CFE 0.5% samples, which exhibited increased springiness compared to other formulations by day 10.

Gumminess is calculated using hardness and cohesiveness, representing the energy required to disintegrate the burger for swallowing, and chewiness, combining hardness, cohesiveness, and springiness, demonstrated notable fluctuations. Formulations with BHT and CFE 0.25% exhibited comparable gumminess and chewiness values, indicating slight alteration in these textural attributes. On the other hand, greater attention is drawn to the high values for the 0.5% CFE treatment after 7 and 10 days of storage.

In general, the addition of chestnut flowers extract in both concentrations (0.25 and 0.5%) as natural preservative in the fish burger formulations appears to have a stabilizing effect on pH, water activity, lipid, protein oxidation and texture parameters during refrigerated storage. These findings suggest that the phenolic-rich extracts from chestnut by-product may contribute to maintaining the physicochemical stability of the fish burgers, which could potentially enhance their shelf life and overall quality.

Further studies could explore the mechanisms underlying the antioxidant effects of chestnut flowers extract in fish burgers and optimize their concentrations for enhanced shelf life extension and quality preservation. Additionally, sensory evaluation could provide valuable insights into the organoleptic properties of the fish burgers formulated with these natural preservatives.

## Shelf-life evaluation

Storage time (days)	Sample	Hardness (kg)	Resilience (%)	Cohesion	Springiness (%)	Gumminess	Chewiness
0	Control	28.31 ± 0.33	8.61 ± 0.53	0.31 ± 0.004	66.74 ± 2.78	8.76 ± 1.00	5.85 ± 0.72
	Control	28.66 ± 0.38a	8.75 ± 0.53a	0.32 ± 0.01ab	56.86 ± 1.06ab	9.17 ± 0.11a	5.21 ± 0.16a
3	BHT	25.44 ± 2.17b	8.25 ± 0.26a	0.32 ± 0.02a	60.69 ± 3.36a	8.11 ± 0.38bc	4.92 ± 0.39a
	CFE 0.25%	22.84 ± 1.31bc	7.29 ± 0.64b	0.29 ± 0.01b	49.33 ± 2.63b	6.54 ± 0.18d	3.23 ± 0.25b
	CFE 0.5%	26.50 ± 0.99ab	7.70 ± 0.18a	0.31 ± 0.01ab	56.89 ± 5.43ab	8.12 ± 0.23b	4.61 ± 0.33a
	Control	28.98 ± 3.69ab	7.71 ± 0.37a	0.29 ± 0.01ab	58.70 ± 4.55a	8.48 ± 1.35ab	4.94 ± 0.48b
7	BHT	26.79 ± 2.25ab	7.39 ± 0.53a	0.28 ± 0.01b	61.97 ± 6.91a	7.53 ± 1.01b	4.66 ± 0.72b
	CFE 0.25%	24.72 ± 0.42b	7.21 ± 0.33a	0.28 ± 0.02b	60.31 ± 1.75a	6.89 ± 0.45b	4.15 ± 0.16b
	CFE 0.5%	33.15 ± 2.00a	7.58 ± 0.77a	0.32 ± 0.01a	65.79 ± 1.62a	10.73 ± 0.89a	7.06 ± 0.64a
	Control	33.27 ± 3.14a	5.79 ± 0.13a	0.28 ± 0.01a	59.32 ± 3.00a	9.16 ± 0.97b	5.45 ± 0.83a
10	BHT	35.38 ± 2.07a	5.04 ± 0.28b	0.29 ± 0.01a	60.41 ± 2.24a	10.27 ± 0.67a	6.19 ± 0.27a
	CFE 0.25%	31.79 ± 3.63a	5.30 ± 0.28b	0.29 ± 0.02a	63.47 ± 10.07a	9.25 ± 1.13ab	5.88 ± 1.18a
	CFE 0.5%	41.08 ± 6.00a	4.35 ± 0.08c	0.30 ± 0.02a	87.18 ± 37.60a	12.55 ± 2.01a	10.74 ± 5.34a

Texture parameters (hardness, adhesiveness, resilience, cohesion, springiness, gumminess, and chewiness) obtained for the different fish burger formulations stored at 4°C for 10 days. Values (mean ± standard deviation, n = 3).

Different letters show significant differences ( $p < 0.05$ ) for the same Control; Butylated hydroxytoluene (BHT) 0.02%; Chestnut Flowers Extract (CFE) 0.25% and 0.5% samples over the storage period (0, 3, 7 and 10 days).

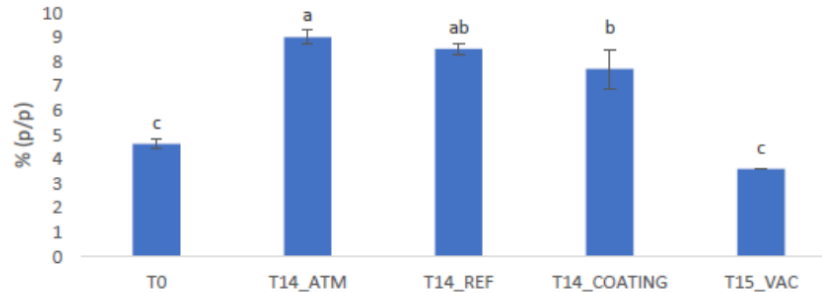


# Evaluation of preservation techniques (vacuum, modified atmosphere, refrigeration, coating) on Sarrajão Burger Properties over 15 days of storage time

## Physicochemical properties

The figure shows the lipid content of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C.

The lipid content of the fillets at Time 0 days was  $4.63 \pm 0.17$  %, after 15 days subjected to the different preservation techniques the value of the lipid content is  $9.02 \pm 0.28$  % for the sample in modified atmosphere,  $8.52 \pm 0.24$  % for the sample in refrigeration,  $7.71 \pm 0.79$  % for the sample with coating,  $3.62 \pm 0.02$  % for the sample in vacuum.

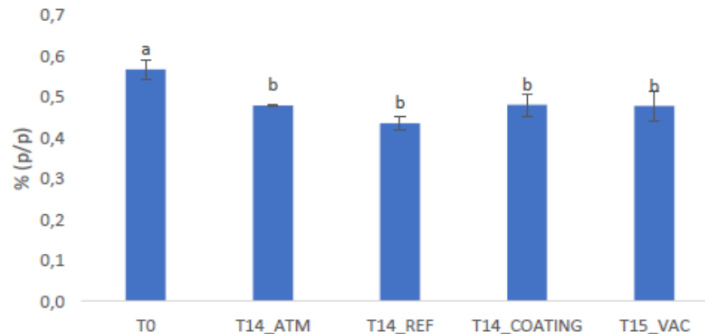


Lipid content of fillets during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum, respectively. Mean values  $\pm$  standard deviation (n-3). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the chloride content of Sarrajão fillets during 15 days of storage in a modified atmosphere, refrigeration, coating, and vacuum at 4°C.

It is possible to observe that the chloride content at Time 0 days is  $0.57 \pm 0.02$  % and, after 15 days of storage in the various techniques indicated, the values are  $0.48 \pm 0.00$  % for the sample in modified atmosphere,  $0.44 \pm 0.02$  % for the sample in refrigeration,  $0.48 \pm 0.03$  % for the sample with coating,  $0.48 \pm 0.04$  % for the sample in vacuum.

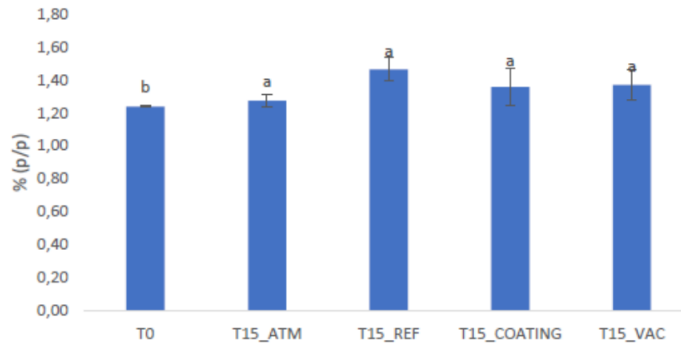


Chloride content of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating and vacuum, respectively.  
Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the ash content of Sarrajão fillets during 15 days of storage in a modified atmosphere, refrigeration, coating and vacuum at 4°C.

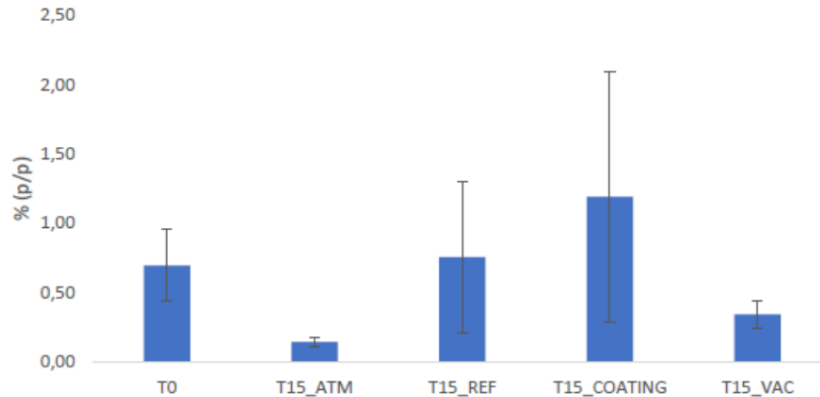
The ash content at Time 0 days is  $1.24 \pm 0.01$  % and after 15 days of storage in the different techniques, the values are  $1.27 \pm 0.04$  % for the sample in modified atmosphere,  $1.46 \pm 0.07$  % for the sample in refrigeration,  $1.36 \pm 0.011$  % for the sample with coating,  $1.37 \pm 0.09$  % for the sample in vacuum.



Ash content Of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, and vacuum, respectively. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the fibre content of Sarrajão fillets during 15 days of storage in a modified atmosphere, refrigeration, coating, and vacuum at 4°C. Fibre content at Time 0 days is  $0.69 \pm 0.26$  % and after 15 days of storage in the different techniques, the values are  $0.14 \pm 0.03$  % for the sample in modified atmosphere,  $0.76 \pm 0.55$  % for the sample in refrigeration,  $1.19 \pm 0.90$  % for the sample with coating,  $0.34 \pm 0.10$  % for the sample in vacuum.

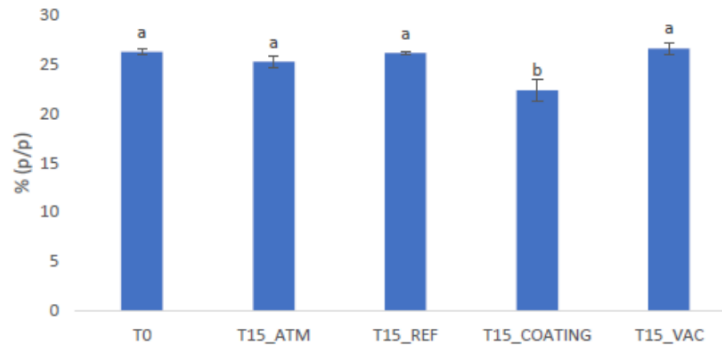


Fibre content of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating and vacuum, respectively.

## Physicochemical properties

The figure shows the protein content of Sarrajão fillets during 15 days of storage in a modified atmosphere, refrigeration, coating, and vacuum at 4°C.

At Time 0 days, the protein content was  $26.26 \pm 0.27$  % and after 15 days of storage in the different techniques the values were  $25.24 \pm 0.56$  % for the modified atmosphere sample,  $26.14 \pm 0.11$  % for the refrigerated sample,  $22.34 \pm 1.09$  % for the coated sample and  $26.60 \pm 0.55$  % for the vacuum sample.

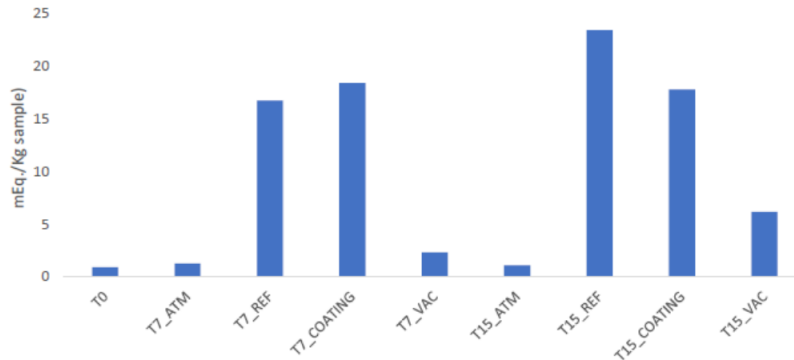


Protein content of fillets during 15 days of storage in modified atmosphere, refrigeration, coating and vacuum, respectively. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the peroxide content of Sarrajão fillets during 15 days of storage in a modified atmosphere, refrigeration, coating and vacuum at 4°C.

It is possible to observe that the peroxide content at Time 0 days is 0.93 (mEq./Kg sample) and, after 7 days of storage in the different techniques the values are, 1.26 (mEq./Kg sample) for the sample in modified atmosphere, 16.72 (mEq./Kg sample) for the sample in refrigeration, 18.41 (mEq./Kg sample) for the sample with coating, 2.31 (mEq. /Kg sample) for the vacuum sample and, after 15 days, the values are: 1.10 (mEq./Kg sample) for the sample in modified atmosphere, 23.40 (mEq./Kg sample) for the sample in refrigeration, 17.77 (mEq./Kg sample) for the sample with coating, 6.15 (mEq./Kg sample) for the vacuum sample.

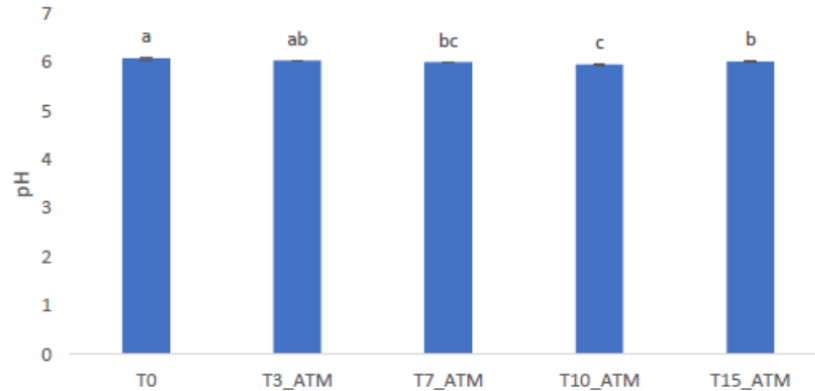


Peroxide content of Sarrajão fillets during 15 days of storage in modified atmosphere, coating and vacuum respectively.

## Physicochemical properties

The figure shows the pH value of Sarrajão fillets during 15 days of storage in a modified atmosphere.

The pH value at Time 0 days is  $6.08 \pm 0.03$ , after 3 days of storage it is  $6.04 \pm 0.01$ , after 7 days it is  $6.01 \pm 0.00$ , after 10 days it is  $5.96 \pm 0.01$  and after 15 days it is  $6.02 \pm 0.01$ .

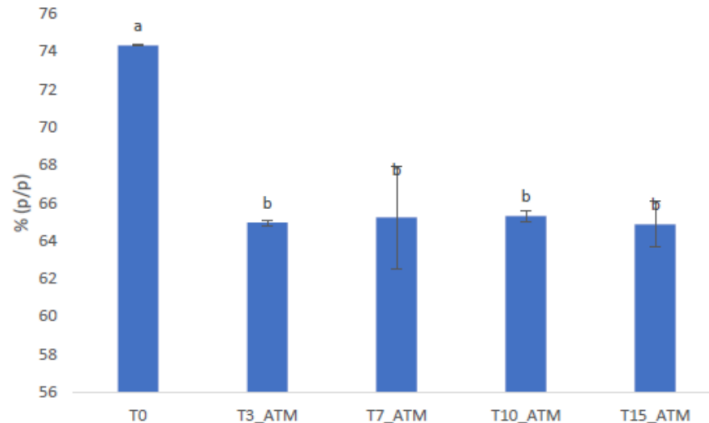


pH value of Sarrajão fillets during 15 days of storage in a modified atmosphere at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the moisture content of Sarrajão fillets during 15 days of storage in a modified atmosphere.

At Time 0, the moisture value was  $74.33 \pm 0.05$  %, after 3 days of storage it was  $64.95 \pm 0.13$  %, after 7 days it was  $65.23 \pm 2.72$  %, after 10 days it was  $65.30 \pm 0.28$  % and after 15 days it was  $64.87 \pm 1.21$  %.



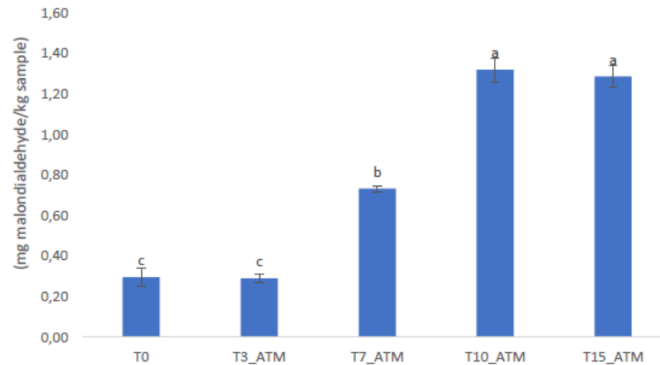
Moisture content of Sarrajão fillets during 15 days of storage in a modified atmosphere at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .



## Physicochemical properties

The figure shows the thiobarbituric acid reactive substances (TBARS) value of Sarrajão fillets during 15 days of storage in a modified atmosphere.

It is possible to observe that the value of TBARS at Time 0 days is  $0.30 \pm 0.05$  mg malondialdehyde/kg sample, after 3 days of storage the value is  $0.29 \pm 0.02$  mg malondialdehyde/kg sample after 7 days the value is  $0.73 \pm 0.02$  mg malondialdehyde/kg sample, after 10 days the value is  $1.32 \pm 0.06$  mg malondialdehyde/kg sample and after 15 days the value is  $1.29 \pm 0.06$  mg malondialdehyde/kg sample.

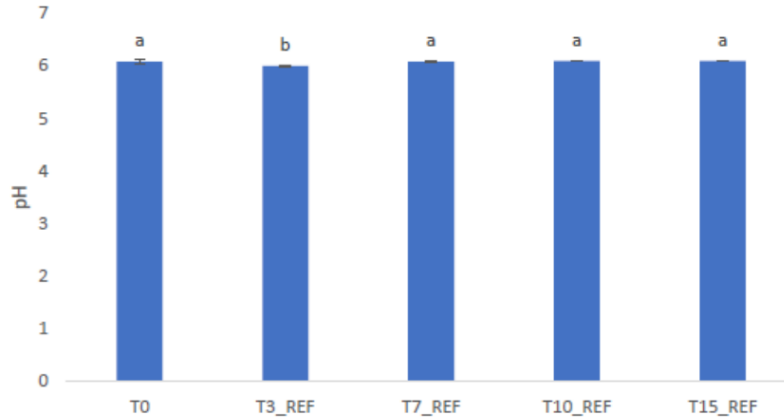


Thiobarbituric acid reactive substances (TBARS) of fillets during 15 days of storage in a modified atmosphere at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the pH value of Sarrajão fillets during 15 days of storage in refrigeration (4°C).

