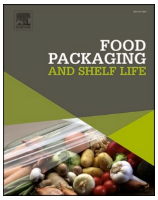
Food Packaging and Shelf Life 50 (2025) 101556

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl



Comparative study of preservation techniques for refrigerated Atlantic bonito Fillets: Effects of modified atmosphere packaging, vacuum packaging, and alginate coating on shelf life and quality

Joana Solinho [a,b,c], Joana Santos [a,b], Manuel Vázquez [c,*], Rita Pinheiro [a,b,**]

[a] School of Technology and Management, Polytechnic Institute of Viana do Castelo, Viana do Castelo, Portugal
[b] Centre for Research and Development in Agri-Food Systems and Sustainability (CISAS) of the Polytechnic Institute of Viana do Castelo, Viana do Castelo, Portugal
[c] Department of Analytical Chemistry, Faculty of Veterinary, Campus Terra, University of Santiago de Compostela, Lugo 27002, Spain

ARTICLE INFO

Keywords:
Atlantic bonito
Preservation techniques
Physicochemical evaluation
Sensory evaluation
Texture

ABSTRACT

Effective preservation strategies are essential to extend fish shelf life, minimize waste, and ensure product quality. Owing to its high perishability, fish is prone to rapid deterioration due to physicochemical changes and microbial growth. This study evaluated the effectiveness of vacuum packaging (50 % vacuum), modified atmosphere packaging (MAP: 80 % N_2 + 20 % CO_2), and sodium alginate coating (1 %), all under refrigeration, compared to refrigeration alone as a control. Atlantic bonito (*Sarda sarda*) fillets were stored at 4 °C for 15 days. Physicochemical parameters (moisture, pH, lipid and protein content, chloride, ash, and fibre), oxidative stability (peroxide index and TBARs), texture, colour, microbiological load, and sensory attributes were analysed at defined intervals. Among the treatments, MAP was most effective in preserving fillet quality, exhibiting the lowest peroxide (1.10 ± 0.05 mEq/kg) and TBARs values (1.29 ± 0.06 mg MDA/kg). MAP samples also demonstrated better retention of texture and colour and maintained microbial counts within acceptable regulatory limits throughout storage. Sensory evaluation confirmed superior freshness, texture, and aroma in MAP-treated fillets, closely resembling freshly processed fish. In contrast, samples stored under vacuum or with sodium alginate coating showed moderate improvements over refrigeration alone but were less effective than MAP. These findings highlight MAP as a promising packaging solution for extending the shelf life of Atlantic bonito, offering enhanced oxidative stability, microbial safety, and sensory quality. The results support the application of MAP in the fish industry to improve product stability and consumer acceptability during refrigerated storage.

1. Introduction

Atlantic bonito *(Sarda sarda)* is a species of marine fish belonging to the Scombridae family, which includes tuna and mackerel. Found in the Atlantic Ocean, the Mediterranean Sea and the Black Sea, it is an epipelagic, neritic, schooling scombrid (Zaboukas et al., 2006). This fish is notable for its nutrient-rich lipid/protein composition, high meat yield, and distinctive flavour (Altan et al., 2022). Despite its importance in sport fishing, it remains underexploited commercially, mainly due to limited research and exploration, even though it offers a rich composition of proteins, lipids, and unique flavour, coupled with ease of preparation (Orsi Relini et al., 2005). Recently, an innovative fish burger made with Atlantic bonito and enriched with chickpea, seaweed (*Spirulina* and *Fucus vesiculosus*), and hydrocolloids (xanthan and carrageenan) has been developed to offer consumers more product options (Solinho et al., 2025).

Overall, fish are an excellent source of polyunsaturated fatty acids, which provide significant benefits for muscle tissue, nutrition, and physiological health. However, these fatty acids are highly susceptible to oxidation when exposed to aerobic conditions. The primary oxidative products in fish oil tissues are unstable peroxide compounds. Due to their inherent instability and rapid breakdown into secondary degradation products, the peroxide value alone is not a reliable indicator of product quality. As oxidation progresses, peroxides decompose into more stable compounds, such as malonaldehyde, a thiobarbituric acid reactive substance (TBARs). The TBARs value generally increases in fish

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* Corresponding author.
** Corresponding author at: School of Technology and Management, Polytechnic Institute of Viana do Castelo, Viana do Castelo, Portugal.
E-mail addresses: manuel.vazquez@usc.es (M. Vázquez), ritapinheiro@estg.ipvc.pt (R. Pinheiro).

https://doi.org/10.1016/j.fpsl.2025.101556
Received 5 April 2025; Received in revised form 25 June 2025; Accepted 29 June 2025
Available online 4 July 2025

and fish products, correlating with changes in flavour and taste quality, including a reduction in overall acceptability (Selmi et al., 2010).

Fish proteins are highly valued for human nutrition due to their easy digestibility. Fish is also a major source of fat-soluble vitamins. According to the FAO, global fish consumption averages 17 kg per person per year, with over 3.2 billion people obtaining at least 15 % of their daily protein intake from fish. The WHO recommends consuming fish at least three times per week, as it provides high-quality proteins and essential nutrients, including fat-soluble vitamins (A, D, and E) and omega-3 fatty acids. Furthermore, fish is an excellent source of key minerals such as potassium, phosphorus, iodine, and selenium, which play a crucial role in energy metabolism and thyroid function, fatty fish is recommended due to its high levels of n-3 fatty acids, however lean fish also contains nutrients that may be beneficial in the prevention of cardiovascular disease (Tørris et al., 2018).

Freshness is a critical factor in the fish market, impacting both consumer satisfaction and economic stability. Several methods are available to assess fish freshness. However, many are expensive or impractical for routine application. Given that a substantial portion of the global population relies on fish for nutrition or subsistence, ensuring its freshness is essential. Nevertheless, the high perishability of fish poses a major challenge, as spoilage begins immediately after harvesting due to the production of metabolites that accelerate deterioration. To address this issue, Innovative technologies such as high hydrostatic pressure, natural preservatives, ozonation, irradiation, pulsed light technology, and retort pouch processing, have been developed to enhance product quality and extend shelf life from harvest to consumption (Prabhakar et al., 2020).

Fish meat is more susceptible to decomposition than other types of meat, making its handling, storage, and distribution more challenging. Traditionally, due to these limitations, fish has been consumed primarily in coastal regions where fishing is common. Given its high sensitivity to environmental conditions, fish must either be consumed immediately after capture or stored under optimal conditions to maintain its nutritional value and ensure it reaches consumers in the best possible state (Kocatepe et al., 2016).