The pH value at Time 0 days is  $6.08 \pm 0.03$ , after 3 days of storage it is  $6.00 \pm 0.01$ , after 7 days it is  $6.08 \pm 0.01$ , after 10 days it is  $6.10 \pm 0.00$  and after 15 days it is  $6.09 \pm 0.00$ .

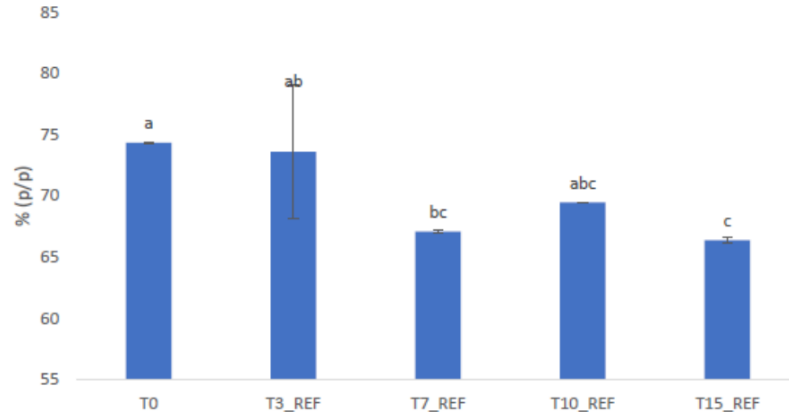


pH value of Sarrajão fillets during 15 days of storage in refrigeration (4°C). Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the moisture content of Sarrajão fillets during 15 days of storage in refrigeration (4°C).

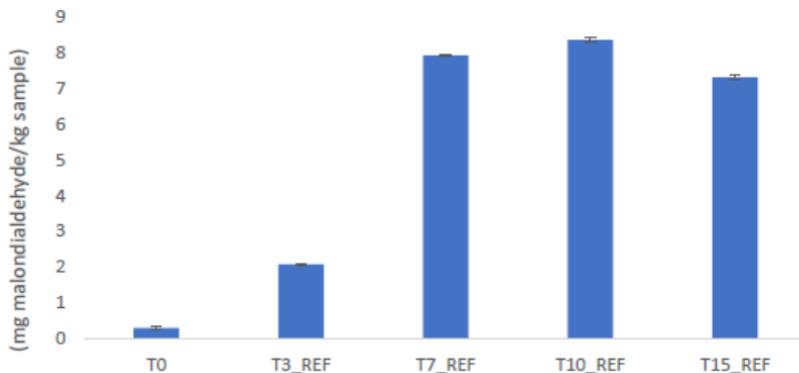
At Time 0, the moisture value was  $74.33 \pm 0.05$  %, after 3 days of storage it was  $73.61 \pm 5.47$  %, after 7 days it was  $67.07 \pm 0.13$  %, after 10 days it was  $69.43 \pm 0.04$  % and after 15 days it was  $66.38 \pm 0.18$  %.



Moisture content of Sarrajão fillets during 15 days of storage in refrigeration (4°C). Mean values  $\pm$  standard deviation (n=3). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the thiobarbituric acid reactive substances (TBARs) value of Sarrajão fillets during 15 days of storage in refrigeration (4°C). It is possible to observe that the value of TBARs at Time 0 days is  $0.30 \pm 0.05$  mg malondialdehyde/kg sample, after 3 days of storage the value is  $2.08 \pm 0.08$  mg malondialdehyde/kg sample after 7 days the value is  $7.93 \pm 0.06$  mg malondialdehyde/kg sample, after 10 days the value is  $8.37 \pm 0.08$  mg malondialdehyde/kg sample and after 15 days the value is  $7.32 \pm 0.10$  mg malondialdehyde/kg sample.

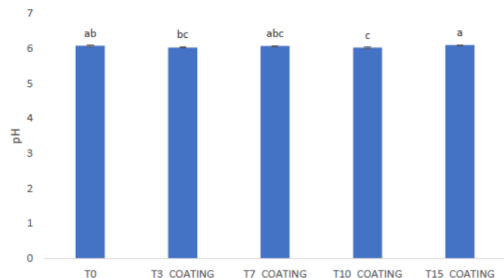


Thiobarbituric acid reactive substances (TBARS) of Sarrajão fillets during 15 days of storage in refrigeration (4°C). Mean values  $\pm$  standard deviation (n=3). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

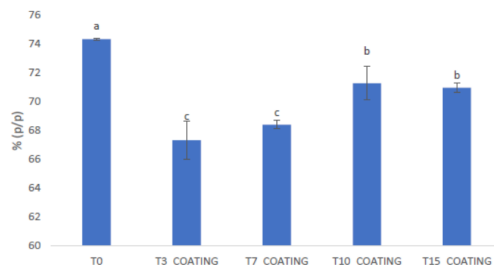
The figure shows the pH value of coated Sarrajão fillets during 15 days of storage at (4°C).

The pH value at Time 0 days is  $6.08 \pm 0.03$ , after 3 days of storage it is  $6.03 \pm 0.01$ , after 7 days it is  $6.08 \pm 0.01$ , after 10 days it is  $6.03 \pm 0.02$  and after 15 days it is  $6.10 \pm 0.01$ .



pH value of coated Sarrajão fillets during 15 days of storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

At Time 0, the moisture value was  $74.33 \pm 0.05$  %, after 3 days of storage it was  $67.32 \pm 1.32$  %, after 7 days it was  $68.40 \pm 0.29$  %, after 10 days it was  $71.27 \pm 1.15$  % and after 15 days it was  $70.96 \pm 0.32$  %.

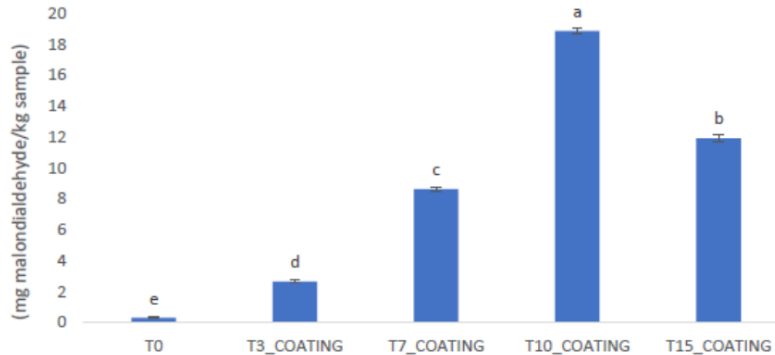


Moisture content of coated Sarrajão fillets during 15 days of storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the thiobarbituric acid reactive substances (TBARS) value of coated Sarrajão fillets during 15 days of storage at (4°C).

It is possible to observe that the value of TBARS at Time 0 days is  $0.30 \pm 0.05$  mg malondialdehyde/kg sample, after 3 days of storage the value is  $2.66 \pm 0.11$  mg malondialdehyde/kg sample after 7 days the value is  $8.63 \pm 0.12$  mg malondialdehyde/kg sample, after 10 days the value is  $18.85 \pm 0.17$  mg malondialdehyde/kg sample and after 15 days the value is  $11.92 \pm 0.22$  mg malondialdehyde/kg sample.

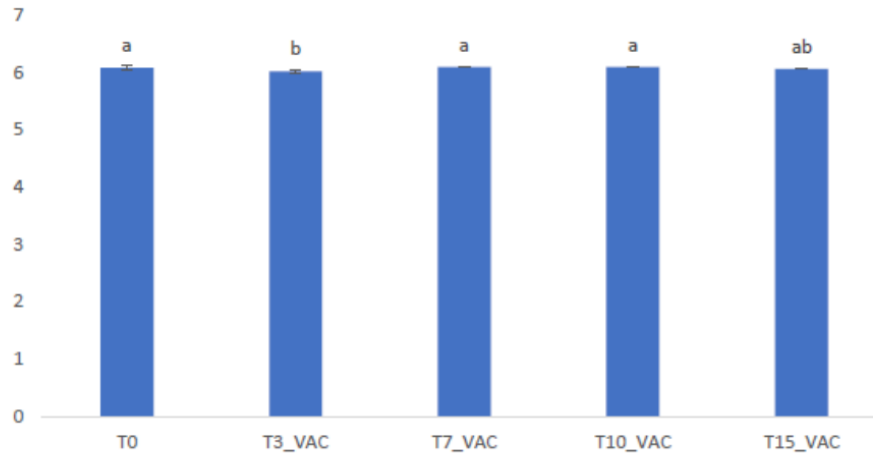


Thiobarbituric acid reactive substances (TBARS) of coated Sarrajão fillets during 15 days of storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the pH value of Sarrajão fillets during 15 days of vacuum storage at 4°C.

The pH value at Time 0 days is  $6.08 \pm 0.03$ , after 3 days of storage it is  $6.02 \pm 0.03$ , after 7 days it is  $6.10 \pm 0.01$ , after 10 days it is  $6.10 \pm 0.01$  and after 15 days it is  $6.06 \pm 0.00$ .

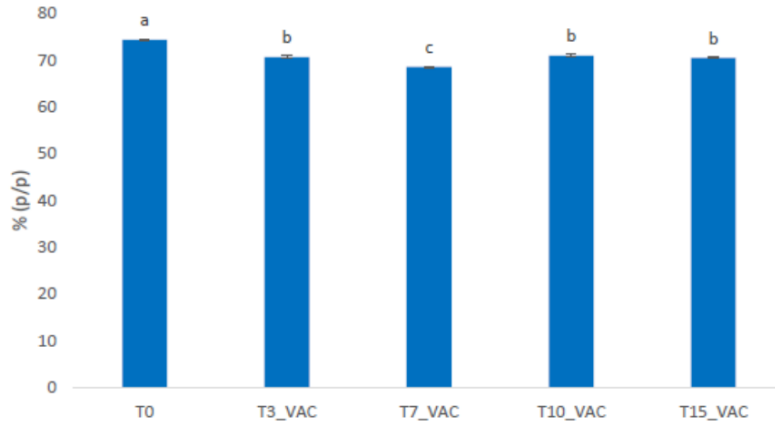


pH value of Sarrajão fillets during 15 days of vacuum storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Physicochemical properties

The figure shows the moisture content of Sarrajão fillets during 15 days of vacuum storage at 4°C.

At Time 0, the moisture value was  $74.33 \pm 0.05$  %, after 3 days of storage it was  $70.65 \pm 0.31$  %, after 7 days it was  $68.54 \pm 0.13$  %, after 10 days it was  $70.96 \pm 0.32$  % and after 15 days it was  $70.50 \pm 0.16$  %.



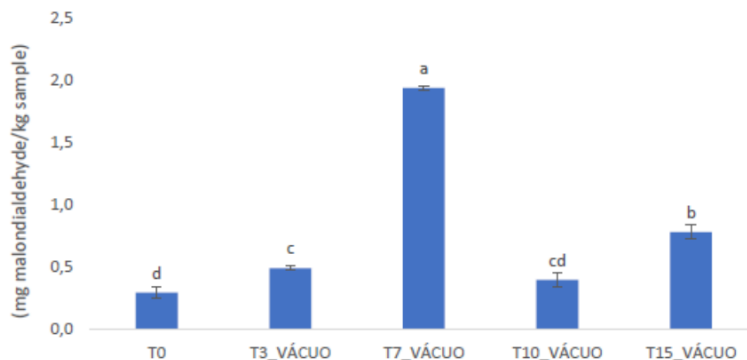
Moisture content of Sarrajão fillets during 15 days of vacuum at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .



## Physicochemical properties

The figure shows the thiobarbituric acid reactive substances (TBARs) value of Sarrajão fillets during 15 days of vacuum storage at 4°C.

It is possible to observe that the value of TBARs at Time 0 days is  $0.30 \pm 0.05$  mg malondialdehyde/kg sample, after 3 days of storage the value is  $0.49 \pm 0.05$  mg malondialdehyde/kg sample after 7 days the value is  $1.94 \pm 0.07$  mg malondialdehyde/kg sample, after 10 days the value is  $0.40 \pm 0.04$  mg malondialdehyde/kg sample and after 15 days the value is  $0.78 \pm 0.06$  mg malondialdehyde/kg sample.

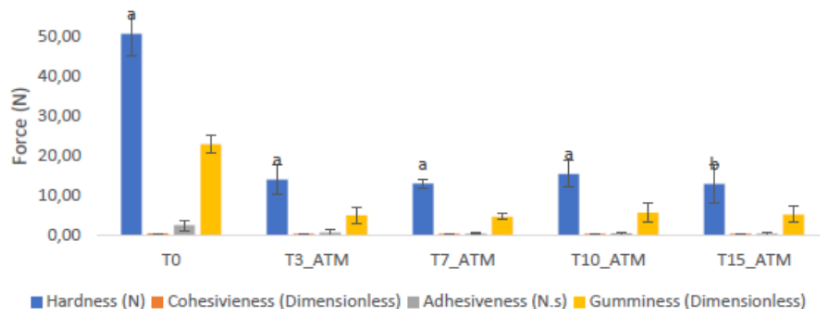


Thiobarbituric acid reactive substances (TBARS) of Sarrajão fillets during 15 days of vacuum storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Texture properties

Texture parameters of Sarrajão fillets samples during 15 days of storage in a modified atmosphere at 4°C are shown in the figure.

At Time 0 the hardness of the Sarrajão fillets was  $50.59 \pm 5.49$  N, the cohesiveness was  $0.45 \pm 0.01$ , the adhesiveness was  $2.52 \pm 1.32$  N.s and the gumminess was  $22.79 \pm 2.16$ , after 3 days of storage the hardness value was  $13.98 \pm 3.73$  N, the cohesiveness was  $0.35 \pm 0.06$ , the adhesiveness was  $0.69 \pm 0.63$  N.s and the gumminess was  $3.73 \pm 1.91$ , after 7 days of storage the hardness value was  $12.92 \pm 1.07$  N, cohesiveness was  $0.36 \pm 0.05$ , adhesiveness was  $0.41 \pm 0.19$  N.s and gumminess was  $4.66 \pm 0.72$ , after 10 days of storage the hardness value was  $15.36 \pm 3.36$  N, cohesiveness was  $0.37 \pm 0.12$ , adhesiveness was  $0.46 \pm 0.41$  N.s and gumminess was  $5.65 \pm 2.38$ , after 15 days of storage, hardness was  $12.86 \pm 4.71$  N, cohesiveness was  $0.41 \pm 0.11$ , adhesiveness was  $0.35 \pm 0.23$  N.s and gumminess was  $5.18 \pm 2.06$ .

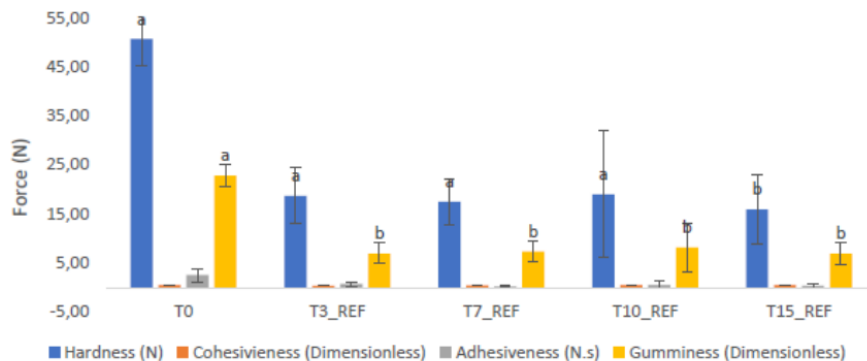


Texture parameters (hardness, cohesiveness, adhesiveness, and gumminess) obtained for the Sarrajão fillets during 15 days of storage in a modified atmosphere at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$

## Texture properties

Texture parameters of Sarrajão fillets samples during 15 days of storage in refrigeration at 4°C are shown in the figure.

At Time 0 the hardness of the Sarrajão fillets was  $50.59 \pm 5.49$  N, the cohesiveness was  $0.45 \pm 0.01$ , the adhesiveness was  $2.52 \pm 1.32$  N.s and the gumminess was  $22.79 \pm 2.16$ , after 3 days of storage the hardness value was  $18.69 \pm 5.66$  N, the cohesiveness was  $0.37 \pm 0.03$ , the adhesiveness was  $0.71 \pm 0.47$  N.s and the gumminess was  $6.92 \pm 2.10$ , after 7 days of storage the hardness value was  $17.44 \pm 4.62$  N, cohesiveness was  $0.42 \pm 0.08$ , adhesiveness was  $0.18 \pm 0.14$  N.s and gumminess was  $7.30 \pm 2.18$ , after 10 days of storage the hardness value was  $18.98 \pm 12.86$  N, cohesiveness was  $0.45 \pm 0.04$ , adhesiveness was  $0.55 \pm 0.67$  N.s and gumminess was  $8.13 \pm 4.90$ , after 15 days of storage, hardness was  $15.87 \pm 7.10$  N, cohesiveness was  $0.46 \pm 0.08$ , adhesiveness was  $0.29 \pm 0.44$  N.s and gumminess was  $6.92 \pm 2.17$ .

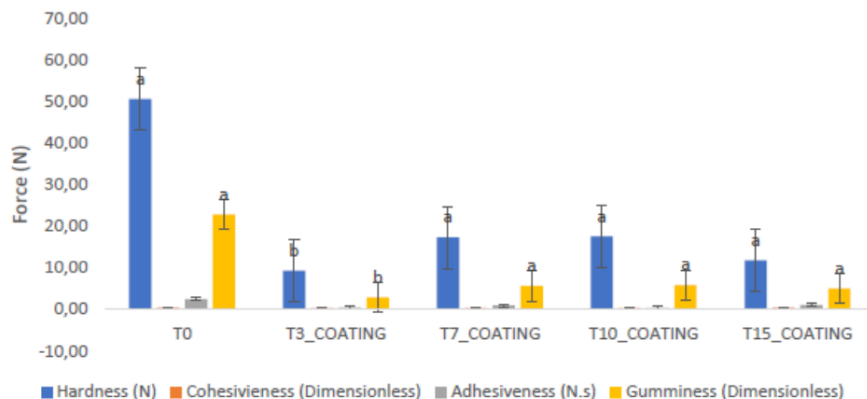


Texture parameters (hardness, cohesiveness, adhesiveness, and gumminess) obtained for the Sarrajão fillets during 15 days of storage in refrigeration at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Texture properties

Texture parameters of coated Sarrajão fillets during 15 days of storage at 4°C are shown in the figure.

At Time 0 the hardness of the Sarrajão fillets was  $50.59 \pm 5.49$  N, the cohesiveness was  $0.45 \pm 0.01$ , the adhesiveness was  $2.52 \pm 1.32$  N.s and the gumminess was  $22.79 \pm 2.16$ , after 3 days of storage the hardness value was  $9.35 \pm 1.77$  N, the cohesiveness was  $0.32 \pm 0.04$ , the adhesiveness was  $0.46 \pm 0.33$  N.s and the gumminess was  $2.96 \pm 0.69$ , after 7 days of storage the hardness value was  $17.34 \pm 8.67$  N, cohesiveness was  $0.37 \pm 0.11$ , adhesiveness was  $0.94 \pm 1.15$  N.s and gumminess was  $5.65 \pm 1.19$ , after 10 days of storage the hardness value was  $17.60 \pm 4.74$  N, cohesiveness was  $0.35 \pm 0.06$ , adhesiveness was  $0.36 \pm 0.25$  N.s and gumminess was  $5.87 \pm 0.84$ , after 15 days of storage, hardness was  $11.79 \pm 3.20$  N, cohesiveness was  $0.42 \pm 0.06$ , adhesiveness was  $1.03 \pm 0.13$  N.s and gumminess was  $4.99 \pm 1.54$ .

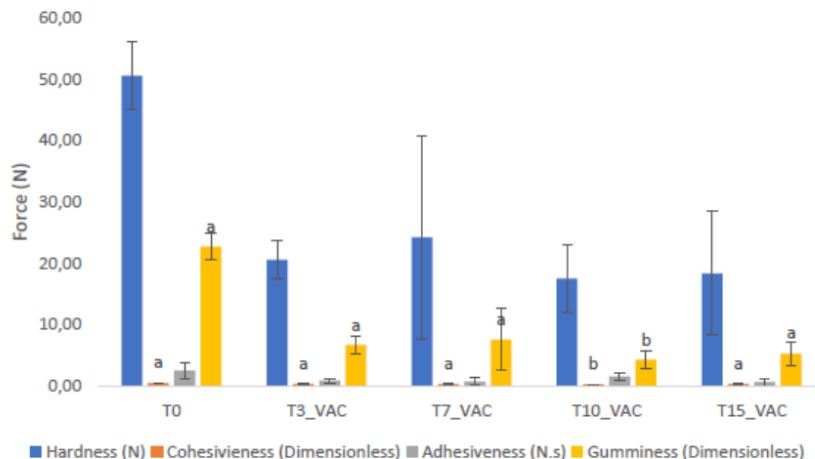


Texture parameters (hardness, cohesiveness, adhesiveness, and gumminess) obtained for the coated Sarrajão fillets during 15 days of storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Texture properties

Texture parameters of Sarrajão fillets during 15 days of vacuum storage at 4°C. are shown in the figure.

At Time 0 the hardness of the Sarrajão fillets was  $50.59 \pm 5.49$  N, the cohesiveness was  $0.45 \pm 0.01$ , the adhesiveness was  $2.52 \pm 1.32$  N.s and the gumminess was  $22.79 \pm 2.16$ , after 3 days of storage the hardness value was  $20.58 \pm 3.15$  N, the cohesiveness was  $0.32 \pm 0.04$ , the adhesiveness was  $0.86 \pm 0.34$  N.s and the gumminess was  $6.67 \pm 1.49$ , after 7 days of storage the hardness value was  $24.26 \pm 16.57$  N, cohesiveness was  $0.32 \pm 0.05$ , adhesiveness was  $0.72 \pm 0.58$  N.s and gumminess was  $7.52 \pm 5.04$ , after 10 days of storage the hardness value was  $17.48 \pm 5.63$  N, cohesiveness was  $0.24 \pm 0.03$ , adhesiveness was  $1.51 \pm 0.61$  N.s and gumminess was  $4.27 \pm 1.51$ , after 15 days of storage, hardness was  $18.38 \pm 10.14$  N, cohesiveness was  $0.63 \pm 0.09$ , adhesiveness was  $5.28 \pm 0.62$  N.s and gumminess was  $10.14 \pm 1.87$ .

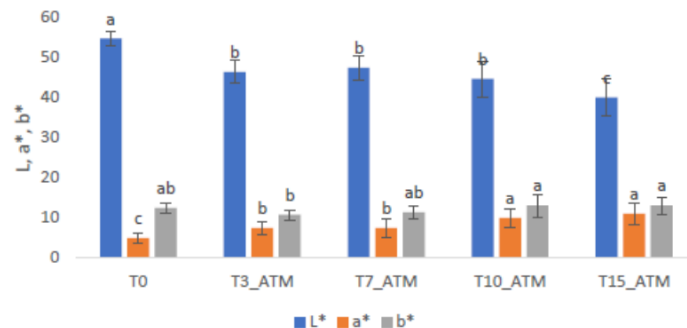


Texture parameters (hardness, cohesiveness, adhesiveness, and gumminess) obtained for the Sarrajão fillets during 14 days of vacuum storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Colour properties

Colour properties of Sarrajão fillets samples during 15 days of storage in a modified atmosphere at 4°C are shown in the figure.

At Time 0 the L parameter of the Sarrajão fillets was  $54.68 \pm 1.94$ , the  $a^*$  was  $4.82 \pm 1.10$ , the  $b^*$  was  $12.42 \pm 1.21$ , after 3 days of storage the L value was  $46.34 \pm 2.83$ , the  $a^*$  was  $7.43 \pm 1.59$  and the  $b^*$  was  $10.69 \pm 1.21$ , after 7 days of storage the L value was  $47.42 \pm 3.11$ , the  $a^*$  was  $7.41 \pm 2.27$  and the  $b^*$  was  $11.35 \pm 1.65$ , after 10 days of storage the L value was  $44.62 \pm 4.50$ , the  $a^*$  was  $9.84 \pm 2.20$  and the  $b^*$  was  $12.97 \pm 2.83$ , after 15 days of storage, the L value was  $42.02 \pm 4.58$  N, the  $a^*$  was  $10.98 \pm 2.68$  and the  $b^*$  was  $12.98 \pm 2.12$  N.s.

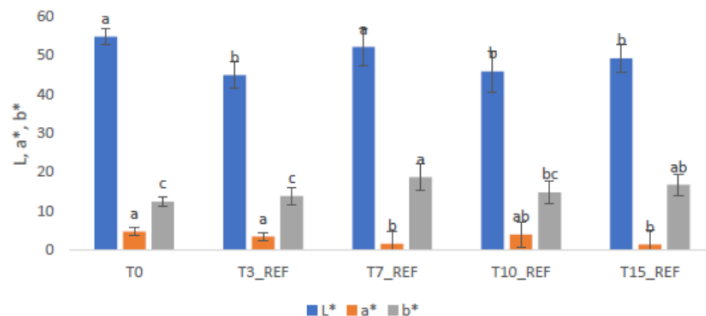


Color properties (L,  $a^*$ ,  $b^*$ ) of Sarrajão fillets during 15 days of storage in a modified atmosphere at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $D < 0.05$ .

## Colour properties

Colour properties of Sarrajão fillets samples during 15 days of storage in refrigeration at 4°C are shown in the figure.

At Time 0 the L parameter of the Sarrajão fillets was  $54.68 \pm 1.94$ , the  $a^*$  was  $4.82 \pm 1.10$ , the  $b^*$  was  $12.42 \pm 1.21$ , after 3 days of storage the L value was  $44.86 \pm 3.45$ , the  $a^*$  was  $3.51 \pm 0.93$  and the  $b^*$  was  $13.83 \pm 2.34$ , after 7 days of storage the L value was  $52.03 \pm 4.85$ , the  $a^*$  was  $1.66 \pm 3.23$  and the  $b^*$  was  $18.68 \pm 3.30$ , after 10 days of storage the L value was  $45.77 \pm 5.40$ , the  $a^*$  was  $4.02 \pm 3.21$  and the  $b^*$  was  $14.82 \pm 2.88$ , after 15 days of storage, the L value was  $49.07 \pm 3.59$  N, the  $a^*$  was  $1.44 \pm 3.55$  and the  $b^*$  was  $16.71 \pm 2.74$  N.s.

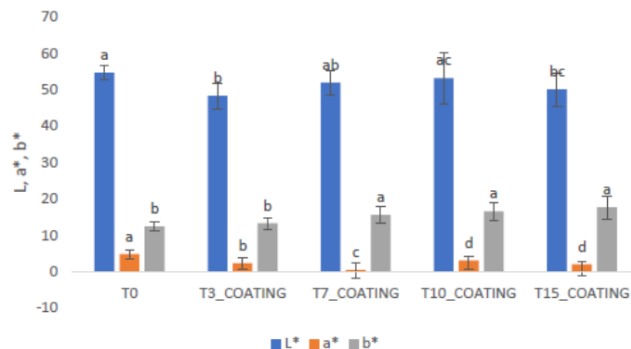


Color properties (L,  $a^*$ ,  $b^*$ ) of Sarrajão fillets during 15 days of storage in refrigeration at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

## Colour properties

Colour properties of coated Sarrajão fillets during 15 days of storage at 4°C are shown in the figure.

At Time 0 the L parameter of the Sarrajão fillets was  $54.68 \pm 1.94$ , the  $a^*$  was  $4.82 \pm 1.10$ , the  $b^*$  was  $12.42 \pm 1.21$ , after 3 days of storage the L value was  $48.31 \pm 3.53$ , the  $a^*$  was  $2.34 \pm 1.37$  and the  $b^*$  was  $13.27 \pm 1.51$ , after 7 days of storage the L value was  $51.97 \pm 3.38$ , the  $a^*$  was  $0.45 \pm 1.98$  and the  $b^*$  was  $15.57 \pm 2.23$ , after 10 days of storage the L value was  $53.23 \pm 7.10$ , the  $a^*$  was  $3.08 \pm 1.04$  and the  $b^*$  was  $16.62 \pm 2.46$ , after 15 days of storage, the L value was  $50.07 \pm 4.52$  N, the  $a^*$  was  $2.04 \pm 0.79$  and the  $b^*$  was  $17.68 \pm 3.21$  N.s.



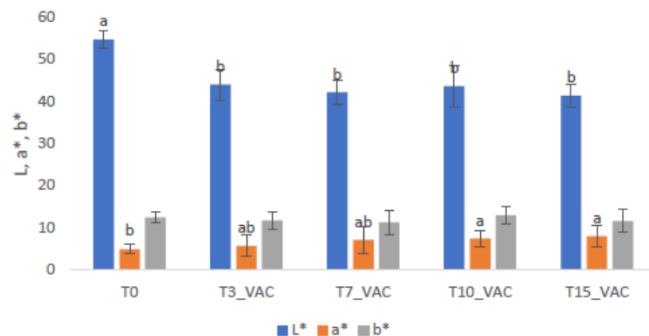
Color properties (L,  $a^*$ ,  $b^*$ ) of coated Sarrajão fillets during 15 days of storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .



## Colour properties

Colour properties of Sarrajão fillets during 15 days of vacuum storage at 4°C. are shown in the figure.

At Time 0 the L parameter of the Sarrajão fillets was  $54.68 \pm 1.94$ , the  $a^*$  was  $4.82 \pm 1.10$ , the  $b^*$  was  $12.42 \pm 1.21$ , after 3 days of storage the L value was  $43.91 \pm 3.77$ , the  $a^*$  was  $5.58 \pm 2.55$  and the  $b^*$  was  $11.66 \pm 1.97$ , after 7 days of storage the L value was  $42.11 \pm 2.91$ , the  $a^*$  was  $7.04 \pm 3.14$  and the  $b^*$  was  $11.21 \pm 2.79$ , after 10 days of storage the L value was  $43.61 \pm 4.95$ , the  $a^*$  was  $7.31 \pm 1.89$  and the  $b^*$  was  $12.91 \pm 2.09$ , after 15 days of storage, the L value was  $41.31 \pm 2.71$  N, the  $a^*$  was  $7.90 \pm 2.54$  and the  $b^*$  was  $11.53 \pm 2.78$  N.s.



Color properties (L, a\*, b\*) of Sarrajão fillets during 15 days of vacuum storage at 4°C. Mean values  $\pm$  standard deviation (n=3). Means within same column with different superscripts are significantly different at  $p < 0.05$ .

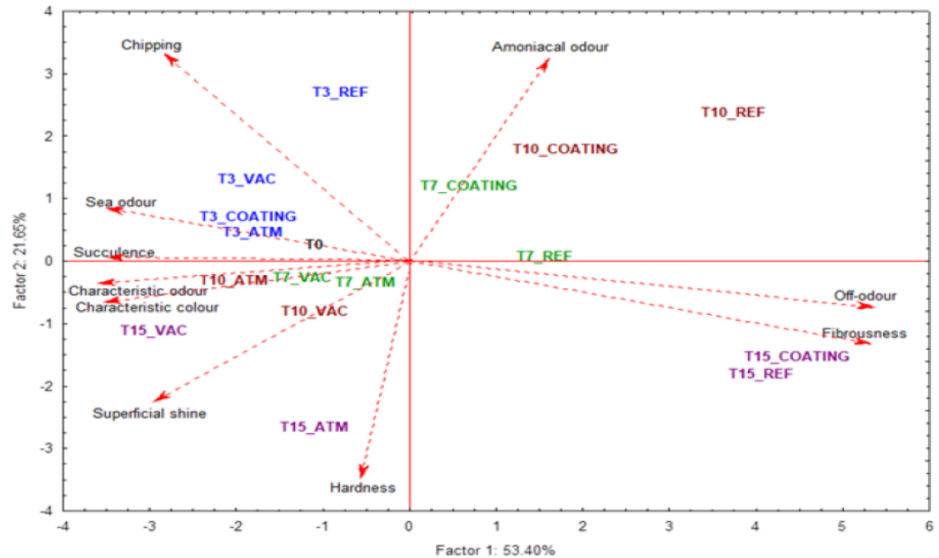
## Sensorial analysis

The results of the sensory analysis of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C are shown in the figure.

Also, PCA was used to investigate relationships between samples, PC1 (Factor 1) and PC2 (Factor 2) summarized almost 53 % and 22 % of this study information, respectively.

A good separation of groups was achieved, with the main differences to be seen in the fillets with T7, T10 and T15 days of storage time, in refrigeration or coated fillets at 4°C.

The Sarrajão fillets from T0, T3, T7 and T15 days of storage in a modified atmosphere and vacuum at 4°C obtained closer overall scores compared to the other samples.



Principal component analysis of sensory evaluation-loading plot of different attributes, performed on Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating and vacuum at 4°C.

## Microbiological properties

According to the microbiological properties, the following microorganisms were analysed: Microorganisms at 30°C, Moulds and Yeasts, Enterobacteriaceae, Staphylococcus coagulase (+), Listeria monocytogenes, Salmonella spp., Sulfite-reducing Clostridium spores, Lactic acid bacteria and Vibrio Parahemolíticos, in accordance with the applicable legislation, Regulation 2073/2005 and the guidelines defined by the Health Protection Agency (HPA).

Table: Microbiological properties of Sarrajão fillets during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C.

The preservation technique with the lowest microbial content was modified atmosphere after 10 days compared to the other preservation techniques, while the least recommended techniques were refrigeration and coating.

Sample	Microorganisms at 30°C (cfu/g)	Moulds (cfu/g)	Yeasts (cfu/g)	Enterobacteriaceae (cfu/g)	Staphylococcus coagulase (+) (cfu/g)	Listeria monocytogenes	Salmonella spp.	Sulfite-reducing Clostridium spores (cfu/g)	Lactic acid bacteria (cfu/g)	Vibrio Parahemolíticos
T0	9,91E+02	<100	<400	<10	<10	<10	Not detected in 25g	<10	2,10E+02	Not detected in 25g
REF_T3	1,18E+03	<100	<100	<10	<40	<10	Not detected in 25g	<10	Ne = 70	Not detected in 25g
VAC_T3	1,32E+02	<100	<100	<10	<10	<10	Not detected in 25g	<10	<40	Not detected in 25g
ATM_T3	6,00E+02	<100	<400	<10	<40	<10	Not detected in 25g	<10	Ne = 90	Not detected in 25g
COATING_T3	1,64E+02	<100	1,20E+03	<20	<10	<10	Not detected in 25g	<10	Ne = 50	Not detected in 25g
REF_T7	1,70E+03	<100	2,09E+03	<10	<10	<10	Not detected in 25g	<10	4,45E+02	Not detected in 25g
VAC_T7	9,59E+02	<100	<400	<10	<10	<10	Not detected in 25g	<10	Ne = 50	Not detected in 25g
ATM_T7	1,36E+02	<100	<100	<10	<10	<10	Not detected in 25g	<10	<40	Not detected in 25g
COATING_T7	8,36E+03	<100	5,36E+03	7,40E+02	<10	<10	Not detected in 25g	<10	1,43E+03	Not detected in 25g
REF_T10	3,68E+05	<100	4,14E+03	4,25E+02	<10	<10	Not detected in 25g	<10	<10	Not detected in 25g
VAC_T10	2,60E+05	<100	<400	1,15E+02	<10	<10	Not detected in 25g	<10	<10	Not detected in 25g
ATM_T10	1,73E+02	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10	Not detected in 25g
COATING_T10	3,60E+07	<100	2,80E+04	1,27E+05	<10	<10	Not detected in 25g	<10	1,40E+04	Not detected in 25g
REF_T14	1,84E+07	Ne= 400	1,36E+03	6,00E+03	<10	<10	Not detected in 25g	<10	2,24E+02	Not detected in 25g
VAC_T14	1,47E+06	<100	8,86E+02	2,00E+04	<10	<10	Not detected in 25g	<10	Ne = 40	Not detected in 25g
ATM_T14	1,36E+02	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10	Not detected in 25g
COATING_T14	>3E+09	<4000	2,80E+04	>1,5E+07	<40	<10	Not detected in 25g	<10	7,73E+03	Not detected in 25g

## Colour properties

Regarding the colour properties of the burger samples submitted to different preservation techniques, modified atmosphere, refrigeration, vacuum, coating at 4°C and freezing at -18°C, the values obtained for the analysed parameters L, a\* and b\* are shown in the table.