Fish spoilage results from a combination of processes, including enzymatic autolysis, oxidation, and microbial growth. It is influenced by intrinsic factors such as endogenous enzyme activity, initial bacterial composition, muscle tissue fragility, as well as extrinsic factors related to water quality, aquaculture practices, food handling, packaging, storage, and transportation conditions, among others (Amaral et al., 2021). Microbial activity is the primary factor responsible for quality deterioration and spoilage of fishery products (Alfaro et al., 2013; Alfaro, Hernández, Marc et al., 2013). The growth of microorganisms, including bacteria, yeasts, and moulds, causes undesirable changes in texture, flavour, odour, and appearance, ultimately making the product unacceptable for consumption (Gould, 1996). Effective control of microbial growth is crucial for maintaining the quality and safety of fishery products. Among the factors influencing spoilage, storage time and temperature play a critical role (Durrani, 2020).

To address this challenge, the development of innovative approaches for extending shelf life has become essential, with the primary aim of maintaining optimal sensory and nutritional quality, as well as safety. Effective methods for preserving fish quality are vacuum packaging (VAC) and modified atmosphere packaging (MAP). These techniques help slow down the natural deterioration process and extend the product's freshness until consumption (Ghaani et al., 2016).

VAC and MAP have remained largely unchanged since their introduction in the early 20th century and are well-established techniques in the food industry. However, increasing concerns about food quality control and preservation have expanded their role beyond conventional use, making them essential for extending shelf life and maintaining product integrity (Bhargava et al., 2020; Ghaani et al., 2016).

The concept of VAC involves removing the air from a package containing the product and sealing it immediately. This method isolates the product from the external environment, reduces the package volume and prevents oxidative spoilage when films with oxygen (O_2) barrier are used (Fletcher, 2012). Due to its low-cost and effectiveness, VAC is widely used in the food industry (Kachele et al., 2017).

MAP is a packaging technique that alters the composition of gases inside the package, typically by reducing oxygen (O_2) levels and increasing the concentration of carbon dioxide (CO_2) and/or nitrogen (N_2). This modified environment helps slow down the growth of microorganisms, delay enzymatic activity, and reduce oxidative spoilage. MAP, particularly with carbon dioxide (CO_2) as an active component, is widely used alongside refrigeration to delay spoilage and extend the shelf life of fresh fishery products (Alfaro et al., 2013). Several studies have been conducted on the effect of MAP in combination with chilled storage on fish and fish products (Goulas & Kontominas, 2007), which conclude that the shelf life of fish in a CO_2 atmosphere can be extended by a factor of 1.5–2.0 (Sivertsvik et al., 2002). This technique, when combined with low storage temperatures, has proven to be highly effective in preserving fish quality (DeWitt & Oliveira, 2016; Lauzon et al., 2009; Liu et al., 2010; Powell et al., 2015; Sivertsvik et al., 2002; Wang et al., 2008). While storage time and temperature are key factors for the product quality, spoilage rates are also influenced by factors such as fish species, sanitary conditions on board, and the quantity of food present in the gastrointestinal tract (Ghaly et al., 2010).

Alginate is a bioactive, biocompatible, non-toxic, and biodegradable polysaccharide with stabilizing, thickening, and gel-forming properties. Its structural diversity and abundance make it a valuable material across various industries (Zia et al., 2015). Due to its biodegradability, gel-forming ability, high encapsulation efficiency, and wide availability, alginate is extensively used in food packaging and pharmaceuticals (Bilal & Iqbal, 2020; Carina et al., 2021). Alginate coating helped reduce lipid oxidation and could not only slow fish deterioration but also improve the fillets' external appearance (Sáez et al., 2020).

This study assessed the effects of VAC, MAP, sodium alginate coating (1 %) combined with refrigeration, and refrigeration alone as a control on the physicochemical, microbiological, and texture properties of Atlantic bonito fillets stored at 4 °C for 15 days. The significance of this research lies in the fact that no studies have specifically focused on Atlantic bonito (*Sarda sarda*), assessing its shelf-life extension using different refrigeration-based preservation techniques.

2. Experimental

2.1. Materials

Atlantic bonito (*Sarda sarda*) was supplied by Docapesca (Viana do Castelo, Portugal) and was captured in the North Atlantic Coast of Portugal, sent to the company Guimarpeixe, where the samples were subjected to ultra-freezing (-80 ºC), transported to the laboratory under freezing temperatures (-18 ºC) and kept at −18 ºC until processing. Ten fish samples were used in this study, with approximately 1–1.5 kg each. Prior to processing, the samples were thawed in a refrigerator for 14 h at 4 °C.

2.2. Processing of fish samples

The samples of Atlantic bonito fillets (*Sarda sarda*) were prepared by gutting the fish, manually filleting them, removing the skin layer, immediately washing them with tap water, and placing them in polyamide/polyethylene plastic bags (PA/PE - 20/70). The properties of the bags are thickness, 90 μm; O_2 permeability, 50 cm^3/m^2 d bar; CO_2 permeability, 150 cm^3/m^2 d bar; N_2 permeability, 10 cm^3/m^2 d bar; Water vapor transmission, 2.8 g/m^2 d). Each package contained approximately 250 g of fish, and the bags provided a total film area of 750 cm^2 per package.

For the REF - refrigeration alone, the samples were placed in polyamide/polyethylene (PA/PE, 20/70) plastic bags, sealed, and stored at

4 °C under refrigerated conditions.

For MAP - modified atmosphere packaging, a mixture of gases (80 % N_2 + 20 % CO_2) was prepared using a mixer (PBI Dansensor - MAP MIX 8000EL, Denmark). The packages were then placed in a packaging machine (Sammic - SU-810GLL, Spain) where the gas mixture was introduced into the bags and sealed. The gas composition of 80 % N_2 and 20 % CO_2 was selected to balance microbial inhibition and product quality.

For VAC - vacuum packaging, the samples were placed in the same packaging machine where 50 % of the air was removed, the vacuum level was set to achieve approximately 50 % vacuum inside the packaging. This setting was selected based on calibration and prior trials, and the bags were then sealed and stored at 4 °C until analysis.

For the COAT - coating technique, 1 % sodium alginate was prepared by dissolving sodium alginate in distilled water under continuous stirring at room temperature until fully hydrated and homogeneous. Fillet samples were immersed in the alginate solution, ensuring full surface coverage and removed immediately. This process created a stable and adherent alginate film acting as a semi-permeable barrier to gases and moisture, thereby helping to slow down oxidation and overall product deterioration.

The samples were then placed on a polystyrene tray before being packed in unsealed PA/PE bags and stored at 4 °C for 15 days. The samples were analysed on days 0, 3, 7, 10, and 15.

2.3. Chemical composition

The pH values were measured using a pH meter (pH 25 +, Crison, Spain), calibrated with pH 4.00, 7.00 and 10.00 standard solutions. The moisture content was determined following the AOAC 925.10:1995 method. A 3 g sample was dried in an oven at 103 ± 2 °C until a constant weight was reached in approximately 340 min.