Samples		L	a*	b*
Refrigeration	T0	31,74±2,54 <sup>c</sup>	5,90±0,83 <sup>a</sup>	6,38±1,06 <sup>a</sup>
	T3	35,05±2,46 <sup>b</sup>	3,83±0,30 <sup>b</sup>	4,40±0,40 <sup>b</sup>
	T7	37,34±0,45 <sup>a</sup>	3,33±0,10 <sup>c</sup>	3,70±0,14 <sup>c</sup>
	T10	36,55±0,90 <sup>ab</sup>	3,07±0,45 <sup>c</sup>	3,42±0,31 <sup>c</sup>
	T15	35,53±0,93 <sup>ab</sup>	3,69±0,26 <sup>c</sup>	4,12±0,29 <sup>c</sup>
Vacuum	T0	31,74±2,54 <sup>c</sup>	5,90±0,83 <sup>a</sup>	6,38±1,06 <sup>a</sup>
	T3	34,80±2,64 <sup>b</sup>	3,74±0,40 <sup>b</sup>	4,63±0,50 <sup>b</sup>
	T7	36,41±0,90 <sup>ab</sup>	3,13±0,28 <sup>c</sup>	3,38±0,32 <sup>c</sup>
	T10	36,74±1,12 <sup>a</sup>	2,93±0,24 <sup>c</sup>	3,39±0,38 <sup>c</sup>
	T15	36,55±0,96 <sup>a</sup>	3,21±0,30 <sup>c</sup>	3,34±0,36 <sup>c</sup>
Modified Atmosphere	T0	31,74±2,54 <sup>c</sup>	5,90±0,83 <sup>a</sup>	6,38±1,06 <sup>a</sup>
	T3	35,41±3,0 <sup>b</sup>	3,83±0,68 <sup>b</sup>	5,044±0,97 <sup>b</sup>
	T7	36,14±2,11 <sup>ab</sup>	3,37±0,29 <sup>c</sup>	4,165±0,57 <sup>c</sup>
	T10	36,56±0,89 <sup>a</sup>	3,08±0,27 <sup>c</sup>	3,707±0,39 <sup>c</sup>
	T15	37,92±3,48 <sup>a</sup>	3,43±0,35 <sup>c</sup>	3,255±0,41 <sup>c</sup>
Coating	T0	31,74±2,54 <sup>d</sup>	5,90±0,83 <sup>a</sup>	6,38±1,06 <sup>a</sup>
	T3	35,53±2,07 <sup>bc</sup>	3,69±0,58 <sup>b</sup>	4,12±0,79 <sup>b</sup>
	T7	37,23±1,41 <sup>a</sup>	3,16±0,20 <sup>c</sup>	3,15±0,24 <sup>c</sup>
	T10	35,67±2,22 <sup>ac</sup>	3,09±0,28 <sup>c</sup>	3,09±0,25 <sup>c</sup>
	T15	35,82±1,07 <sup>ab</sup>	3,15±0,14 <sup>c</sup>	3,30±0,36 <sup>c</sup>
Freezing	T0	31,74±2,54 <sup>c</sup>	5,90±0,83 <sup>a</sup>	6,38±1,06 <sup>a</sup>
	T10	36,66±0,96 <sup>a</sup>	3,71±0,26 <sup>c</sup>	3,96±0,44 <sup>b</sup>
	T15	34,58±2,48 <sup>b</sup>	4,97±0,59 <sup>b</sup>	4,50±0,59 <sup>b</sup>

Color properties (L, a\*, b\*) of Sarrajão burger during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C. Mean values ± standard deviation (n=3). Means within same column with different superscripts are significantly different at p<0,05.

## Texture properties

The texture properties of the hamburger samples that were submitted to different preservation techniques (modified atmosphere, refrigeration, vacuum, coating at 4°C and freezing at -18°C) are shown in the table. The values obtained for the parameters analysed (hardness, cohesiveness, adhesiveness, and gumminess) are shown in the table.

Samples		Hardness (N)	Cohesiveness (Dimensionless)	Adhesiveness (N.s)	Gumminess (Dimensionless)
Refrigeration	T0	1,51±0,10 <sup>b</sup>	0,39±0,03 <sup>ab</sup>	3,00±0,065 <sup>a</sup>	0,59±0,07 <sup>c</sup>
	T3	1,98±0,22 <sup>b</sup>	0,42±0,03 <sup>a</sup>	2,92±0,29 <sup>ab</sup>	0,83±0,10 <sup>bc</sup>
	T7	2,52±0,28 <sup>a</sup>	0,41±0,03 <sup>a</sup>	2,98±0,72 <sup>a</sup>	1,03±0,08 <sup>ac</sup>
	T10	2,85±0,43 <sup>a</sup>	0,40±0,02 <sup>a</sup>	3,17±0,87 <sup>a</sup>	1,14±0,18 <sup>ab</sup>
	T15	2,52±0,46 <sup>a</sup>	0,33±0,07 <sup>b</sup>	1,97±0,42 <sup>b</sup>	0,86±0,28 <sup>c</sup>
Vacuum	T0	1,51±0,10 <sup>c</sup>	0,39±0,03 <sup>a</sup>	3,00±0,065 <sup>ab</sup>	0,59±0,07
	T3	1,93±0,26 <sup>bc</sup>	0,39±0,02 <sup>a</sup>	2,97±0,76 <sup>ab</sup>	0,76±0,12
	T7	2,12±0,26 <sup>b</sup>	0,40±0,04 <sup>a</sup>	2,93±0,89 <sup>ab</sup>	0,85±0,08
	T10	2,21±0,24 <sup>b</sup>	0,39±0,03 <sup>a</sup>	3,33±0,74 <sup>a</sup>	0,85±0,12
	T15	2,75±0,46 <sup>a</sup>	0,29±0,11 <sup>b</sup>	1,86±0,59 <sup>b</sup>	0,84±0,43
Modified Atmosphere	T0	1,51±0,10 <sup>b</sup>	0,39±0,03 <sup>a</sup>	3,00±0,065 <sup>abc</sup>	0,59±0,07
	T3	2,15±0,52 <sup>ab</sup>	0,38±0,03 <sup>a</sup>	2,37±0,53 <sup>abc</sup>	0,82±0,20
	T7	2,06±0,28 <sup>a</sup>	0,39±0,04 <sup>a</sup>	2,31±0,78 <sup>abc</sup>	0,85±0,16
	T10	2,31±0,33 <sup>a</sup>	0,40±0,04 <sup>a</sup>	2,48±0,87 <sup>c</sup>	0,91±0,11
	T15	1,54±0,26 <sup>c</sup>	0,35±0,12 <sup>b</sup>	2,67±0,14 <sup>b</sup>	0,72±0,21
Coating	T0	1,51±0,10 <sup>b</sup>	0,39±0,03 <sup>ab</sup>	3,00±0,065 <sup>ac</sup>	0,59±0,07 <sup>c</sup>
	T3	1,51±0,31 <sup>b</sup>	0,32±0,7 <sup>a</sup>	1,04±0,63 <sup>abc</sup>	0,46±0,08 <sup>ac</sup>
	T7	2,44±0,43 <sup>a</sup>	0,40±0,04 <sup>a</sup>	1,88±0,79 <sup>ac</sup>	0,97±0,19 <sup>ab</sup>
	T10	2,44±0,31 <sup>a</sup>	0,40±0,03 <sup>a</sup>	1,88±0,76 <sup>ac</sup>	0,89±0,16 <sup>ab</sup>
	T15	1,63±0,29 <sup>a</sup>	0,32±0,08 <sup>b</sup>	1,79±0,25 <sup>bc</sup>	0,53±0,18 <sup>ac</sup>
Freezing	T0	1,51±0,10	0,39±0,03 <sup>a</sup>	3,00±0,065	0,59±0,07 <sup>ab</sup>
	T10	1,54±0,24	0,41±0,03 <sup>a</sup>	2,67±0,78	0,63±0,12 <sup>a</sup>
	T14	1,56±0,19	0,28±0,11 <sup>b</sup>	2,31±0,19	0,43±0,16 <sup>b</sup>

Texture parameters (hardness, cohesiveness, adhesiveness, and gumminess) obtained for of Sarraão burger during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C. Mean values ± standard deviation (n=3). Means within same column with different superscripts are significantly different at p<0.05.

## Physicochemical properties

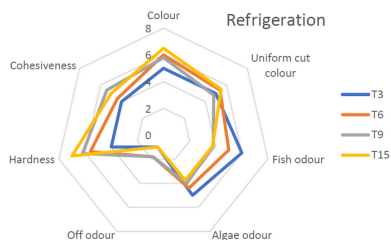
In terms of the physicochemical properties of the hamburger samples subjected to different preservation techniques, modified atmosphere, refrigeration, vacuum, coating at 4°C and freezing at -18°C, the values obtained for the parameters analysed (humidity, pH, Tbars) are shown in the table.

Samples		Moisture % (p/p)	pH	TBARs (mg malondialdehyde/kg sample)
Refrigeration	T0	68,30±1,13	6,16±0,02 <sup>b</sup>	0,17±0,24
	T3	68,74±0,37	6,16±0,00 <sup>b</sup>	0,36±0,44
	T7	73,67±8,33	6,19±0,00 <sup>ac</sup>	0,44±0,58
	T10	66,09±0,25	6,17±0,00 <sup>abc</sup>	0,05±0,07
	T15	67,95±0,36	6,17±0,00 <sup>abc</sup>	0,09±0,13
Vacuum	T0	68,30±1,13	6,16±0,02 <sup>c</sup>	0,17±0,24
	T3	63,31±0,58	6,16±0,00 <sup>bc</sup>	0,23±0,28
	T7	66,39±1,17	6,19±0,00 <sup>a</sup>	0,19±0,25
	T10	67,08±0,28	6,17±0,00 <sup>ab</sup>	0,26±0,33
	T15	67,37±0,73	6,16±0,00 <sup>b</sup>	0,05±0,05
Modified Atmosphere	T0	68,30±1,13	6,16±0,02 <sup>ab</sup>	0,17±0,24
	T3	69,08±1,05	6,11±0,00 <sup>ab</sup>	0,24±0,28
	T7	67,67±0,16	6,12±0,00 <sup>c</sup>	0,26±0,31
	T10	66,14±1,75	6,16±0,00 <sup>bc</sup>	0,04±0,04
	T15	76,92±0,23	6,12±0,00 <sup>ab</sup>	0,08±0,09
Coating	T0	68,30±1,13	6,16±0,02 <sup>a</sup>	0,17±0,24
	T3	70,42±0,32	6,12±0,00 <sup>a</sup>	0,44±0,56
	T7	68,98±0,43	6,13±0,00 <sup>b</sup>	0,24±0,30
	T10	48,64±23,41	6,12±0,00 <sup>ab</sup>	0,05±0,06
	T15	71,42±0,52	6,25±0,00 <sup>ab</sup>	0,08±0,10
Freezing	T0	68,30±1,13	6,16±0,02 <sup>ac</sup>	0,17±0,24
	T10	68,74±4,62	6,24±0,00 <sup>b</sup>	0,04±0,06
	T15	65,47±0,77	6,14±0,00 <sup>ac</sup>	0,07±0,09

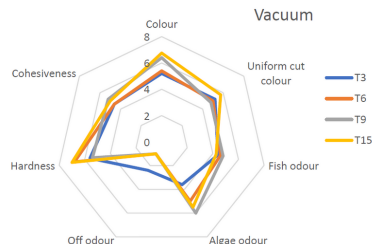
Physicochemical properties of Sarrajão burger during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C. Mean values ± standard deviation (n=3). Means within same column with different superscripts are significantly different at p<0.05.

## Sensorial properties

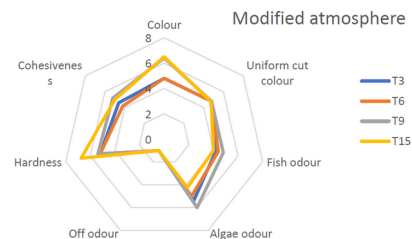
The sensory properties of the hamburger samples that have been submitted to different preservation techniques, modified atmosphere, refrigeration, vacuum, coating at 4°C and freezing at -18°C, the values obtained for the parameters analysed (colour, uniform cut colour, fish odour, algae odour, off odour, hardness, cohesiveness) are shown in the figures.



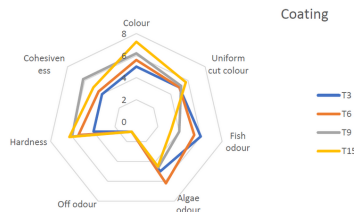
Sensorial properties of Sarrajão burger during 15 days of storage in refrigeration at 4°C.



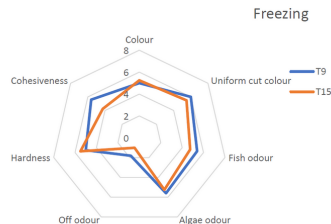
Sensorial properties of Sarrajão burger during 15 days in vacuum storage at 4°C



Sensorial properties of Sarrajão burger during 15 days of storage in a modified atmosphere at 4°C.



Sensorial properties of coated Sarrajão burger during 15 days of storage at 4°C.



Sensorial properties of coated Sarrajão burger during 15 days of storage at -18°C.

# Microbiological properties

According to the microbiological properties, the following microorganisms were analysed: Microorganisms at 30°C, Moulds and Yeasts, Enterobacteriaceae, Staphylococcus coagulase (+), Listeria monocytogenes, Salmonella spp., Sulfite-reducing Clostridium spores, Lactic acid bacteria and Vibrio Parahemoliticus, in accordance with the applicable legislation, Regulation 2073/2005 and the guidelines defined by the Health Protection Agency (HPA).

Sample	Microorganisms at 30°C (cfu/g)	Moulds (cfu/g)	Yeasts (cfu/g)	Enterobacteriaceae (cfu/g)	Staphylococcus coagulase (+) (cfu/g)	Listeria monocytogenes (cfu/g)	Salmonella spp.	Sulfite-reducing Clostridium spores (cfu/g)	Lactic acid bacteria (cfu/g)	Psychotropic (cfu/g)
T0	4,18E+04	<4000	<100	9,73E+03	<10	<10	Not detected in 25g	<10	<10	3,65E+02
REF_T3	4,73E+04	<100	<100	1,30E+04	1,15E+02	<10	Not detected in 25g	<10	<10	4,64E+04
VAC_T3	4,64E+04	<100	<100	8,64E+03	<10	<10	Not detected in 25g	<10	<10	1,49E+04
ATM_T3	5,32E+04	<100	<400	1,01E+04	<40	<10	Not detected in 25g	<10	<10	3,46E+04
COATING_T3	7,41E+04	<100	3,40E+03	2,25E+04	<10	<10	Not detected in 25g	<10	4,05E+02	6,23E+04
REF_T7	1,02E+05	<100	2,19E+04	3,25E+04	<10	<10	Not detected in 25g	<10	<10	3,08E+04
VAC_T7	5,59E+04	<100	<400	2,70E+04	<10	<10	Not detected in 25g	<10	<10	1,02E+04
ATM_T7	8,95E+04	<100	<100	5,00E+04	<10	<10	Not detected in 25g	<10	<10	1,15E+04
COATING_T7	2,55E+06	<100	2,00E+04	9,60E+04	<10	<10	Not detected in 25g	<10	<10	1,65E+06
REF_T10	3,70E+06	<100	2,05E+04	3,00E+05	<10	<10	Not detected in 25g	<10	<10	6,90E+06
VAC_T10	6,90E+05	<100	<400	6,10E+05	<10	<10	Not detected in 25g	<10	<10	6,41E+05
ATM_T10	8,45E+04	<100	<100	1,02E+05	<10	<10	Not detected in 25g	<10	1,30E+02	7,21E+04
COATING_T10	1,46E+07	<100	7,50E+03	>1,5E+06	<10	<10	Not detected in 25g	<10	6,65E+02	6,56E+07
Freezing_T10	3,95E+04	Ne=400	7,73E+03	1,60E+04	<10	<10	Not detected in 25g	<10	<40	2,55E+04

Microbiological properties obtained for Sarrajão burgers during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C.



## Microbiological properties

Since the burgers will not be eaten raw, microbiological analyses were carried out on the burgers after cooking at 180°C for 8 min until the thermal center reached 85°C.

The results show that the most appropriate preservation technique for the burgers is vacuum for 3 days.

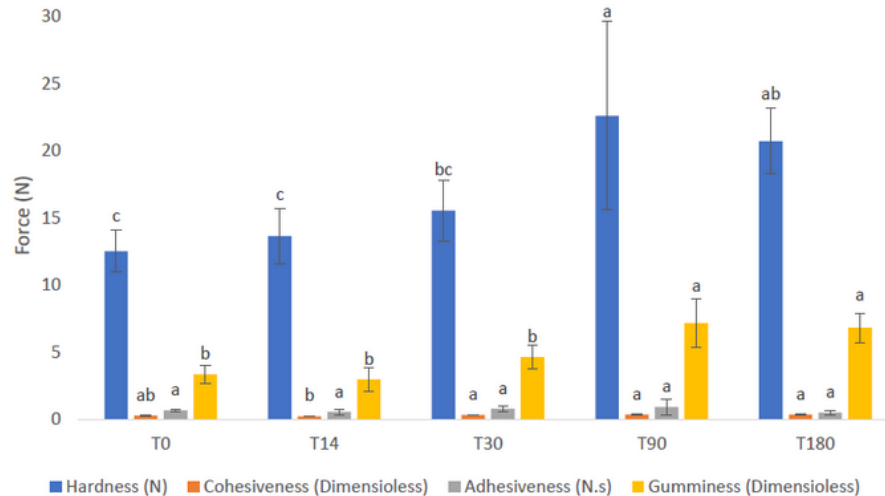
Sample	Microrg anisms at 30°C (cfu/g)	Moulds (cfu/g)	Yeasts (cfu/g)	Enterobacteri aceae (cfu/g)	Staphyloco ccus coagulase (+) (cfu/g)	Listeria monocytog enes (cfu/g)	Salmonella spp.	Sulfite- reducing Clostridium spores (cfu/g)	Lactic acid bacteria (cfu/g)
REF_T3	1,27E+0 2	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
VAC_T3	1,09E+0 2	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
ATM_T3	4,55E+0 1	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
COATING _T3	4,55E+0 1	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
REF_T10	9,09E+0 0	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
VAC_T10	9,09E+0 0	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
ATM_T10	<10	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
COATING _T10	<10	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10
Freezing_ T10	<10	<100	<100	<10	<10	<10	Not detected in 25g	<10	<10

Microbiological properties obtained for cooked Sarrajão burgers during 15 days of storage in modified atmosphere, refrigeration, coating, and vacuum at 4°C.

# Evaluation of the preservation techniques (freezing) on Sarrajão fillet properties over 180 days of storage time

## Texture properties

Concerning the texture analysis, hardness, cohesiveness, and gumminess increased 1,65-fold, 1,2-fold, 2-fold, respectively.

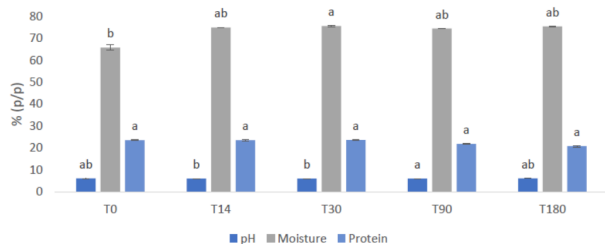


Texture properties of Sarrajão fillets during 180 days of storage in freezing at -18°C. Mean values  $\pm$  standard deviation (n=3). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

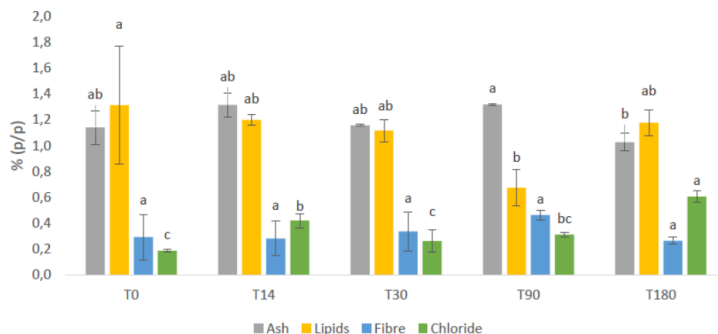
## Physicochemical properties

Chemical results showed that lipid content decreased and the peroxide index and TBARs increased 24-fold and 8-fold, respectively, after 180 days of storage time. On the contrary there were no significant differences on fibre and protein content of fish fillets. Also, moisture, ash, chloride content and pH showed differences during storage time.

Sample	PV (mEq./Kg sample)	Tbars (mg malondialdehyde/kg sample)
T0	0,93±0,01 <sup>b</sup>	0,30±0,05 <sup>b</sup>
T180	22,33±5,49 <sup>a</sup>	2,45±0,18 <sup>a</sup>



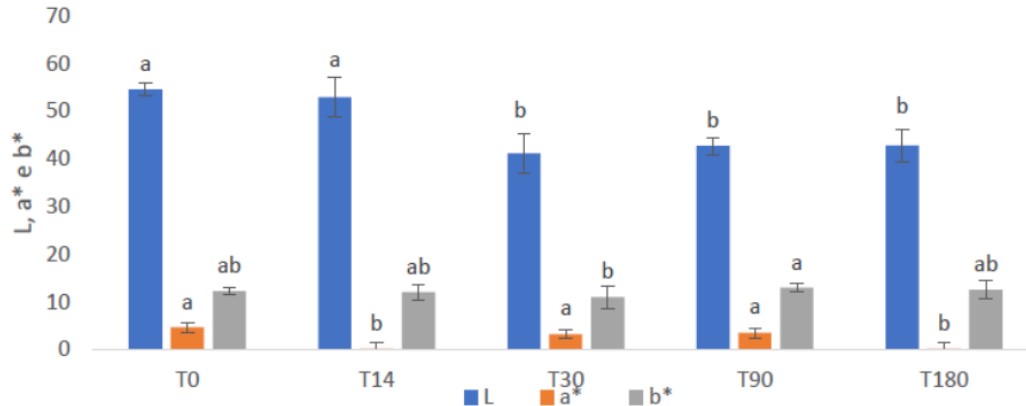
Physicochemical properties (PV and Tbars) of Sarrajão burger during 180 days of storage at -18°C. Mean values ± standard deviation (n=3). Means within the same column with different superscripts are significantly different at p<0.05.



Physicochemical properties (ash, lipids, fibre, chloride) of Sarrajão fillets during 180 days of storage in freezing at -18°C. Mean values ± standard deviation (n=3). Means within the same column with different superscripts are significantly different at p<0.05.

## Colour properties

Regarding colour parameters it was observed that the fillets showed slightly differences in Luminosity,  $a^*$  and  $b^*$  parameters from day zero to the 180-day storage period.



Colour properties of Sarrajão fillets during 180 days of storage in freezing at  $-18^{\circ}\text{C}$ . Mean values  $\pm$  standard deviation ( $n=3$ ). Means within the same column with different superscripts are significantly different at  $p < 0.05$ .

## Microbiological properties

A microbiological analysis was made according to the Regulation nº2073/2005 and the guidelines defined by the Health Protection Agency (HPA). Fish fillets presented satisfactory microbiological quality regarding the parameters analysed (Microorganisms at 30 °C, moulds and yeasts, *Salmonella* spp., *Escherichia coli*, Enterobacteriaceae, Coagulase staphylococci (+), *Listeria monocytogenes*, Lactic Acid Bacteria, *Vibrio parahaemolyticus*, sulfite-reducing *Clostridium* spores), during 180 days of storage time.

According to WP4, the purpose of this report is to present the results of the characterisation of the products produced with Sarraão.

Sample	Microorganisms 30°C (cfu/g)	E. Coli (cfu/g)	Moulds (cfu/g)	Yeasts (cfu/g)	Enterobacteriaceae (UFC/g)	Staphylococcus coagulase (+) (cfu/g)	Listeria monocytogenes	Salmonella spp.	Sulfite- reducing Clostridium spores (cfu/g)	Lactic acid bacteria (cfu/g)	Vibrio parahaemoliticus (cfu/g)
T0	2,3E+04	<10	<100	7,0E+02	9,0E+02	<10	Not detected in 25g	Not detected in 25g	<10	3,2E-01	Not detected in 25g
T14	4,0E+02	<10	<100	<100	1,7E+02	<10	Not detected in 25g	Not detected in 25g	<10	1,0E+01	Not detected in 25g
T30	1,3E+03	<10	<100	<100	4,0E+01	<10	Not detected in 25g	Not detected in 25g	<10	<10	Not detected in 25g
T90	2,5E+03	<10	<100	<100	3,0E+01	<10	Not detected in 25g	Not detected in 25g	<10	<10	Not detected in 25g
T180	2,0E+03	<10	<400	<100	5,00E+00	<10	Not detected in 25g	Not detected in 25g	<10	<10	Not detected in 25g

# Technical specification of components to be used in the Sarraão products development

## Sarraão products characterization

### Paté

#### Nutritional/chemical characterization

The table shows the results of the physicochemical characterisation of the formulation of Sarraão pâté.

Regarding protein content it was found that the pâté presents 16,09 ±0,47 %, for moisture content showed a value of 64,65 ±0,45%, regarding the ash content, a value of 3,44 ±0,02 % was obtained, the pH showed a value of 6,27±0,01 %, concerning water activity, an average value of 0.958 was obtained.

Heating Temperature  (°C)	Cabbage  (%)	Hydrocolloid  (%)	Protein  (%, p/p)	Moisture  (%, p/p)	Ash  (%, p/p)	pH	a <sub>w</sub>
115	2%	1%	16,09±0,47	64,65 ±0,45	3,44 ±0,02	6,27±0,01	0,958

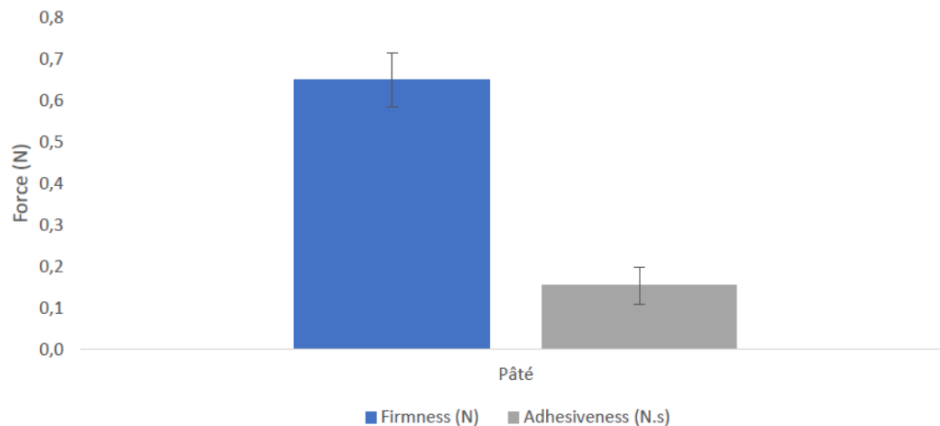
Results of the chemical characterisation of the pâté (average ± standard deviation).

# Sarrajão products characterization

## Pâté

### Texture Characterization

The texture analysis showed that the pâté has a firmness of 0,648 N, an adhesiveness value of -0.153 N.s.



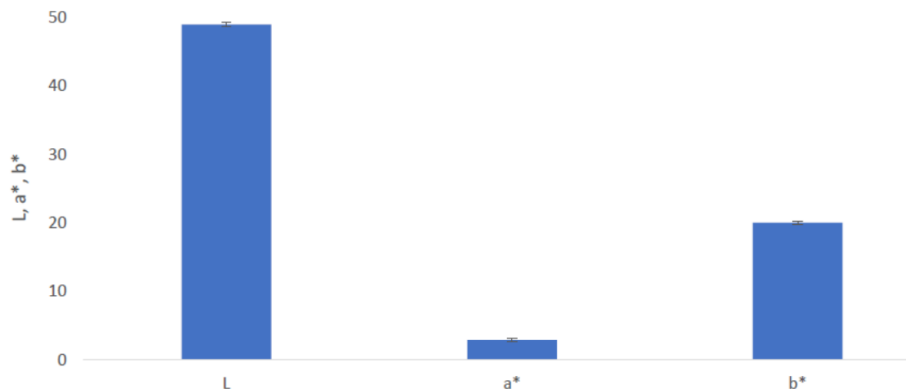
Results obtained for firmness and adhesiveness according to the texture analysis carried out on the pâté.

# Sarrajão products characterization

## Paté

### Colour Characterization

The figure shows the values obtained for the various parameters analysed during the colour analysis (L, a\* and b\*). The Sarrajão fillets presents a value of  $49,00 \pm 0,329$  for the L coordinate, for the a\* coordinate the value is  $2,90 \pm 0,234$  and for the b\* coordinate the value is  $20,04 \pm 0,182$ .



Results obtained from the colour analysis carried out on the paté, for the parameters L, a\* and b\*.



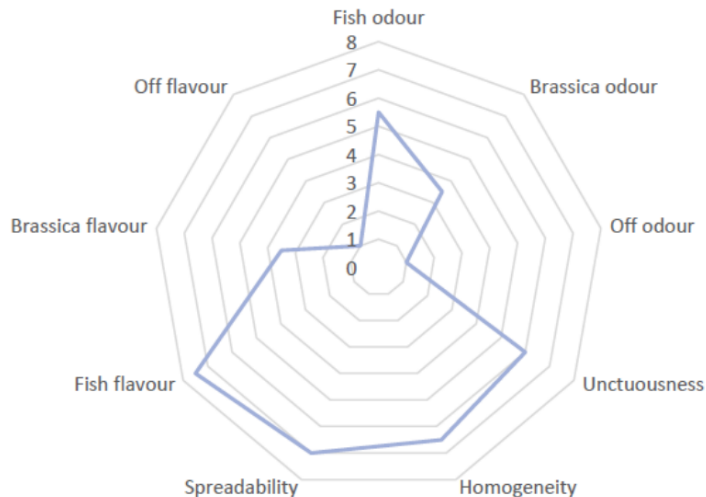
# Sarrajão products characterization

## Paté

### Sensory Characterization

Regarding the sensory analysis, a quantitative descriptive analysis (ADQ®) was carried out with a panellist of semi-trained panellists in order to classify several attributes, namely fish odour, cabbage odour, off-odour, unctuousness, homogeneity, spreadability, fish flavour, brassica flavour and off-flavour. These attributes were evaluated on an intensity scale of 10 points (1 - lowest intensity, 10 - higher intensity).

Results show that the panellists scored the pâté with a score of 6 for the " fish odour", " fish flavour", with a score of 8, "unctuousness", with a score of 6, " spreadability" and " homogeneity", with a score of 7, obtaining the maximum classification in the global appreciation, with a score of 5. Regarding the attributes, "odour" and "brassica flavour" scored 4. The remaining attributes scored 1, as they referred to unfavourable attributes, i.e. off-odour and off-flavour.



Sensory analysis of pâté.

— 115\_2\_1

# Sarrajão products characterization

## Hamburger

### Nutritional/chemical characterization

The table shows the results of the physicochemical characterisation of the formulation of Sarrajão burger.

Regarding to the physicochemical characterization of Sarrajão burger, it was found that the protein content was  $15,38 \pm 0,36$  %, the results for moisture content showed a value of  $68,30 \pm 1,13$  %, the ash content, a value of  $1,73 \pm 0,09$  % was obtained, the pH showed a value of  $6,16 \pm 0,02$  %, the lipid content showed a value of  $1,62 \pm 0,05$  % and for the fibre content the value was  $3,49 \pm 0,22$  %.

Sample	Spirulina	<i>Fucus vesiculosos</i>	Xanthan	Carrageenan	Moisture (%, p/p)	pH	Fibre (%, p/p)	Lipids (%, p/p)	Protein (%, p/p)	Ash (%, p/p)
Burger	2%	3%	3%	0%	68,30±1,13	6,16±0,02	3,49±0,22	1,62±0,05	15,38±0,36	1,73±0,09

Results of the chemical characterization of the burger (average ± standard deviation).

## Sarrajão products characterization

### Hamburger

#### Antioxidant activity and Oxidative Degradation Indicators

Regarding antioxidant activity, the value obtained for total phenolic compounds was  $0,166 \pm 0,034$  mg GAE/g, for ABTS the value was  $0,4300 \pm 0,0802$  (mg TE/g), and for DPPH the value was  $0,0868 \pm 0,0294$  (mg TE/g).

In relation to oxidative degradation indicators, TBARs obtained the value  $0,17 \pm 0,24$  (mg malondialdehyde/kg sample) and peroxides were  $19,618 \pm 0,00$  (mEq./Kg sample).

Sample	TPC (mg GAE/g)	ABTS (mg TE/g)	DPPH (mg TE/g)	TBARs (mg malondialdehyde/kg sample)	PV (mEq./Kg sample)
Burger	$0,166 \pm 0,034$	$0,4300 \pm 0,0802$	$0,0868 \pm 0,0294$	$0,17 \pm 0,24$	$19,618 \pm 0,00$

TPC – Total Phenolic Content; ABTS - 2,2'-azino-bis (3-ethylbenzothiazolyl)-6-sulfonic acid); TPC - Total phenolic content; DPPH - 2,2-diphenyl-1-picrylhydrazyl; PV – Peroxide Value; TBARs – Thiobarbituric Acid Reactive Substances.

Results of the Antioxidant activity and lipidic oxidation of the Sarrajão burger r(average  $\pm$  standard deviation).

# Sarrajão products characterization

## Hamburger

### Microbiological Characterization

According to the values shown on the table the microbiological analyses demonstrate that were lower than <10 cfu/g for Staphylococcus coagulase (+), Sulfite-reducing Clostridium spores and Lactic Acid Bacteria, respectively. Listeria monocytogenes and Salmonella spp. were not detected in 25g of product. For the parameter Microorganisms at 30°C the value  $4 \times 10^2$  cfu/g was obtained, as well as for the psychotrophs, for Enterobacteriaceae  $1 \times 10^3$  cfu/g was obtained and for moulds and yeasts the value obtained was  $2,9 \times 10^3$  cfu/g.

Microorganisms at 30°C	Psychotrophic	Moulds and Yeasts	Enterobacteriaceae	Staphylococcus coagulase (+)	Listeria monocytogenes	Salmonella spp.	Sulfite- reducing Clostridium spores	Lactic acid bacteria
$4 \times 10^2$ cfu/g	$4 \times 10^2$ cfu/g	$2,9 \times 10^3$ cfu/g	$1 \times 10^3$ cfu/g	<10 cfu/g	Not detected in 25g	Not detected in 25g	<10 cfu/g	<10 cfu/g

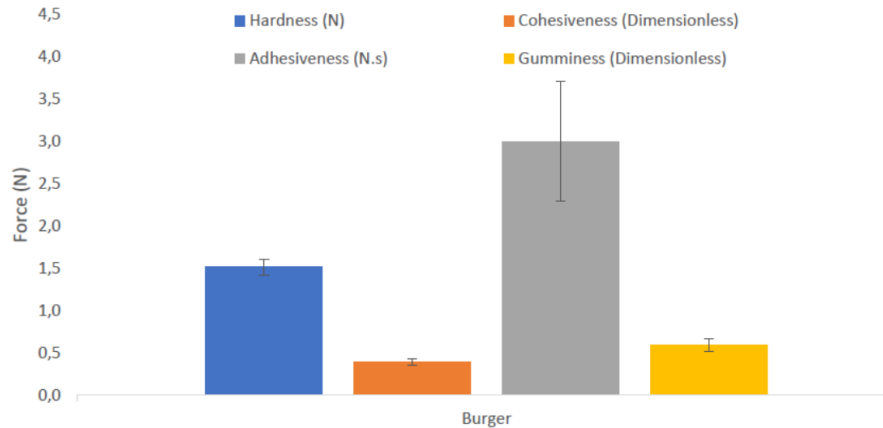
Information on microbiological analysis data on fresh Sarração.

# Sarrajão products characterization

## Hamburger

### Texture Characterization

The texture analysis showed that the pâté has a hardness of 1,51 N, an adhesiveness value of -0.39 N.s, a cohesiveness value of 3,00 and a gumminess value of 0,59.

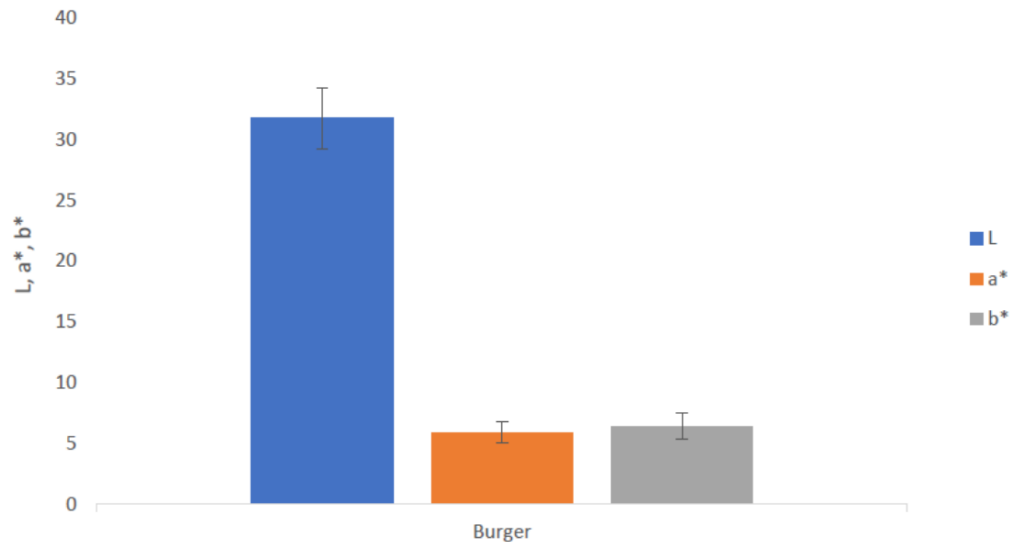


Texture analysis of hardness, cohesiveness, adhesiveness, and gumminess of Sarrajão burger.

# Sarrajão products characterization

## Hamburger

### Colour Characterization



Results obtained from the colour analysis carried out on the burger, for the parameters L, a\* and b\*.

# Sarrajão products characterization

## Hamburger

### Sensory Characterization

Regarding the sensory analysis, a quantitative descriptive analysis (ADQ®) was carried out with a panellist of semi-trained panellists to classify several attributes, namely colour, uniform cut colour, fish odour, algae odour, off odour, hardness, cohesiveness. These attributes were evaluated on an intensity scale of 10 points (1 - lowest intensity, 10 - higher intensity).