Crude fibre was determined using the AOAC 962.09:1995 Method. A 2 g sample placed in a reflux balloon and treated with H_2SO_4 and KOH. After each boiling, the sample was filtered. The filters (FiltraTech - Glass microfibre 1.2 µm, France) were then dried in an oven at 130 ± 2 °C for 2 h, weighed, and subsequently ashed in a muffle furnace (Heraeus, M110, Hanau, Germany) at 600 ± 15 °C for 30 min before weighing again.

Sodium chloride content was determined using the AOAC 937.09:1995 method. Approximately 1 g of sample was weighed into an Erlenmeyer flask, followed by the addition of 0.1 N $AgNO_3$ and concentrated HNO_3. After boiling for 15 min, the solution was cooled to room temperature, and water along with iron (II) ammonium sulphate was added. Finally, the excess $AgNO_3$ was titrated until a permanent light brown colour was observed. Ash content was determined using the AOAC 938.08:1995 method. A 3 g sample was weighed and placed in the muffle furnace oven (Heraeus, M110, Hanau, Germany) at a temperature of ≤ 550 ºC for 30 min, after they were weighed to determine the ash content.

Protein content was determined using the AOAC 955:04:1995 method by the Kjeldahl method. The sample was digested at 420 °C for 90 min after adding 98 % H_2SO_4 and a catalyst, using a DK6 heating digester (Velp Scientifica, Usmate (MI), Italy). The digested sample was then distilled after adding 40 % NaOH, and the distillate being was collected in a 4 % boric acid solution using a Velp UDK 140 distillation unit (Velp Scientifica, Usmate (MI), Italy). The nitrogen concentration was obtained by titration with 0.1 N HCl. The protein content was determined by multiplying the nitrogen content by the factor of 6.25.

Lipid content was determined using AOAC Method 920.39:1995. A 5 g fish sample was weighed and 4 N HCl was added. The sample was then heated to boiling on a heating plate for 1 h. After boiling, the contents were filtered under vacuum, and the filters (Labbox - Qualitative filter paper 90 mm, Whatman 93, Espanha) were placed in an oven at 102 ± 2 ºC for 1 h. Once dried, the lipids were extracted for 4–6 h. After the extraction stage, the solvent was evaporated in a rotary evaporator, and the sample was further dried in an oven at 102 ± 2 ºC until a constant weight was achieved. Finally, the retained fat was weighed.

Thiobarbituric acid reactive substances (TBARS) method was carried out as reported elsewhere (Pinela et al., 2012) and the peroxide values (PV) were determined using the iodometric method with visual endpoint detection, as described in ISO 3960:2017. All the analysis were performed in triplicate.

2.4. Sensory analysis

Initially, the panellists defined the anchors and attributes for evaluating the sensory characteristics of the raw fillets during storage. Subsequently, a Quantitative Descriptive Analysis (QDA®) was conducted according to ISO 6658:2017, with seven previously trained panellists. The evaluated attributes included characteristic colour, superficial shine, chipping, characteristic odour, off-odour, ammoniacal odour, sea odour, hardness, succulence, and fibrousness. These attributes were rated on a 10-point intensity scale (1 = lowest intensity, 10 = highest intensity). Additionally, the samples were assessed for overall taste on a 5-point scale (1 = very bad, 5 = excellent). Each sample was served on an individual plate, coded with a three-digit number, and evaluated by the panellists.

2.5. Texture and colour determination

The texture analysis was performed using a penetration test using the TA-XT2i Texture Analyser (Stable Micro Systems Ltd, United Kingdom), equipped with a stainless-steel cylindrical a probe (P/10). The texture properties measured included firmness, cohesiveness and adhesiveness. Fish fillets were subjected to deformation at room temperature (25 °C). The probe punched into a constant crosshead test velocity of 0.5 mm/s and a test distance of 8 mm. Eight replicates were performed for each run, using different areas of the sample. The colour of the samples was assessed using a Minolta CR300 (Konica Minolta, Japan) with the colour system CIE L*, a*, b*. Lightness (L *), redness to greenness (+a* to -a*) and yellowness to blueness (+b* to -b*). Fifteen replicates were performed, using different areas of the sample.

2.6. Microbiological analysis

Fish fillet samples (25 g) were aseptically taken from different parts, homogenised for 60 s in a stomacher, and serially diluted for subsequent analysis. The primary diluent used for the mother dilution was Peptone Buffered Water (PBW) for *Salmonella spp*. and *Listeria monocytogenes*, while Alkaline Saline Peptone Water (ASPW) was used for *Vibrio parahaemolyticus*. Peptone salt (PS) was used for subsequent dilutions.

The total bacteria count was performed according to EN ISO 4833–1:2013 and amendment 1:2022. Yeasts and moulds were quantified following ISO 21527–1:2008, while Enterobacteriaceae counts were determined using ISO 21528–2:2017. Coagulase-positive staphylococci were enumerated according to ISO 6888–1:2021 and amendment 1:2023. *Listeria monocytogenes* was detected and enumerated using ISO 11290–1:2017, and *Salmonella* detection followed ISO 6579–1:2017 and amendment 1:2020. Sulphite-reducing Clostridia spores were enumerated following Portuguese Standard NP 2262:1986S. Lactic acid bacteria were quantified according to ISO 15214:1998, and *Vibrio parahaemolyticus* detection was performed following ISO 21872–1:2017. All analyses were performed in triplicate.

2.7. Statistical analysis

Statistical analysis was conducted by TIBCO® Statistica®, v.14.0.0, TIBCO Software Inc, Palo Alto, CA, USA. Results are presented as mean values ± standard deviation (SD) for each determination. Data were subjected to analysis of variance (ANOVA), a Tukey HSD test.

Additionally, Principal Component Analysis (PCA) was applied to assess differences between means. Significant differences were set at $p < 0.05$.

3. Results and discussion

3.1. Proximate analysis and evaluation of lipid oxidation

Table 1 presents the results of the proximate analysis (lipids, protein, chlorides, ash, and fiber) of Atlantic bonito fillets subjected to different preservation techniques combined with refrigeration (VAC, MAP, sodium alginate coating 1 %) as well as refrigeration alone as a control. The fillets were stored at 4 °C for 15 days. Proximate analysis of Atlantic bonito fillets revealed protein, ash, and lipid contents of 26.26 ± 0,27 g/100 g, 1.24 ± 0.01 g/100 g, and 4.63 ± 0.17/100 g, respectively. These results are consistent with findings reported by (Oksuz, 2008; Özden, 2010) for Atlantic bonito fillets, indicating a similar biochemical composition across different studies.

Regarding lipid content, fresh fillets exhibited a value of 4.63 ± 0.17 g/100 g at day 0. After 15 days of storage, no significant difference was observed in the VAC (3.62 ± 0.02 g/100 g) compared with the control. In contrast, the other techniques resulted in significantly higher values ($p < 0.05$). The protein content was 26.26 ± 0.27 g/100 g at the beginning of the experiment (day 0). After 15 days of storage, it remained relatively stable in the refrigerated control sample (26.14 ± 0.11 g/100 g). However, it significantly decreased to 25.24 ± 0.56 g/100 g in the MAP sample and to 22.34 ± 1.09 g/100 g in the coated samples, ($p < 0.05$). Notably, the VAC samples showed a statistically significant difference, retaining the highest protein content (26.6 ± 0.55 g/100 g) compared to the other preservation techniques.