Results show that the panellists scored the burger with a score of 5 for the "colour", " uniform cut colour", "fish odour", "algae odour", "hardness" and "cohesiveness", and for the "off odour" the score was 6 points.



Sensory analysis of Sarrajão burger.

# Sarrajão products characterization

## Results of the acceptability test

### Consumer acceptability test (fillet)

Regarding the acceptability test of the Sarração fillets carried out in the atrium of the ESTG-IPVC, the evaluation form provided to the participants is represented in Figure 10. This form is made up of 11 questions, 4 of which are mandatory and aim to characterise the public (questions 1, 2, 3 and 4), then there are 3 questions that aim to characterise the participants' consumption habits (questions 5, 6, 7), questions 8 and 9 refer to the appreciation and justification of the product and finally, question 10 relates to the possible future purchase of the product provided in the test.



### CONSUMER TEST

#### Acceptability of Sarração fillets

The aim of this consumer test is to collect important information about the acceptability of a product developed as part of the BlueProject.

Please complete the questions below. There are no right or wrong answers, so please answer all the questions honestly and spontaneously. Please note that the survey is anonymous. Questions marked with \* are mandatory.

Thank you for your collaboration 😊

#### 1. Gender\*

☐ Female ☐ Male ☐ Other

#### 2. Age\*

☐ <18 ☐ 19-25 ☐ 26-35 ☐ 36-45 ☐ 46-55 ☐ >55

#### 3. District\*

☐ Aveiro ☐ Beja ☐ Braga ☐ Bragança ☐ Castelo Branco ☐ Coimbra  
☐ Évora ☐ Faro ☐ Guarda ☐ Leiria ☐ Lisboa ☐ Porto  
☐ Portalegre ☐ Santarém ☐ Setúbal ☐ Viana do Castelo ☐ Vila Real ☐ Viseu  
☐ Açores ☐ Madeira

#### 4. Do you usually eat fish?\*

☐ Yes ☐ No

If not, why? \_\_\_\_\_

If you answered NO, go to point 8!

#### 5. What cooking method do you usually use for fish? (You can select several options)

☐ Fried ☐ Boiled ☐ Roast (oven) ☐ Grilled

#### 6. How often do you eat fish?

☐ Daily ☐ Many times a week ☐ Once a week  
☐ Once a month ☐ Rarely

#### 7. How do you usually eat fish? (You can select several options)

☐ Fried fillet  
☐ Grilled fillet  
☐ Goldfish  
☐ Nuggets  
☐ Boiled fillet  
☐ "Massada"

#### 8. Please rate overall the sample presented, using the scale below.

☐ Like extremely  
☐ Like very much  
☐ Like moderately  
☐ Like slightly  
☐ Neither like or dislike  
☐ Dislike slightly  
☐ Dislike moderately  
☐ Dislike very much  
☐ Dislike extremely

#### 9. If you felt it was disagreeable, please explain why.

#### 10. Would you be disposed to consume this product?

☐ Yes ☐ No

Evaluation form provided to the participants during the acceptability test.



# Sarrajão products characterization

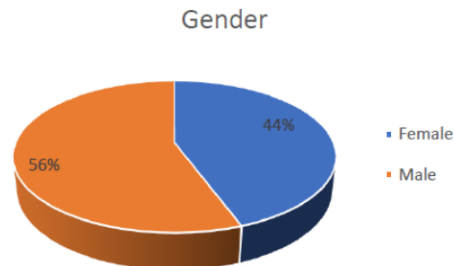
## Results of the acceptability test

### Consumer characterisation

Concerning the answers of the participants in the acceptability test, a total of 115 individuals participated, and 56 % of the participants were male and 44 % female.

Gender	Number of participants
Female	51
Male	64
Total	115

Number of female and male participants, respectively.



Percentage of female and male participants, respectively.

# Sarrajão products characterization

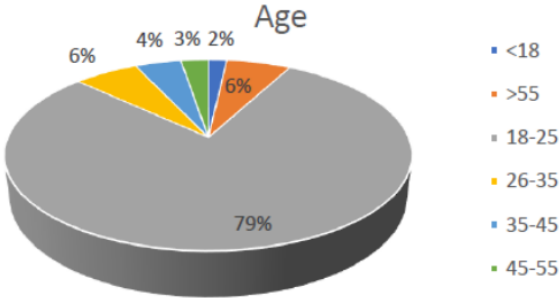
## Results of the acceptability test

### Consumer characterisation

The most common age group was 18–25 years old, with 79 % of the participants, followed by >55 years old (6 %), as well as 18-36 years old (6 %), then 35-45 years old (4 %), 45-55 years old (3 %) and finally the <18 years old group (2 %).

Age	Number of participants
<18	2
>55	7
18-25	91
26-35	7
35-45	5
45-55	3
Total	115

Number of participants in each group.



Percentage of participants in each age group.

# Sarração products characterization

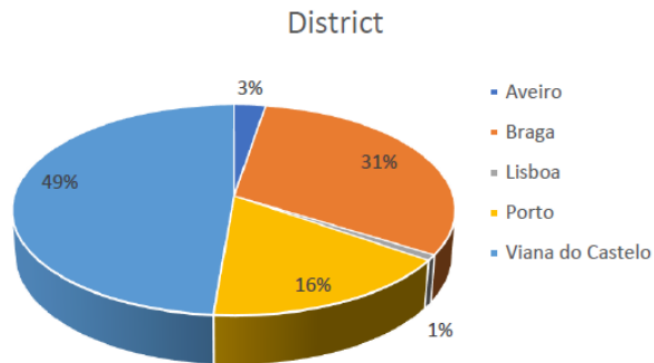
## Results of the acceptability test

### Consumer characterisation

In terms of districts, around 49 % were from Viana do Castelo, 31 % from Braga and 16 % from Porto, 3 % from Aveiro and 1 % from Lisbon.

District	Number of participants
Aveiro	3
Braga	36
Lisboa	1
Porto	19
Viana do Castelo	56
Total	115

Number of participants from different districts.



Percentage of participants from different districts.

# Sarração products characterization

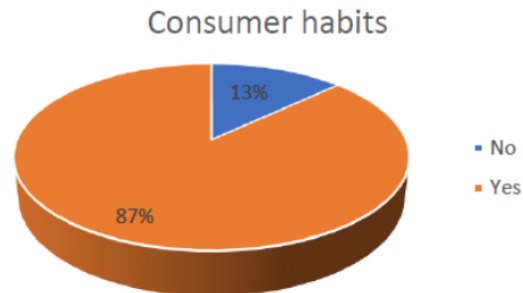
## Results of the acceptability test

### Consumer characterisation

In terms of consumption habits, around 87% of the participants consume fish, while only 13% do not.

Consume habits	Number of participants
No	15
Yes	100
Total	115

Number of participants who consume fish or not.



Percentage of participants who consume fish or not.

# Sarrajão products characterization

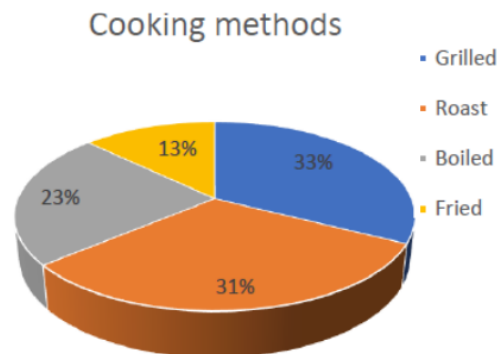
## Results of the acceptability test

### Consumer characterisation

Regarding cooking methods, the most common is grilled (33 %), followed by roasted (31 %), next boiled (23 %) and then fried (13 %).

Cooking methods	Number of participants
Grilled	63
Roast	61
Boiled	45
Fried	25

Number of participants and their cooking methods.



Percentage of participants and their cooking methods.

# Sarrajão products characterization

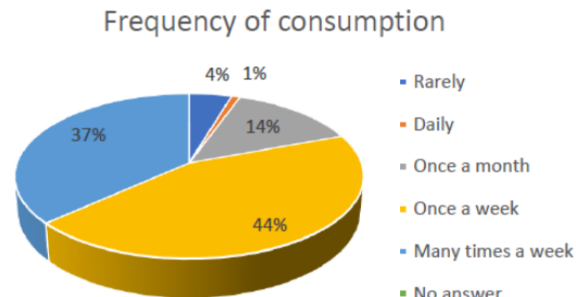
## Results of the acceptability test

### Consumer characterisation

And the frequency of consumption varies between one time a week (44%) and many times a week (37%).

Frequency of consumption	Number of participants
Rarely	5
Daily	1
Once a month	15
Once a week	48
Many times a week	40
No answer	6
Total	109

Number of participants and their frequency of fish consumption.



Percentage of participants and their frequency of fish consumption.

# Sarrajão products characterization

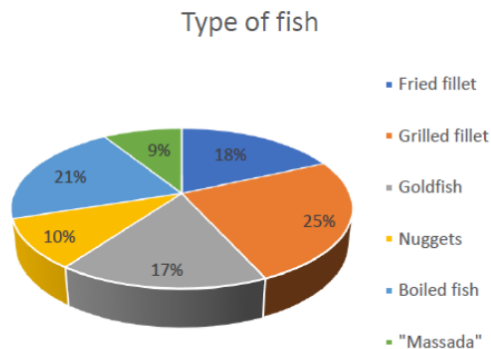
## Results of the acceptability test

### Consumer characterisation

According to the answers, the majority of participants consume grilled fish fillets (25 %), followed by boiled fish (21 %), fried fish (18 %), goldfish (17 %), nuggets (10 %) and "massada" (9 %).

Type of fish	Number of participants
Fried fillet	51
Grilled fillet	70
Goldfish	47
Nuggets	28
Boiled fish	59
"Massada"	25

Number of participants and the type of fish they consume.



Percentage of participants and the type of fish they consume.

# Sarrajão products characterization

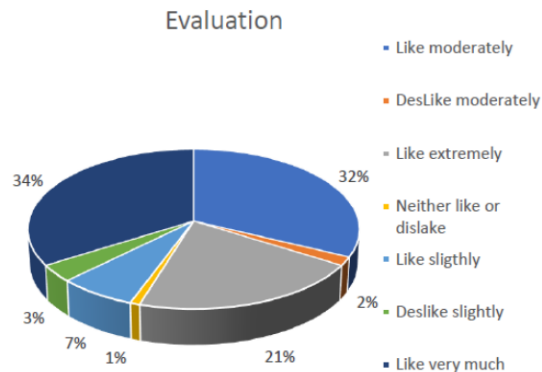
## Results of the acceptability test

### Consumer characterisation

Regarding the acceptability of the product: 21 % like it very much, 34 % like it a lot and 32 % like it moderately, so that, in total, 87 % of the participants had a very good acceptability of the Atlantic bonito fillets.

Evaluation	Number of participants
Like moderately	37
Dislike moderately	2
Like extremely	24
Neither like or dislike	1
Like slightly	8
Dislike slightly	4
Like very much	39
Total	115

Number of participants and their evaluation in the acceptability test.



Percentage of participants and their evaluation in the acceptability test.



# Sarração products characterization

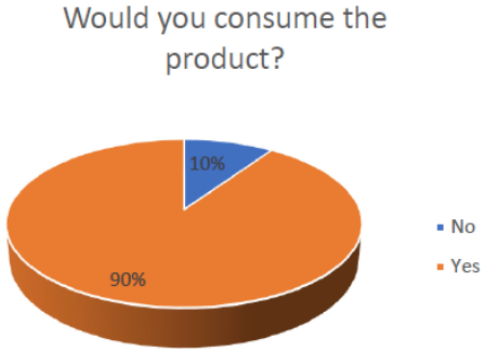
## Results of the acceptability test

### Consumer characterisation

When asked if they would consume the product, 90% of the participants said yes, with only 10% saying no.

Would you consume the product?	Number of participants
No	11
Yes	104
Total Geral	115

Number of participants who would consume the product in the future and those who would not.



Percentage of participants who would consume the product in the future and those who would not.




## **Chapter 6**

# **Main scientific results – Consumer test in schools**

# Main scientific results – Consumer test in schools

According to WP5, which aims to find out about consumer behavior and eating habits, obtaining feedback on the quality of the product supplied and consumer perceptions, a consumer test was performed with children from two schools located in the Municipality of Esposende.

Sensory testing with children can provide valuable data in basic research or product development. The consumer test with the children was carried out at the Esposende Basic School and the Forjães School Centre. The ages of the participants were between 6 and 10 years old. A total of 192 students were selected to take part in the test, and the food provided was "Arroz de Sarrajão" (Esposende) and "Massada de Sarrajão" (Forjães). In Figure 1 it is possible to observe the evaluation form provided to the participants during the consumer test.






 **Blue Project**

Iceland  
Liechtenstein  
Norway grants

How old are you? \_\_\_\_\_ What grade of school are you in? \_\_\_\_\_







How many times do you eat fish in a week?

Rarely ☐ Once a week ☐ Every other day ☐ Every day ☐

Dislike extremely	Dislike moderately	Neither like nor dislike	Like moderately	Like extremely
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What kind of fish do you eat most often?

 ☐  ☐  ☐  ☐  ☐  ☐

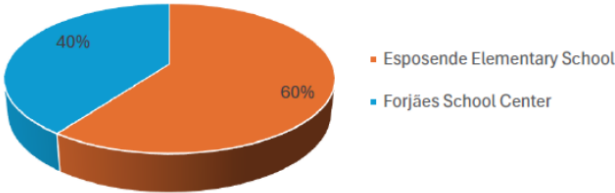
Evaluation form provided to the participants during the consumer test.

# Consumer characterisation

The table and figure shows the number and the percentage of participants from each of the schools that took part in the consumer test. A total of 192 tasters participated in the consumer test, 115 students are (60%) from the Esposende Elementary School and 77 students, (40%) from the Forjães School Center.

School	Number of participants
Esposende	115
Forjães	77
Total	192

Number of students from each school analysed, who participated in the consumer test.



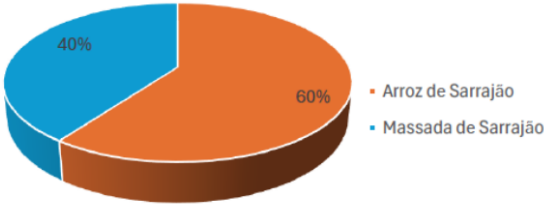
Percentage of students from each school analysed, who participated in the consumer test.

# Consumer characterisation

In the table and figure, it is possible to observe that the meals provided were different in each of the schools that participated in the consumer test. At Esposende Elementary School, the 115 students who participated in the consumer test had lunch "Arroz de Sarrajão" (60%), while at Forjães School Center the 77 participants had lunch "Massada de Sarrajão" (40%).

Meal	Number of participants
Arroz de Sarrajão	115
Massada de Sarrajão	77
Total	192

Number of participants who consumed the different meals provided at each school.



Percentage of participants who consumed the different meals provided at each school.

## Consumer characterisation

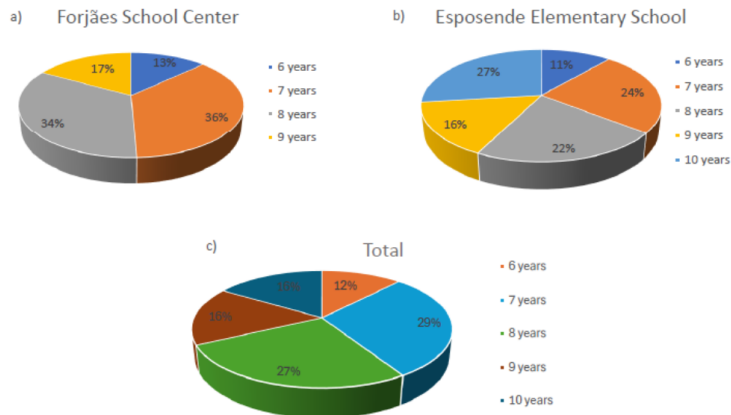
Regarding the table and figure, it is observed that the age of 192 participants varies between 6 and 10 years, namely, 23 panelists (12%) had 6 years, 56 panelists (29%) 7 years, 51 panelists (27%) 8 years, 31 panelists (16%) 9 years and 31 panelists (16%) 10 years.

At the Forjães School Center, the ages of the participants were between 6 and 9 years, 10 students were 6 years old, 28 were 7 years old, 26 were 8 years old and 13 were 9 years old.

In Esposende Elementary School the age of the participants is between 6 and 10 years, 13 students were 6 years old, 28 were 7 years old, 25 were 8 years old, 18 were 9 years old and 31 were 10 years old.

Age	Number of participants	Forjães School Center	Esposende Elementary School
6 years	23	10	13
7 years	56	28	28
8 years	51	26	25
9 years	31	13	18
10 years	31	0	31
Total	192	77	115

Age of the participants of each school of the consumer test.



a) Percentage of the participants of Forjães School Center; b) Percentage of the participants of Esposende Elementary c) Percentage of the participants of both schools of the consumer test.

## Consumer characterisation

Concerning the level of education of the participants, 41 (21%) were in 1st grade, 66 (34%) were in 2nd grade, 43 (23%) were in 3rd grade, 18 (9%) were in 4th grade and 24 (13%) were in 5th grade.

At the Forjães School Centre, the level of education of the group that participated in the consumer test was between 1st and 3rd grade, with 17 (22%) attending 1st grade, 31 (40%) attending 2nd grade and 29 (38%) attending 3rd grade.

At Esposende Elementary School, the participants level of education varies from 1st to 5th grade, with 25 (21%) attending 1st grade, 35 (30%) attending 2nd grade, 14 (12%) attending 3rd grade, 18 (16%) attending 4th grade and 24 (21%) attending 5th grade.



## Characterisation of consumption habits

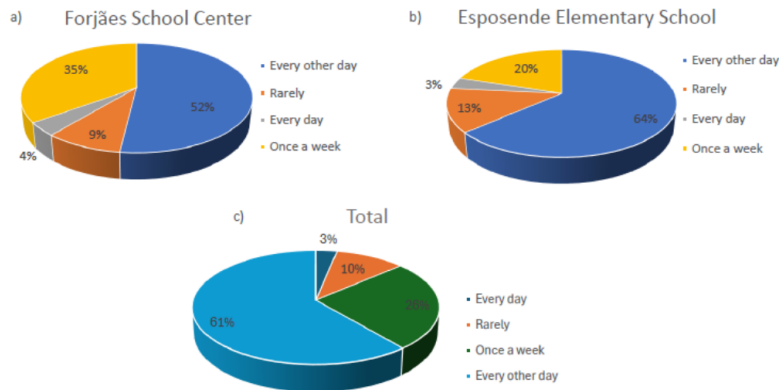
According to the frequency of consumption of the participants in the consumer test, it is possible to verify that the majority of students consume fish every other day (61%), followed by once a week (26%), followed by rarely (10%) and every day (3%).

At the Forjães School Centre, around 52% of the participants in the consumer test eat fish every other day, 35% only once a week, 9% rarely and 4% every day.