The chloride content was 0.57 ± 0.02 g/100 g at the start of the experiment (0 days). After 15 days of storage with different preservation techniques, it decreased to 0.48 ± 0.00 g/100 g under MAP, to 0.48 ± 0.03 g/100 g with coating, and 0.48 ± 0.04 g/100 g under VAC. No significant differences were observed compared to the control under refrigeration alone (0.44 ± 0.02 g/100 g). The ash content of Atlantic bonito fillets was 1.24 ± 0.01 g/100 g at the start of the experiment (0 days). After 15 days of storage under various preservation methods, the values increased significantly ($p < 0.05$) to 1.46 ± 0.07 g/100 g for the refrigeration sample, 1.27 ± 0.04 g/100 g for the MAP sample, 1.36 ± 0.11 g/100 g for the coated sample, and 1.37 ± 0.09 g/100 g for the VAC sample.

At the beginning of the experiment (day 0), the fibre content was 0.69 ± 0.26 g/100 g. After 15 days of storage, it decreased significantly ($p < 0.05$) to 0.14 ± 0.03 g/100 g in the MAP sample and to 0.34 ± 0.10 g/100 g in the VAC sample. In contrast, it remained relatively stable in the refrigerated control sample (0.76 ± 0.55 g/100 g). Notably, the fibre content increased to 1.19 ± 0.90 g/100 g in the coated sample, likely due to the alginate coating, which was quantified as fibre.

Table 2 presents the variations in pH and moisture content observed during storage. The initial pH of fillets stored under modified atmosphere packaging was 6.08 ± 0.03. A gradual decrease was observed over time, reaching 5.96 ± 0.01 by day 10, followed by a slight increase to 6.02 ± 0.01 at day 15. This trend suggests an initial acidification phase, likely due to CO_2 accumulation in the packaging, followed by a stabilization phase as equilibrium was reached.

Fillets stored under refrigeration alone exhibited slight pH fluctuations, ranging from 6.00 ± 0.01 on day 3–6.10 ± 0.00 on day 10. This variation could be attributed to microbial metabolic activity, which generates alkaline compounds such as ammonia, counteracting the initial acidification. In VAC-stored fillets, pH remained relatively stable, fluctuating slightly between 6.02 ± 0.03 (day 3) and 6.10 ± 0.01 (day 10). The absence of oxygen under vacuum conditions likely contributed to reduced microbial activity, slowing metabolic changes in the fillets. Similarly, coated fillets displayed pH fluctuations between 6.03 ± 0.01 (day 3) and 6.10 ± 0.01 (day 15). The alginate coating may have influenced microbial and enzymatic activity, delaying acidification compared to other preservation methods.

Over time, a gradual pH decline was observed across all preservation techniques, reflecting ongoing biochemical changes. Among them, MAP packaging exhibited the slowest pH decrease, reaching 6.02 ± 0.01 on day 15. In contrast, refrigeration alone (6.09 ± 0.00) and VAC packaging (6.06 ± 0.00) maintained slightly higher pH values than MAP. Notably, the coating treatment preserved the pH at 6.10 ± 0.01, likely due to the protective properties of alginate, which may have helped regulate microbial and enzymatic activity.

In terms of moisture content, MAP packaging led to a noticeable decrease, from 74.33 ± 0.05 g/100 g (initial) to 64.87 ± 1.21 g/100 g (day 15), likely due to gas exchange and dehydration effects associated with this storage method. Samples stored under refrigeration alone also showed a decline in moisture, reaching 66.38 ± 0.18 g/100 g by day 15, suggesting gradual water loss through evaporation and potential enzymatic degradation of muscle structure. In contrast, VAC-stored fillets maintained relatively higher moisture content, decreasing from 74.33 ± 0.05 g/100 g to 70.50 ± 0.16 g/100 g over 15 days. Vacuum conditions helped retain moisture by minimizing air exposure and reducing oxidative processes that could lead to dehydration. Coated fillets demonstrated the best moisture retention, with values stabilizing at 70.96 ± 0.32 g/100 g by day 15. This suggests that the sodium alginate coating acted as an effective barrier, limiting water loss and helping preserve both texture and freshness. These results align with previous studies (Özden, 2010; Vurat & Kocatepe, 2023) here moisture content values around 66.8 ± 0.53 g/100 g and pH values near 6.03 ± 0.08 for Atlantic bonito fillets were reported. The observed variations among preservation methods emphasize the role of packaging in maintaining the biochemical integrity of fish fillets during storage.

To assess the impact of these storage methods on the oxidative stability of the fillets, the peroxide value (PV) and thiobarbituric acid reactive substances (TBARs) were analysed. The peroxide value serves as a key indicator of primary lipid oxidation, reflecting the formation of hydroperoxides, which can subsequently degrade into secondary oxidation products detected by the TBARs analysis. These oxidation products compromise both the quality and sensory attributes of the fish. Table 2 presents the peroxide content of Atlantic bonito fillets stored for 15 days under various preservation techniques, including modified

Table 1
Proximate composition (lipids, protein, chlorides, ash and fibre) of Atlantic bonito fillets over 15 days of storage using different preservation techniques combined with refrigeration (modified atmosphere, vacuum and coating) as well as refrigeration alone as a control.

Parameters (g/100 g)	Time (0 day) (Fresh fillets)	Time (15 days)			
		REF (Control)	MAP	VAC	COAT
Lipids	4.63 ± 0.17^{c}	8.53 ± 0.24^{ab}	9.02 ± 0.28^{a}	3.62 ± 0.02^{c}	7.71 ± 0.79^{b}
Protein	26.26 ± 0.27^{a}	26.14 ± 0.11^{a}	25.24 ± 0.56^{b}	26.6 ± 0.55^{a}	22.34 ± 1.09^{b}
Chlorides	0.57 ± 0.02^{a}	0.44 ± 0.02^{b}	0.48 ± 0.00^{b}	0.48 ± 0.04^{b}	0.48 ± 0.03^{b}
Ash	1.24 ± 0.01^{b}	1.46 ± 0.07^{a}	1.27 ± 0.04^{a}	1.37 ± 0.09^{a}	1.36 ± 0.11^{a}
Fibre	0.69 ± 0,26^{a}	0.76 ± 0.55^{a}	0.14 ± 0.03^{b}	0.34 ± 0.10^{b}	1.19 ± 0.90^{a}

REF- Refrigeration; MAP – modified atmosphere packaging; VAC – Vacuum Packaging; COAT-Coating. Mean values ± standard deviation (n = 3). Means within the same row with different superscripts are significantly different at $p < 0.05$.

Table 2

pH value, Moisture content, Peroxide value and Thiobarbituric acid reactive substances (TBARS) of Atlantic bonito fillets during 15 days of storage using different preservation techniques combined with refrigeration (modified atmosphere, vacuum and coating) as well as refrigeration alone as a control.

Parameters	Time (days)	REF (Control)	MAP	VAC	COAT
pH	0	6.08 ± 0.03^{a}	6.08 ± 0.03^{a}	6.08 ± 0.03^{a}	6.08 ± 0.03^{ab}
	3	6.00 ± 0.01^{b}	6.04 ± 0.01^{ab}	6.02 ± 0.03^{b}	6.03 ± 0.01^{bc}
	7	6.08 ± 0.01^{a}	6.01 ± 0.00^{bc}	6.10 ± 0.01^{a}	6.08 ± 0.01^{abc}
	10	6.10 ± 0.00^{a}	5.96 ± 0.01^{c}	6.10 ± 0.01^{a}	6.03 ± 0.02^{c}
	15	6.09 ± 0.00^{a}	6.02 ± 0.01^{b}	6.06 ± 0.00^{ab}	6.10 ± 0.01^{a}
Moisture (g/100 g)	0	74.33 ± 0.05^{a}	74.33 ± 0.05^{a}	74.33 ± 0.05^{a}	74.33 ± 0.05^{a}
	3	73.61 ± 5.47^{ab}	64.95 ± 0.13^{b}	70.65 ± 0.31^{b}	67.32 ± 1.32^{c}
	7	67.07 ± 0.13^{bc}	65.23 ± 2.72^{b}	68.54 ± 0.13^{c}	68.40 ± 0.29^{c}
	10	69.43 ± 0.04^{abc}	65.30 ± 0.28^{b}	70.96 ± 0.32^{b}	71.27 ± 1.15^{b}
	15	66.38 ± 0.18^{c}	64.88 ± 1.21^{b}	70.50 ± 0.16^{b}	70.96 ± 0.32^{b}
Peroxide value (mEq/kg sample)	0	0.93 ± 0.005^{b}	0.93 ± 0.005^{b}	0.93 ± 0.005^{b}	0.93 ± 0.005^{b}
	7	16.72 ± 0.03^{ab}	1.26 ± 0.01^{ab}	2.31 ± 0.02^{ab}	18.46 ± 0.04^{a}
	15	23.3 ± 0.10^{a}	1.01 ± 0.05^{b}	6.15 ± 0.02^{ab}	17.73 ± 0.04^{ab}
Thiobarbituric acid reactive substances (mg malondialdehyde/kg sample)	0	0.30 ± 0.05^{c}	0.30 ± 0.05^{c}	0.30 ± 0.05^{b}	0.30 ± 0.05^{e}
	3	2.08 ± 0.08^{b}	0.29 ± 0.02^{c}	0.49 ± 0.05^{b}	2.66 ± 0.11^{d}
	7	7.93 ± 0.06^{a}	0.73 ± 0.02^{ab}	1.94 ± 0.07^{a}	8.63 ± 0.08^{c}
	10	8.4 ± 0.08^{a}	1.3 ± 0.06^{a}	0.4 ± 0.04^{b}	18.8 ± 0.17^{a}
	15	7.32 ± 0.10^{a}	1.29 ± 0.06^{a}	0.78 ± 0.06^{ab}	11.92 ± 0.22^{b}

REF- Refrigeration alone; MAP – modified atmosphere; VAC – Vacuum Packaging; COAT-Coating. Mean values ± standard deviation (n = 3). Means within same column (in each variable) with different superscripts are significantly different at $p < 0.05$.

atmosphere, refrigeration, coating, and vacuum packaging at 4 °C.

The peroxide value was 0.93 mEq/kg at time 0 (fresh fillets), indicating minimal oxidation. Over the storage period, a significant increase ($p < 0.05$) in PV was observed, with variations across the different preservation techniques. This highlights the influence of the applied preservation methods on the oxidative stability of the fillets.

Refrigeration alone showed a sharp increase in PV, reaching 16.72 mEq/kg at day 7 and 23.40 mEq/kg by day 15. This substantial rise suggests that oxygen exposure and microbial activity under simple refrigeration accelerate lipid peroxidation. The lack of oxygen-barrier packaging likely facilitated oxidative degradation, resulting in higher peroxide accumulation compared to other preservation methods.

MAP storage showed a slight increase in PV, reaching 1.26 mEq/kg by day 7, before stabilizing at 1.10 mEq/kg by day 15. The relatively low peroxide formation in MAP can be attributed to the high nitrogen (N_2) concentration, which limits oxygen availability and thus reduces oxidative reactions. Additionally, the presence of carbon dioxide (CO_2) in the gas mixture likely contributed to microbial inhibition, indirectly slowing down the oxidation process.

Vacuum packaging showed moderate peroxide formation, with values reaching 2.31 mEq/kg on day 7 and 6.15 mEq/kg by day 15. The oxygen-depleted environment provided by vacuum packaging likely slowed down oxidation; however, residual oxygen and enzymatic reactions may have still contributed to gradual peroxide formation over the storage period.

Coating with sodium alginate resulted in a peak peroxide value of 18.41 mEq/kg on day 7, which remained relatively high at 17.77 mEq/kg by day 15. Although edible coatings can act as protective barriers, their effectiveness largely depends on moisture retention and gas permeability properties. In this case, the alginate coating may have trapped moisture, providing a favourable environment for microbial activity and enzymatic oxidation, which ultimately contributed to increased lipid degradation.

These findings emphasize the improved oxidative stability offered by modified atmosphere packaging, followed by vacuum storage, while refrigeration alone and coating resulted in higher lipid peroxidation rates, which could negatively impact sensory quality and shelf life. The observed trends are consistent with studies that highlight the critical role of oxygen exposure and storage conditions in influencing lipid oxidation in fishery products. the peroxide value showed a gradual tendency to increase in the samples in all packaging systems studied. according to (Alice et al., 2020).

Fig. 1 illustrates the variation in TBARs values in Atlantic bonito fillets over 15 days of storage under different preservation techniques studied. TBARs analysis is a widely used method for evaluating secondary lipid oxidation, a process that significantly impacts food quality. This method measures the concentration of malondialdehyde (MDA), a key secondary product formed during the oxidation of polyunsaturated fatty acids (PUFAs), which serves as an indicator of oxidative degradation in fishery products . Higher TBARs values indicate greater lipid degradation, which can lead to rancidity and undesirable sensory changes, such as off-flavours and altered texture. These changes are particularly important for consumers' perception of freshness and overall quality in fishery products. Therefore, monitoring TBARs levels is crucial for assessing the shelf life and acceptability of stored fish.

In the case of refrigeration alone (control), a rapid and significant increase in TBARs was observed. The value reached 2.08 ± 0.08 mg MDA/kg by day 3 and peaked at 8.37 ± 0.08 mg MDA/kg on day 10, before slightly decreasing to 7.32 ± 0.10 mg MDA/kg by day 15. The high oxygen exposure under refrigeration promotes autoxidation of lipids, accelerating rancidity. The slight decrease after day 10 may be attributed to the further degradation of malondialdehyde (MDA) into volatile compounds, which reduces its measurable concentration, thus explaining the observed drop in TBARs values toward the end of the storage period.

In MAP storage, the TBARs value remained relatively low throughout the storage period. It started at 0.30 ± 0.05 mg MDA/kg at time 0, slightly decreased to 0.29 ± 0.02 mg MDA/kg on day 3, and then gradually increased to 1.32 ± 0.06 mg MDA/kg by day 10, before slightly stabilizing at 1.29 ± 0.06 mg MDA/kg on day 15. The limited oxidative increase observed in MAP is likely due to the reduced oxygen availability, which slows down lipid oxidation. Additionally, the presence of CO_2 in the gas mixture may have contributed to microbial inhibition, thereby indirectly preserving lipid stability by reducing the microbial activity that can accelerate oxidation.

For VAC packaging, oxidative stability was better maintained compared to refrigeration, with TBARs values rising moderately from 0.30 ± 0.05 mg MDA/kg at time 0–1.94 ± 0.07 mg MDA/kg on day 7. The values then decreased slightly to 0.40 ± 0.04 mg MDA/kg on day 10 and reached 0.78 ± 0.06 mg MDA/kg by day 15. The lower oxidation levels observed in vacuum-stored samples can be attributed to the absence of oxygen, which significantly slows down lipid peroxidation. However, enzymatic oxidation, which does not require oxygen, may still occur at a reduced rate, contributing to the moderate increase in TBARs over time.

Coated samples exhibited the highest levels of lipid oxidation, with

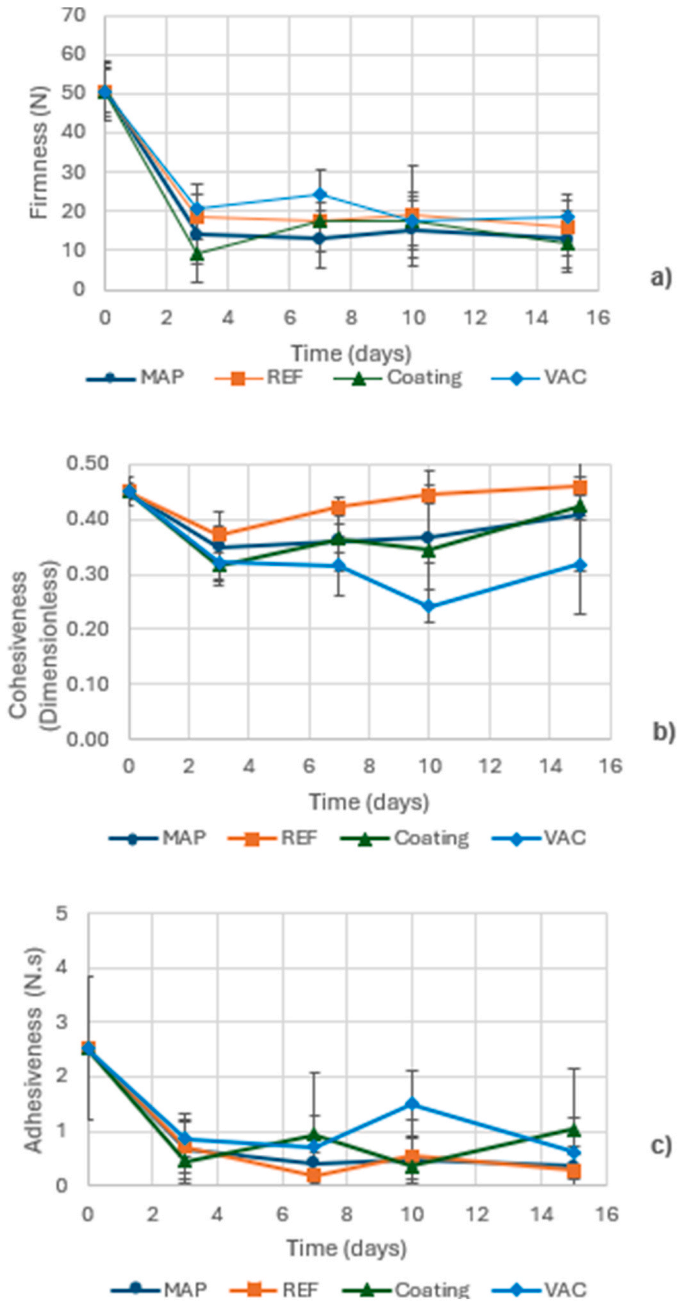

Fig. 1. Texture parameters obtained for the Atlantic bonito fillets during 15 days of storage at 4 °C in different preservation techniques (MAP - modified atmosphere, REF – refrigeration alone (control), VAC - vacuum and Coating): a) Firmness; b) Cohesiveness c) Adhesiveness.

TBARs increasing sharply from 2.66 ± 0.11 mg MDA/kg on day 3–18.85 ± 0.17 mg MDA/kg on day 10, before decreasing to 11.92 ± 0.22 mg MDA/kg on day 15. The significant oxidation in coated samples suggests that while the coating may have provided a physical barrier, it could have also trapped moisture, creating favourable conditions for microbial activity and enzymatic oxidation. Furthermore, if the coating was not an effective oxygen barrier, it may have allowed oxidative processes to continue without restriction, leading to accelerated lipid degradation.

Overall, TBARs values exhibited a pattern of initial stability followed by a sharp increase, particularly in refrigeration and coating treatments, until day 10. After day 10, a slight decrease in TBARs values was observed in some treatments, likely due to the breakdown of malondialdehyde (MDA) into volatile compounds that are not detected by the TBARs assay (Alice et al., 2020)). Fish fillets are considered of excellent quality when TBARs values remain below 3 mg MDA/kg, while values exceeding 5 mg MDA/kg indicate quality deterioration (Schormüller, 1968)). The threshold for human consumption is typically set between 7 and 8 mg MDA/kg. Based on these criteria, only the refrigerated alone and coated samples exceeded the acceptability limit after 7–10 days, suggesting that these preservation techniques are less effective at maintaining lipid quality during prolonged storage.

These findings reinforce the effectiveness of MAP and VAC packaging in slowing lipid oxidation and preserving the freshness of Atlantic bonito fillets. In contrast, refrigeration and coating accelerated oxidation, reducing shelf life and increasing the likelihood of undesirable sensory changes, such as rancidity.

3.2. *Evaluation of texture properties*

Fig. 2 illustrates the results of the texture profile analysis (TPA) for Atlantic bonito fillets stored under different preservation techniques over 15 days at 4 °C. The texture parameters analyzed include firmness, cohesiveness, and adhesiveness, which are essential indicators of the fillets' structural integrity and sensory quality.

At Time 0, all samples exhibited similar initial values for firmness (50.59 ± 5.49 N), cohesiveness (0.45 ± 0.01), and adhesiveness (2.52 ± 1.32 N·s). However, as storage progressed, noticeable differences emerged among the preservation techniques.

In refrigerated-only fillets (control), firmness also declined significantly, reaching 18.69 ± 5.66 N by day 3 and stabilizing around 15.87 ± 7.10 N by day 15. Refrigeration alone does not provide structural support to the fillets, leading to protein degradation and moisture loss, both of which negatively impact texture. Cohesiveness remained relatively stable throughout the storage period, suggesting that, although there was some breakdown of the protein matrix, a degree of water retention was still maintained. However, adhesiveness showed a progressive decrease, likely due to the gradual drying of the fillet surface in the absence of protective barriers.

In fillets stored under MAP, firmness decreased sharply within the first 3 days (13.98 ± 3.73 N) and remained relatively low throughout the storage period, reaching 12.86 ± 4.71 N by day 15. This decline in firmness can be attributed to protein denaturation and enzymatic activity, which progressively weaken the muscle structure. The gradual reduction in adhesiveness suggests a loss of surface moisture, likely due to the gas exchange dynamics within the modified atmosphere packaging, which impacts the water-binding capacity of proteins.

The VAC-packaged fillets showed a moderate decrease in firmness in the first 3 days (20.58 ± 3.15 N), followed by a temporary increase to 24.26 ± 16.57 N on day 7, possibly due to protein cross-linking or moisture redistribution within the muscle fibres. By day 15, firmness had decreased to 18.38 ± 10.14 N, indicating progressive softening. Cohesiveness showed a notable increase on day 15 (0.63 ± 0.09), which may be related to gel formation or protein aggregation under low-oxygen conditions. Interestingly, adhesiveness exhibited a sharp increase on day 15 (5.28 ± 0.62 N·s), which could be associated with higher surface hydration, as vacuum packaging reduces dehydration but does not eliminate enzymatic activity.

Coated fillets showed the most pronounced initial loss in firmness, dropping to 9.35 ± 1.77 N after 3 days. However, firmness partially recovered to 17.34 ± 8.67 N on day 7 and 17.60 ± 4.74 N on day 10 before declining again to 11.79 ± 3.20 N by day 15. This fluctuation suggests that the coating initially softened the texture by increasing moisture retention, but as the coating dried or degraded, some firmness was regained before ultimately declining due to continued enzymatic activity and structural breakdown. Cohesiveness remained relatively stable, while adhesiveness showed moderate variations, indicating that the coating formed a semi-permeable barrier, reducing dehydration but not completely preventing enzymatic degradation.

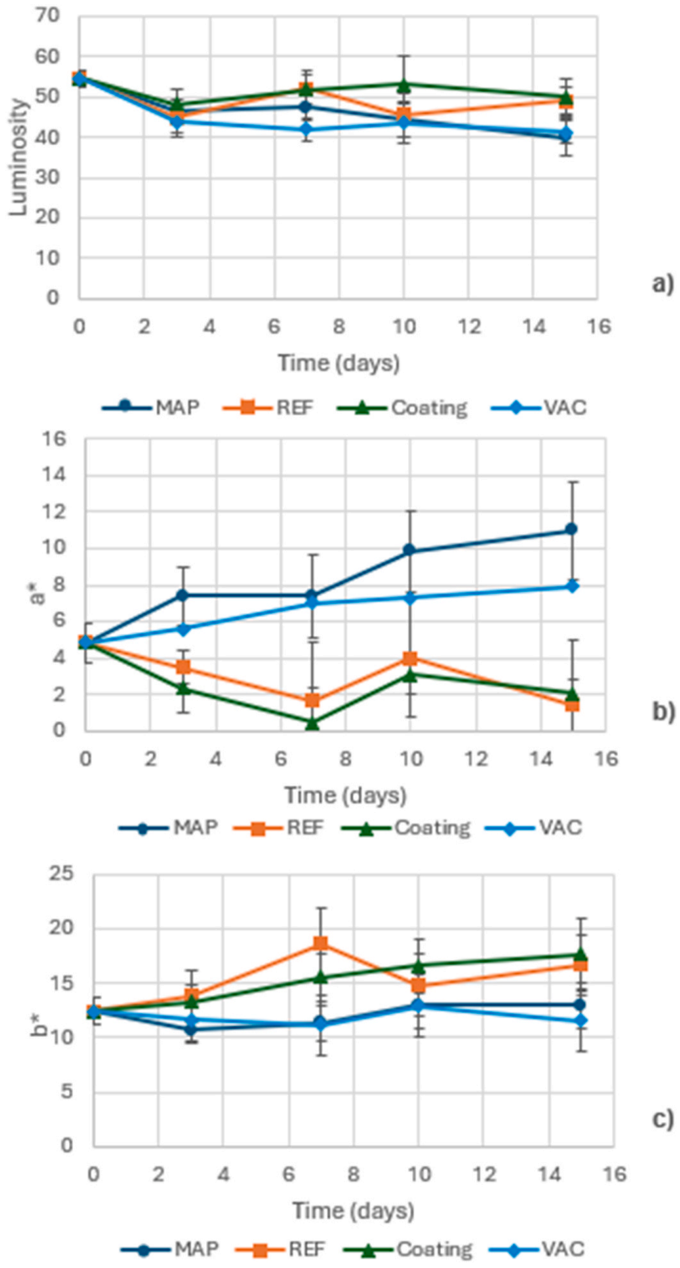

Fig. 2. Results of the colour characterisation of the Atlantic bonito fillets during 15 days of storage at 4 °C in different preservation techniques (MAP - modified atmosphere, REF – refrigeration alone (control), VAC - vacuum and Coating): a) (L - Luminosity); b) a* parameter; c) b*parameter.

Overall, all preservation methods resulted in a decrease in firmness over time, though modified atmosphere and vacuum storage were more effective in maintaining texture compared to refrigeration and coating. The variations in cohesiveness and adhesiveness reflect complex interactions between protein denaturation, moisture migration, and enzymatic activity throughout storage. These findings align with previous studies, which suggest that vacuum packaging and modified atmosphere techniques help maintain muscle structure for longer periods by minimizing oxidative damage, whereas refrigeration alone leads to faster protein breakdown and moisture loss (Goulas & Kontominas, 2007). Although coatings can act as a protective barrier, excessive moisture retention may negatively impact texture, emphasizing the need for further optimization of edible coatings for fish preservation.

The decline in firmness is consistent with findings by (Opara et al., 2022), who reported a similar trend in hake fillets, where prolonged storage led to a progressive softening of muscle structure. The loss of firmness can be attributed to protein degradation, enzymatic activity, and moisture redistribution within the muscle fibres, all of which contribute to textural deterioration over time.

3.3. *Evaluation of colour properties*

The colour properties of Atlantic bonito fillets stored under different preservation techniques—modified atmosphere, refrigeration, vacuum, and coating—over a 15-day period at 4 °C are presented in Fig. 2. These properties are essential for assessing the visual quality and consumer acceptance of fish products. Changes in colour, particularly in terms of lightness (L*), redness (a*), and yellowness (b*), can indicate biochemical alterations such as lipid oxidation, microbial growth, and enzymatic activity. Monitoring these colour changes helps in understanding how different storage methods impact the overall appearance and perceived freshness of the fillets.

For refrigerated alone samples (control), L values exhibited greater variability, initially dropping to 44.86 ± 3.45 by day 3, then fluctuating before reaching 49.07 ± 3.59 by day 15. This variability may be attributed to progressive protein denaturation and moisture loss, which affect light reflection properties. The a* values showed a significant decline, from 4.82 ± 1.10 at Time 0–1.44 ± 3.55 at day 15, indicating a shift towards a greener tone, which could result from oxidation of pigment molecules such as myoglobin. Conversely, b* values increased from 12.42 ± 1.21–16.71 ± 2.74, suggesting a yellowing effect, possibly due to lipid oxidation and accumulation of secondary oxidation products that contribute to discoloration.

For MAP, L values decreased steadily, from 54.68 ± 1.94 at Time 0–42.02 ± 4.58 by day 15, indicating a gradual darkening of the fillets, which is commonly associated with oxidative changes and myoglobin oxidation in fish tissue. The a* values increased from 4.82 ± 1.10 at Time 0–10.98 ± 2.68 on day 15, reflecting a redder hue, likely due to oxidative reactions involving heme proteins and lipid oxidation byproducts. The b* values remained relatively stable, with minor fluctuations, suggesting that yellowness was less affected by MAP compared to other preservation techniques

VAC fillets also experienced a gradual decrease in L values, from 54.68 ± 1.94 at Time 0–41.31 ± 2.71 at day 15, indicating progressive darkening. However, the a* parameter increased moderately, from 4.82 ± 1.10 at Time 0–7.90 ± 2.54 at day 15, signifying a retention of reddish coloration compared to refrigerated samples. This may be due to lower oxygen exposure, which slows down oxidative browning processes. The b* parameter remained relatively stable throughout storage, suggesting that vacuum packaging helped maintain yellowness, likely by reducing oxidative degradation of carotenoid pigments and lipid-derived colour compounds.

For coated fillets, L values remained relatively stable, with only a minor reduction from 54.68 ± 1.94 at Time 0–50.07 ± 4.52 at day 15. This suggests that the coating provided a protective barrier against dehydration and oxidative changes, preserving overall brightness. The a* values exhibited a decreasing trend, reaching 2.04 ± 0.79 at day 15, indicating a shift towards a greener tone, similar to refrigerated samples. This could be attributed to moisture retention effects from the coating, which may have altered the chemical environment and pigment oxidation pathways. Meanwhile, b* values increased from 12.42 ± 1.21–17.68 ± 3.21, suggesting an intensification of yellowness, possibly due to lipid oxidation byproducts interacting with the coating material.

Overall, lightness (L) decreased across all preservation methods, with the most significant darkening observed in vacuum and MAP samples. This trend is consistent with studies indicating that protein oxidation and moisture loss contribute to reduced reflectance, leading to a darker appearance. The a* parameter (redness) increased in vacuum and MAP samples, indicating a redder hue, while it decreased in refrigerated and coated samples, suggesting a shift towards a greenish tone. The preservation of red coloration in VAC and MAP samples may be due to reduced oxygen availability, which slows pigment oxidation, while in refrigerated and coated fillets, increased oxidation likely led to the formation of brown metmyoglobin, causing a loss of red pigmentation. The b* parameter (yellowness) showed an increasing trend in coated and refrigerated samples, suggesting a greater accumulation of lipid oxidation byproducts, which contribute to yellowish discoloration. In contrast, MAP and vacuum-stored fillets maintained more stable b* values, likely due to slower lipid oxidation rates in reduced-oxygen environments. These findings highlight the influence of oxygen exposure, moisture retention, and lipid oxidation on colour stability in Atlantic bonito fillets, with VAC and MAP proving more effective in maintaining colour integrity compared to refrigeration and coating techniques.

3.4. *Evaluation of microbiological properties*

The microbiological evaluation of fishery products is a valuable tool for assessing quality and detecting potential contamination (Fig. 3). In this study, a microbiological analysis was conducted over a 15-day period to determine the most effective preservation method and establish the maximum storage duration for the fillets. Samples were analysed at four time points: days 3, 7, 10, and 15. The following microorganisms were assessed based on microbiological criteria: total bacteria count, moulds and yeasts, Enterobacteriaceae, coagulase-positive *Staphylococcus*, *Listeria monocytogenes*, *Salmonella* spp., sulphite-reducing Clostridia spores, lactic acid bacteria, and *Vibrio parahaemolyticus*. The analyses followed the applicable legislation, specifically Regulation (EC) No 2073/2005, and the guidelines established by the Health Protection Agency (HPA).

The results showed that *Salmonella* spp., *Vibrio parahaemolyticus*, and *Listeria monocytogenes* were not detected in 25 g samples. Sulphite-reducing Clostridia spores were present, though their quantity could not be determined.

According to the results obtained for the total bacteria count (TBC), the initial microbial load was approximately 3 log CFU/g. Over the 15-day storage period, distinct differences emerged in the ability of each treatment to control microbial growth. In the refrigeration group, TBC increased steadily, surpassing the recommended limit for raw fish consumption between days 10 and 15 (6–7 log CFU/g, as stated in the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market, HPA, 2024). By day 15, it had reached 7.26 log CFU/g. The coating treatment was even less effective in inhibiting microbial growth, with TBC rising more rapidly and exceeding the spoilage threshold before day 15, ultimately reaching 9.46 log CFU/g at the end of the storage period.

In contrast, the vacuum treatment resulted in a slower increase in microbial load, with TBC reaching 6.17 log CFU/g by day 15 of storage. Notably, the MAP treatment proved to be the most effective in controlling microbial growth. In MAP-stored samples, TBC remained stable and well below the spoilage threshold throughout the storage period, showing a slight decline over time and reaching 2.13 log CFU/g on day 15. Regarding moulds, the initial values at T0 was 2 log CFU/g, and this value remained stable over time, showing no significant fluctuations. In contrast, the initial yeast count at T0 was 2.60 log CFU/g and increased as time progressed, however, when using the MAP preservation technique, the yeast count after 15 days was 2 log CFU/g.

In relation to