At the Esposende Primary School, 64% of the participants consume fish every other day, 20% only once a week, 13% rarely and only 3% every day.

Frequency of consumption in a week	Number of participants	Forjães School Center	Esposende Elementary School
Every day	7	3	4
Rarely	22	7	15
Once a week	50	27	23
Every other day	113	40	73
Total	192	77	115

Frequency of consumption of the participants in the consumer test.



a) Percentage of consumption frequency of the participants of Forjães School Center; b) Percentage of consumption frequency of the participants of Esposende Elementary School; c) Percentage of consumption frequency of the participants of both schools in the consumer test.



## Characterisation of consumption habits

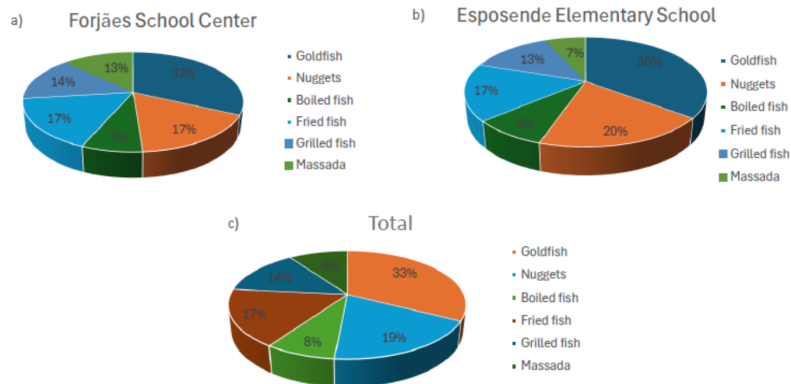
In the table it can be summarised the type of fish consumed by the participants in the consumer test. It is possible to observe that goldfish is the most consumed type of fish (33%), followed by nuggets (19%), then fried fillet (17%), grilled fillet (14%), "massada" (9%) and boiled fish (8%).

At Forjães School Centre, the consumption habits of the participants follow the same trend, with the goldfish representing the most consumed type of fish (32%), followed by nuggets (17%), then fried fillet (17%), grilled fillet (14%), "massada" (13%) and boiled fish (7%).

In relation to the Esposende Elementary School, the consumption habits of the participants are identical to those mentioned above, with goldfish being the most consumed type of fish (35%), followed by nuggets (20%), followed by fried fillet (17%), followed by grilled fillet (13%), followed by boiled fish (8%) and finally "massada" (7%).

Types of Fish	Number of participants (Both Schools)	Forjães School Center	Esposende Elementary School
Goldfish	126	52	74
Nuggets	72	30	42
Boiled fish	31	13	18
Fried fish	66	30	36
Grilled fish	53	25	28
Massada	37	22	15

Types of fish consumption of the participants in the consumer test.



a) Percentage of the types of fish consumed by the participants of Forjães School Center; b) Percentage of the types of fish consumed by the participants of Esposende Elementary School; c) Percentage of the types of fish consumed by the participants in the consumer test.

## Results of the acceptability test

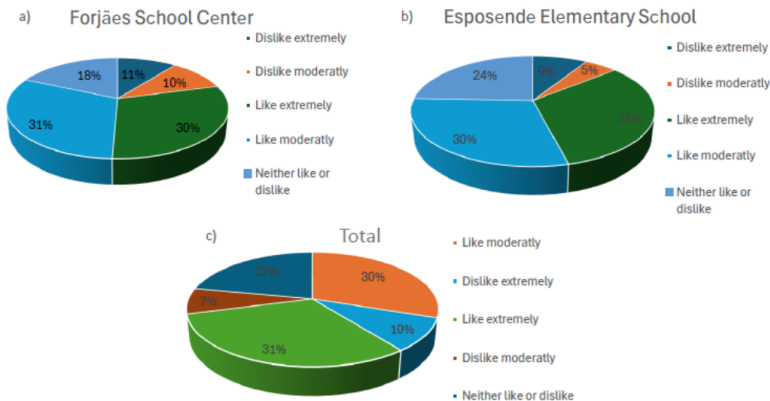
In terms of the overall appreciation of the product provided to the participants of the consumer test, in general there was a good acceptance of the product, since, about 31% liked extremely, followed by 30% who liked moderately, then 22% did not like or dislike, 10% disliked extremely and 7% disliked moderately.

In the School Center of Forjães, 30% liked extremely, followed by 31% who liked moderately, then 18% did not like or dislike, 11% disliked extremely and 10% disliked moderately.

In the Esposende Elementary School about 32% of the participants of the consumer test liked extremely, followed by 30% who liked it moderately, then 24% did not like or dislike, 9% disliked extremely and 5% disliked moderately. Thus, it can be concluded that the acceptance of the product was 61%.

Consumer Appreciation	Number of participants	Forjães School Center	Esposende Elementary School
Liked moderately	58	24	34
Dislike extremely	18	8	10
Like extremely	60	23	37
Dislike moderately	14	8	6
Neither like or dislike	42	14	28
<b>Total</b>	<b>192</b>	<b>77</b>	<b>115</b>

An overall evaluation of the consumer test.



a) Percentage of overall appreciation of the participants of Forjães School Center; b) Percentage of overall appreciation of the participants of Esposende Elementary School; c) Percentage of overall appreciation of the participants of both schools in the consumer test.



## **Chapter 7** **Dissemination and Outreach**

**The evidence produced by the Blue Project was disseminated at several national and international events.**

## **Participation in scientific meetings and conferences**



**Joana T. Martins**  
**Universidade do Minho**  
“Blue Project - Bioeconomy, People, Sustainability, Health”  
Biotechnology in the Food Sector,  
Porto, Portugal

## Participation in scientific meetings and conferences



**Joana Solinho and Rita Pinheiro, IPVC**  
“Fortification/enrichment of Sarrajão (Sarda sarda) fillets with hydrocolloid and vegetable and animal protein-based coatings”

S4Agro 2023, Castelo Branco, Portugal

## Participation in scientific meetings and conferences

[illegible]

**Ítala Marx, Universidade do Minho**  
 “Application of by-products from the  
 agricultural industry as natural preservatives  
 for fish-based product”

European Researchers' Night 2023,  
Coimbra, Portugal



## Participation in scientific meetings and conferences



**Ítala Marx, Joana Martins, Fernanda Ludtke, Jorge Vieira, António Vicente, Universidade do Minho**

**“Sustainable growth of the blue economy in Portugal: The Blue Project”**

**Circular Economy: Make It Happen, Braga, Portugal**

# Participation in scientific meetings and conferences



## Effect of the seasonality of Atlantic Bonito (*Sarda sarda*) on chemical, nutritional and sensory characteristics

Joana Solinho<sup>1</sup>, Ricardo Pereira-Pinto<sup>1,2</sup>, Joana Martins<sup>1,4</sup>, Jorge Vieira<sup>1,4</sup>, Rita Pinheiro<sup>1,5,6</sup>

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<sup>5</sup> [joanadolinho@hotmail.com](mailto:joanadolinho@hotmail.com), [rtapinheiro@ipvc.pt](mailto:rtapinheiro@ipvc.pt), [spereira@ipvc.pt](mailto:spereira@ipvc.pt), [joanasmartins@ipvc.pt](mailto:joanasmartins@ipvc.pt), [jorgevieira@ipvc.pt](mailto:jorgevieira@ipvc.pt)

### Abstract

Portugal is one of the major fish consumers in Europe and the World. According to the FAO and the World Health Organization, fish consumption should be increasingly recommended as a healthy and sustainable diet, not only for the diversity of species but also for its health benefits to consumers. Fish is an essential source of nutrients, as it is low in fat content and high level of protein, vitamins, and minerals. In recent decades, this food group's consumption has increased and become available to consumers far from coastal areas. This study aimed to compare Atlantic Bonito samples captured in two seasons (Spring and Autumn) to characterize the seasonality of this species regarding nutritional, chemical and sensorial properties. Parameters such as pH, water activity, moisture, protein, lipids, carbohydrates, chlorides, fibre, and ash were determined using the fish's muscular tissue. A quantitative descriptive analysis (QDA) was carried out with a panel of six semi-trained panelists. The fish attributes evaluated by the panelists were: characteristic colour, superficial shine, dripping, characteristic odour, off-odour, ammoniacal and sea odour, hardness, juiciness, fibrousness, characteristic flavour, acid, bitter and scallop taste, and off-flavour, evaluated on an intensity scale of 10 points (1 - lowest intensity, 10 - highest intensity). Also, samples were classified in terms of overall liking (1 to 5 points: 1 - very bad, 5 - excellent). Fish samples were cooked in a convective oven at 180°C. In order to compare not only seasonality but also the effect of the time during cooking, samples were divided into two batches: 5 and 10 min of cooking time. Results showed no significant differences on pH, water activity, moisture, protein, carbohydrates, chlorides, fibre and ash, between Spring and Autumn samples ( $p > 0.05$ ). Concerning lipids content, samples collected in the Autumn were 6.32-fold higher than Spring samples ( $p < 0.05$ ), with 7.55% and 1.21%, respectively. As for sensory analysis, panelists noticed some differences between Spring and Autumn samples regarding the following characteristics: superficial shine, dripping, characteristic and sea odour, hardness, juiciness, fibrousness, characteristic flavour and acid taste. These differences were more pronounced on 10 min batch samples, regardless the seasonality. The results of sensory analysis showed that fish Spring sample scored higher points than Autumn samples for all the attributes evaluated. The same behavior was found for 5 min cooked Spring sample fish, since even cooking (10 min) lead to lower satisfaction scores. It can be concluded that the Atlantic Bonito seasonality has an influence on sensory characteristics and nutritional properties, mainly lipids content. The panel preferred the Spring fish cooked at 180°C during 5 min.

**Acknowledgements** The authors thank the Esporanda Town Hall for accepting the master's work. The authors thank the Blue Project, Bioeconomy People, Sustainability, Health, Island-Land Interactions Norway grants, Blue Growth Programme, Call2 – Business, Development, Innovation and SMEs.

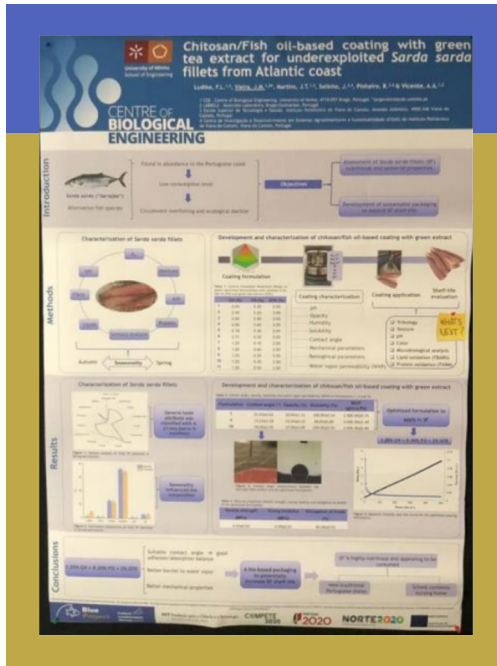
## Joana Solinho and Rita Pinheiro, IPVC

“Effect of the seasonality of Atlantic Bonito (*Sarda sarda*) on chemical, nutritional and sensory characteristics”

EFF23, Florence, Italy



## Participation in scientific meetings and conferences



**Jorge Vieira, Universidade do Minho**  
**“Chitosan/Fish oil-based coating with green tea extract for underexploited**  
**Sarda sarda fillets from Atlantic coast”**  
**DOF2023, Melbourne, Australia**

# Participation in scientific meetings and conferences

CONGRESSO  
INTERNACIONAL DA  
AGROINDÚSTRIA  
26 e 27 de Julho



CIAGRO 2023

AGROINDÚSTRIA  
4.0: DESAFIOS E  
OPORTUNIDADES\*

**CARACTERIZAÇÃO DAS PROPRIEDADES FÍSICO-QUÍMICAS, DE TEXTURA, COR, SENSORIAIS E MICROBIOLÓGICAS DO SARRAJÃO (S.ARD4 S.ARD4) FRESCO**

**CARACTERIZACIÓN DE LAS PROPIEDADES FÍSICOQUÍMICAS, DE TEXTURA, COLOR, SENSORIALES Y MICROBIOLÓGICAS DEL SARRAJÃO (S.ARD4 S.ARD4) FRESCO**

**CHARACTERISATION OF PHYSICO-CHEMICAL, TEXTURE, COLOUR, SENSORY AND MICROBIOLOGICAL PROPERTIES OF FRESH SARRAJÃO (S.ARD4 S.ARD4)**

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DOI: <https://doi.org/10.31682/TVCIAGRO.0209>

**RESUMO**

Segundo a Food and Agriculture Organization of United Nations (FAO) e a Organização Mundial de Saúde (OMS), o consumo de peixe é recomendado numa alimentação saudável e equilibrada, uma vez que contribui para colmatar uma parte importante das necessidades dos seres humanos, no nível dos ácidos gordos, omega-3 e vitaminas, prevenindo o risco de doenças cardiovasculares devido ao seu teor de colesterol, e favorecendo o desenvolvimento cognitivo normal. Portugal tem a maior consumo per capita (77 kg per capita/ano) de peixe da União Europeia (23 kg per capita/ano), classificando-o como um dos maiores do mundo. Os produtos de origem marinha possuem um papel primordial na alimentação humana, cerca de 14% da proteína animal consumida pelo ser humano em todo o mundo tem origem na pesca (Blinkiewicz e Kolakowska, et al., 2003).

O objetivo deste trabalho foi a caracterização das propriedades físico-químicas, de textura, cor, sensoriais e microbiológicas do Sarrajão (Gadus aeglefinus) fresco, inteiro e em formato de filé. Para tal determinou-se o teor de proteínas, lipídios, hidratos de carbono, cinzas, fibra, cinzas, humidade, a atividade da água e o pH. Efetuou-se uma análise do perfil de textura (TPA) aos filés e a firmeza do peixe inteiro, assim como a análise à cor. Foi também realizada uma análise descritiva quantitativa (QDA®) com um painel de sete provadores não-treinados. Foi feita uma análise microbiológica utilizando o Regulamento nº2073/2005 e as diretrizes definidas pela Agência de Proteção da Saúde (APS).

De acordo com os resultados obtidos constatou-se que o Sarrajão é um peixe com alto teor proteico, 23,4 e 0,32 % (p/p). Em relação à textura, os filés de peixe apresentam uma dureza de 3,55 N, 2,33, de coesividade e -0,17 N/mm<sup>2</sup>, de aderência e 11,38 N de granulidade. Ao nível da firmeza no peixe inteiro, verificou-se que a Zona 3 (cheefatura caudal) revelou ser a zona com maior firmeza. Relativamente à análise da cor foi possível verificar que os filés de Sarrajão apresentam uma baixa humidade (77 °) e uma cor amarelada (L\*, 38,76 e 5,4, respectivamente).

Em relação à análise sensorial, os atributos analisados foram: cor, brilho superficial, laca, odor característico, odor desagradável, odor marinho e a marinha, dureza, maciez, fibrosidade, sabor característico, sabor ácido, amargo e a marinha ligeira e sabor desagradável. O painel atribuiu maior pontuação aos atributos odor e marinha, maciez e sabor característico. Os Resultados das análises

**Joana Solinho and Rita Pinheiro, IPVC**

**“Characterisation of physico-chemical, texture, colour, sensory and microbiological properties of fresh Sarrajão (Sarda sarda)”**

**Ciagro 2023, Online Event**

# Participation in scientific meetings and conferences

**Combining Chitosan-Fish Oil-Green Tea Extract as A Potential Active Coating for Fresh Atlantic Bonito Fillet Preservation**

**Joana T. Martins<sup>1,2\*</sup>, Fernanda L. Ludtke<sup>1,2</sup>, Jorge M. Vieira<sup>1,2</sup>, Ítala Marx<sup>1,2</sup>, Joana Solinho<sup>3,4</sup>, Rita Pinheiro<sup>3,4</sup>, António A. Vicente<sup>1,2</sup>**

<sup>1</sup>Centre of Biological Engineering (CEB), University of Minho, Portugal; <sup>2</sup>LABELLS Associate Laboratory, Braga/Guimarães, Portugal; <sup>3</sup>Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo (IPVC), Portugal; <sup>4</sup>Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentabilidade (CISAS) do Instituto Politécnico de Viana do Castelo, Portugal

**Abstract:**

Sustainable bio-based packaging materials are gaining increasing attention by food manufacturers and consumers, as possible substitutes for synthetic plastic materials. In particular, edible biopolymer coatings/films could be applied to highly perishable food products to preserve their freshness and increase shelf life. Atlantic bonito (*Sarda sarda*) is a well-known fish from Atlantic Ocean with pleasant taste and high nutritional value. However, it is susceptible to lipid oxidation and spoilage. Therefore, a sustainable and active coating formulation composed by chitosan (CH)-fish oil (FO)-green tea extract (GTE) was developed to be applied on Atlantic bonito fillets' surface to extend its shelf life. CH, FO and GTE were selected due to their well-known antioxidant and antimicrobial effects. A Central Composite Rotational Design was developed to evaluate the effect of coating/film compounds' concentration – CH (0.5-2.31%, w/w), FO (0.20-0.44%, w/w) and GTE (2%, w/w)

FCT-2023  
November 27-29, 2023 | Paris, France

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– on their physicochemical and functional properties (e.g., mechanical, barrier, surface properties). The changes in quality of fish fillets were also assessed, such as pH and textural analysis. Based on surface contact angle results, 1.25% CH-0.30% FO-2% GTE formulation (78°) was the most promising one due to good coating adhesion ability on fish fillets' surface. Also, this formulation showed to be a good barrier to water vapor ( $2.29 \times 10^{-4}$  g/(m.s.Pa)) as well as good mechanical properties comparing to other tested formulations. The developed CH-FO-GTE coating/film displays properties that allow its use as an environmentally friendly active food packaging system to be applied to Atlantic bonito fillets.

**Joana T. Martins,**  
**Universidade do Minho**  
“Combining chitosan-fish oil-green tea extract as a potential active coating for fresh Atlantic Bonito fillet preservation”

9th International Conference on Food Chemistry & Technology (FCT-2023), Paris, France

## Participation in scientific meetings and conferences



**Joana Solinho, Sofia Machado  
and Rita Pinheiro**

“Valorisation of Atlantic Bonito and Brassica  
by-products in the development of a nutritionally  
rich pâté through factorial design”

5th Campus Sustainability Conference,  
Viana do Castelo, Portugal

## Participation in scientific meetings and conferences



**Fernanda L. Lüttke, Universidade do Minho**

**"Oregano oil emulsion-based coatings for food application: Impact of droplet size on Atlantic bonito (Sarda sarda) fillets' shelf-life"**

**19th Food Thessaloniki, Greece**

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## ESTG's World Food Day, Viana do Castelo, Portugal

## Participation in scientific meetings and conferences



**António A. Vicente, Universidade do Minho**

**“Development of sustainable strategies to extend Sarrajo fillets and products shelf-life”**

**ExpoBiotec'24, Braga, Portugal**

# Participation in scientific meetings and conferences



## PROGRAMA DAS 11ª JORNADAS DE CIÊNCIAS BIOTECNOLÓGICAS 20 DE NOVEMBRO DE 2023

### Enquadramento e objetivos

As Ciências da Vida e a Biotecnologia têm suscitado um interesse crescente por parte do público, em geral, devido às suas potencialidades para, nas próximas décadas, passarem a constituir áreas de ponta nos domínios da ciência, da indústria e do emprego.

A Biotecnologia assume-se como uma Tecnologia Facilitadora Essencial, cuja investigação e inovação têm como objetivos desenvolver produtos e processos industriais competitivos, sustentáveis e inovadores e contribuir como motor da inovação em variados setores tais como agricultura, alimentação, química e saúde. Outras fontes chave de inovação estão na interface entre biotecnologia e outras tecnologias facilitadoras e de convergência, em particular as nanotecnologias e as TICs, nomeadamente com aplicações em sensores e diagnóstico.

No quadro atual de uma população global crescente, que depende de recursos naturais, a Biotecnologia ajudará, certamente, a enfrentar os desafios com que a sociedade se depara, em particular, a sustentabilidade da agricultura e a segurança alimentar, a economia de baixo carbono, a inovação no abastecimento e tratamento de água, e a valorização dos resíduos em recursos, promovendo a bioeconomia circular, sustentável e baseada no conhecimento.

Como resposta a estes desafios a ESA-IPVC, através de projetos de ensino, como o curso de Licenciatura em Biotecnologia e o curso Técnico Superior Profissional em Industrias Biotecnológicas, e de I&D+i, pretende acompanhar os avanços científicos e tecnológicos nos domínios da Biotecnologia e contribuir para o desejável desenvolvimento sustentado de territórios e Organizações, promovendo processos de produção sustentáveis, baseados em aplicações biotecnológicas que visem a otimização dos processos, a utilização responsável dos recursos naturais e o tratamento e valorização de resíduos e efluentes, assim como a inovação em processos com vista ao desenvolvimento de novos produtos para os setores alimentar, cosmético, farmacêutico, entre outros.

No âmbito da 8ª Semana das Ciências da Vida e da Terra desenvolvem-se as 11as Jornadas de Ciências Biotecnológicas promovidas pelas Comissões do Curso de Licenciatura em Biotecnologia e Curso Técnico e Superior Profissional em Industrias Biotecnológicas, nas quais se pretendem apresentar e discutir temas atuais que incidem sobre as principais áreas de competência destes cursos, respondendo aos interesses e expectativas dos alunos destes cursos e dos restantes alunos de Academia, visando a participação dos docentes, investigadores e técnicos do IPVC, profissionais e entidades do setor.

O evento contará, no dia 20 de novembro de 2023, com sessões técnico-científicas organizadas em dois painéis temáticos: Domínios de Aplicação da Biotecnologia e Biotecnologia e Sustentabilidade.

### Comissão Organizadora

Ana Cristina Rodrigues, Ana Isabel Ferraz, Álvaro Queiroz, Isabel Afonso Paula, Júlio César Lopes, Ana Patrícia Guedes

**Rita Pinheiro, IPVC**

**“Blue Project - Bioeconomy, People, Sustainability, Health”**

**Biotechnological Sciences Conference, Viana do Castelo, Portugal**



## Participation in scientific meetings and conferences



**Joana Solinho, Sofia Machado and Rita Pinheiro**  
“Effect of heat treatment, hydrocolloid and Brassica  
concentration on texture properties of a Sarrajão pâté”  
XII FIRMA 2023, Online

## Participation in scientific meetings and conferences



**Associação  
iAPVC**  
Instituto de Alimentos  
Petrópolis e Saúde

**Resumo  
Resumo**

**Solinho, J.S.; Gonçalves, A.S.; Machado, S.V.; Pedreira, R.S.**

**Unidade de Produção e Manipulação de Alimentos, Instituto de Alimentos, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil**

## Enrichment of an Atlantic bonito burger with chickpea, Spirulina and *Fucus vesiculosus*: antioxidant activity, physicochemical and texture properties with different hydrocolloids

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### INTRODUCTION

The Consumer Market Orientation for Fish and Aquaculture Products report reveals the fish consumption in 2021 increased by 2.2 billion for the European region. It is a major industry development to improve the nutritional and organoleptic qualities of fish while adding the average weight of 100g of protein, which are added to the sustainability of the food environment.

The dietary needs in an abundant and cultured fish, composed of other benefits, makes it an ideal source of protein, which can be incorporated in it in 50g and can contribute to a characteristic taste.



### AIM

The objective of this work was to identify and optimize the addition of fish burger based on Atlantic bonito with the addition of plant-based ingredients such as chickpea and algae, and hydrocolloids to the formulation.

### METHODOLOGY

Matrix design: effect of adding the hydrocolloids (alginate, carrageenan, and xanthan gum) to the formulation of the Atlantic bonito burger. The matrix design was composed of 100g of Atlantic bonito burger, 10g of chickpea, 10g of algae, 10g of hydrocolloid, 10g of oil, 10g of salt, 10g of pepper, 10g of onion, 10g of garlic, 10g of tomato, 10g of carrot, 10g of celery, 10g of parsley, 10g of basil, 10g of oregano, 10g of thyme, 10g of rosemary, 10g of sage, 10g of dill, 10g of fennel, 10g of coriander, 10g of cumin, 10g of paprika, 10g of chili powder, 10g of onion powder, 10g of garlic powder, 10g of tomato powder, 10g of carrot powder, 10g of celery powder, 10g of parsley powder, 10g of basil powder, 10g of oregano powder, 10g of thyme powder, 10g of rosemary powder, 10g of sage powder, 10g of dill powder, 10g of fennel powder, 10g of coriander powder, 10g of cumin powder, 10g of paprika powder, 10g of chili powder, 10g of onion powder, 10g of garlic powder, 10g of tomato powder, 10g of carrot powder, 10g of celery powder, 10g of parsley powder, 10g of basil powder, 10g of oregano powder, 10g of thyme powder, 10g of 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**Joana Solinho, Sofia Gonçalves, Sofia Machado  
and Rita Pinheiro, IPVC**

## “Enrichment of an Atlantic bonito burger with chickpea, Spirulina and Fucus vesiculosus: antioxidant activity, physicochemical and texture properties with different hydrocolloids”

19th Food Colloids Conference Place, Thessaloniki, Greece

## Evidences from activities in schools

Matis visited schools in the Municipality of Esposende for a workshop on Sustainable Food



## Evidences from activities in schools

The Blue Project was integrated into the "Fish Chef" initiative, a competition in which students develop innovative fish-based recipes.



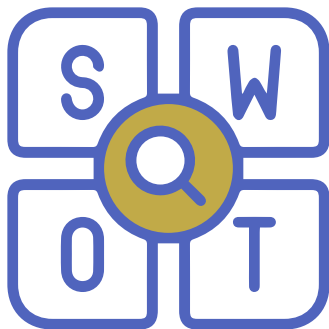




## **Chapter 8** **SWOT Analysis**

# SWOT Analysis

The Blue Project intends to build a strategic line of action, which has as its starting point the promotion of the consumption of local fish, guaranteeing catch quotas, promoting the reuse of excess of the Portuguese coast and reduce food waste.



## Strengths

- Innovation and sustainability that allowed collaboration, complementarity and synergies.
- High capacity in terms of infrastructure, resources and human capital.
- Scientific research expertise and knowledge of the consortium's universities.
- Offering a less common and more sustainable alternative.
- Exploration of maritime resources in Portugal's Exclusive Economic Zone and a species with high nutritional benefits.
- Creating consumption habits among new generations of consumers: children; as well as the practice of healthy and sustainable eating.
- Alignment with the UN's SDGs.
- Good dissemination of the project information and results.
- Promotion of the municipality of Esposende in terms of sustainability and external image.
- Adoption of sarrajão fillets by Esposende hotels and catering establishments.
- Involvement of schools and students in project activities.
- Increase in the number of schools serving sarrajão meals.
- Maximisation of waste management associated with the industry.
- Sarrajão skin used industrially.
- Scientific results achieved by the project.

## Weaknesses

- Challenges related to bureaucratic complexity, especially in licensing and regulatory processes.
- Complexity logistical in managing and transporting perishable products, as well in the fish fillet production and textile material development.
- Challenges associated with manual filleting of Atlantic Bonito.
- Sarrajão skin is sometimes difficult to work with



## Opportunities

- Promote sustainable consumption literacy among Portuguese consumers.
- Establishing partnerships to raise awareness of sarrajão (school canteens, hotel chains, restaurants, chefs, sushi restaurants).
- Development of sub-products derived from Atlantic Bonito.
- Developing projects with the Portuguese canning industry.
- Develop waste reduction strategies for other fish species and food sector product.
- Results of the scientific research for this project to be used in other fields.
- Being a reference project for the implementation of blue economy practices in the fishing community.
- Implementing local consumption and a local economy.
- Collaborations and partnerships with international institutions and companies to share knowledge and resources.
- Innovation for textile product design
- Integrating Sarrajão fish into the promotion of sustainable tourism, attracting consumers concerned with ethical practices.
- Positioning sarrajão products and by-products in sustainable product markets.
- Increasing concern about environmental issues and sustainability.
- Favorable environment for initiatives like the Blue Project, which promote responsible practices. The possibility of replicating the Blue Project model in other regions of Portugal or countries opens opportunities to broaden the project's impact and visibility.
- Growing research and development, strengthening the project's knowledge base.





## Threats

- Seasonal availability and migratory movements of the fish.
- Consumers' lack of knowledge of Sarrajão fish.
- Low exploitation and lack of knowledge of commercial practices for Sarrajão fish.
- There are few recipes and cooking practices using this product.
- Difficulty in implementing sustainable exploitation of sarrajão in the long term.
- Challenges relating to long-term economic viability.
- Not being an attractive product for consumers now.
- Lack of large-scale preservation techniques that do not jeopardize the quality of the product (texture, color, acidity (ph), nutritional values, smell).
- Difficulties in entering a competitive market which could jeopardize the commercial viability of the product.
- Other competitors entering the market or other initiatives that can pose a challenge to the differentiation and sustainability of the Project in the market.
- Competition from other sustainable protein sources, such as vegetarian alternatives and lab-grown meat.
- Impact of climate change and pollution on the migration routes of the sarrajão fish.
- Risk of environmental accidents, such as spills from cargo ships, directly affecting marine resources.
- Fluctuations and economic cycles can affect the availability of resources and investments necessary for the continuity of the Blue Project.
- Amendments to environmental or food safety legislation can impact the project's operations.
- Threat of competitive commercialization of sarrajão.





## **Chapter 9** **Blue Project impact**

# Milestones



• In the municipality of Esposende, the project allowed the increase of the consumption of sarrajão fish in the municipality's schools.



• With Matis' visit to Esposende schools, the children had the opportunity to carry out activities with the aim of being involved in the project, in order to involve the solar community in this dynamic.



• Through the University of Minho and IPVC, there was a collaboration of scientific work that allowed the design of innovative solution.

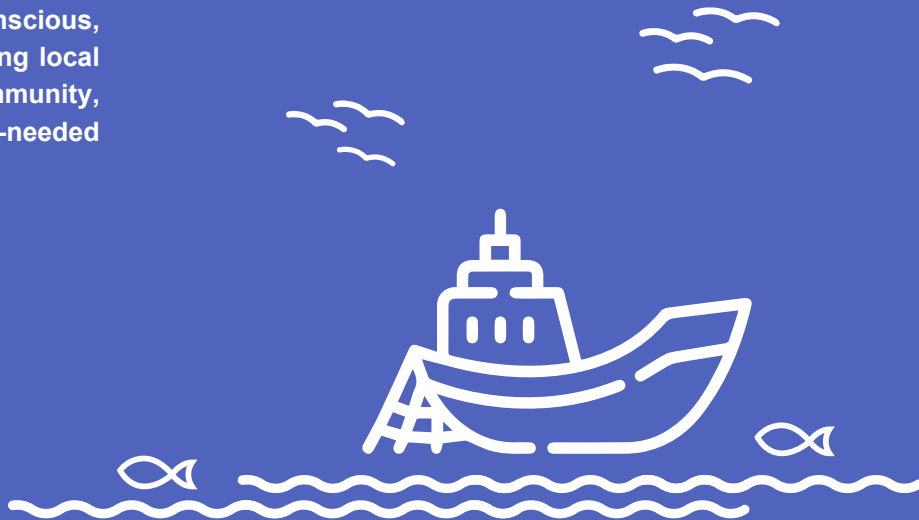


• Great collaboration between Guimarpeixe and Tintex, allowing the number of jobs in the respective companies to be boosted, due to the project.

# Impact

Blue Project is not limited to the implementation of sustainable fishing practices, but also takes part in the global aspirations to promote a circular economy and a more sustainable lifestyle.

The Blue Project will be able to promote a change in attitudes and behavior in the face of current global problems, educating consumers to make conscious, informed and sustainable choices. By promoting local fishing, it aims to involve the scientific community, industry and society in a bold and much-needed project.



# Social impact

- Introducing consumers to an alternative species of fish - Atlantic Bonito.
- Promotion of healthy and sustainable food consumption practices and habits among a new generation of consumers.
- Improving health and well-being of the local communities.
- Access to nutritious food and sustainable practices can enhance the quality of life for local residents.
- Encouraging competitiveness and innovation among stakeholders.
- Creation of employment opportunities in the fishing, processing, and distribution sectors.
- Commitment with the Sustainable Development Goals of 2030 Agenda of the United Nations: 2 - Zero Hunger, 3 - Ensure Healthy Lives and Promote Well-being for All at All Stages, 4 - Quality Education, 10 - Reduced Inequalities, 12- Responsible Consumption and Production, and 14 - Life Below Water.
- Growth of Investment (hours and amounts) in internal training of employees to participate in the project.
- Scientific publications inherent to the project.
- Feasibility of applying the scientific results obtained in future research or related projects.
- Very important research work to achieve goals and develop innovative solutions.
- Impact of participation in the project on municipal policies.
- Image and reputation in the area of sustainability of the municipality.
- Improving the quality of life of citizens.
- Involvement of schools in the municipality where children had the opportunity to participate in the project
- The Blue Project implemented food literacy strategies, with the aim of promoting fish consumption and reducing food waste.
- Students from primary schools in Esposende and Forjães had the opportunity to evaluate some dishes in which the main product was sarração fish.
- This initiative in schools intend to be continued, in order to promote good nutrition in children.



# Economic impact

- Adoption of blue and circular economy practices.
  - Increase the fishing communities income and the fishing sector's contribution to the national economy.
  - Strengthening the local economy by explore and innovate through a local natural resource.
  - Economic exploitation of surpluses, transforming waste into sub-products.
  - By utilizing leftovers from sarrajão fish and creating new products, the project can generate additional income streams for stakeholders.
  - Supporting local businesses and promoting sustainable practices can enhance economic resilience in the region.
  - Strengthening the value chain can lead to greater competitiveness, market access, and economic growth.
  - Growth of Guimarpeixe's investment in the Innovation area due to participation in the project.
  - Positioning of developed products on the market
- Adoption of blue and circular economy practices.



# Environmental impact

- Reducing ocean waste
- Reducing food waste
- Promote sustainability and the circular economy
- Promotion of food literacy
- Development of new products from food by-products to reduce waste.
- Implementation of awareness-raising and education actions for food sustainability.
- Conscientious management of local fishing activity to ensure environmental sustainability.





## **Chapter 10**

# **Cost-Benefit Analysis**



# Cost-Benefit Analysis

This report is a document that evaluates the costs and benefits of a project, policy, or program over a one-year period. It compares the expected outcomes of the intervention with the resources required to implement it.

The report helps decision-makers to assess the feasibility, efficiency, and effectiveness of the intervention and to identify potential trade-offs or alternatives.

Social	Economical	Environmental
<ul style="list-style-type: none"><li>• Introducing consumers to an alternative species of fish - Atlantic Bonito.</li><li>• Promotion of healthy and sustainable food consumption practices.</li><li>• Consumption of local products in a local economy approach.</li></ul> <p>Create healthy consumption habits among a new generation of consumers.</p> <ul style="list-style-type: none"><li>• Commitment with the Sustainable Development Goals of 2030 Agenda of the United Nations: 2- Zero Hunger, 3- Ensure Healthy Lives and Promote Well-being for All at All Stages, 4- Quality Education, 10- Reduced Inequalities, 12- Responsible Consumption and Production, and 14- Life Below Water.</li></ul>	<ul style="list-style-type: none"><li>• Increase the fishing community's income.</li><li>• Increase the fishing sector's contribution to the national economy.</li><li>• Encouraging competitiveness and innovation among consortium companies.</li><li>• Strengthening the local economy by explore and innovate through a local natural resource.</li><li>• Economic exploitation of surpluses, transforming waste into sub-products.</li><li>• Adoption of blue and circular economy practices.</li></ul>	<ul style="list-style-type: none"><li>• Sustainable exploitation of Atlantic Bonito.</li><li>• Diversification of fish species consumption.</li><li>• Non-polluting practices by the industries participating in this project (processes; waste management; materials).</li><li>• New uses for surplus fish, avoiding waste.</li><li>• Smaller ecological footprint in terms of distribution, by addressing a local and proximity economy.</li></ul>

# Assessment of costs and benefits

- The initial project costs can be significant, but the long-term benefits, such as health promotion, innovation and sustainability, can outweigh these costs.
- Intangible benefits, such as environmental awareness and nutritional education, must also be considered in the analysis.
- The assessment of return on investment must take into account not only financial aspects, but also social and environmental impacts.

## Performance indicators

- Indicators such as schools' adherence to the program, consumer acceptance of products and the impact on reducing food waste can be used to evaluate project performance.
- Cost-benefit analysis can therefore help determine the efficiency of the project in achieving its objectives.
- In short, based on these considerations, the project's cost-benefit analysis demonstrates significant potential to generate social, economic and environmental benefits, despite the initial costs involved. Ongoing evaluation is recommended throughout project implementation to monitor and adjust costs and benefits as necessary.



# Conclusion

- The Annual Cost-Benefit Analysis highlights a compelling cost-benefit scenario for stakeholders.
- The project demonstrates a strong alignment with sustainability goals, including promoting healthy consumption habits, increasing income for fishing communities, and fostering innovation in the fishing sector.
- The report emphasizes the economic benefits of the project, such as boosting the local economy and encouraging competitiveness and innovation among participating companies.
- Furthermore, the project's environmental benefits, such as sustainable exploitation of fish species and non-polluting practices, underscore its commitment to environmental stewardship.
- Overall, the project presents a robust case for investment, offering a favorable balance of costs and benefits across social, economic, and environmental dimensions.



Iceland  
Liechtenstein  
Norway grants



Promotor:



Parceiros:



TINTEX

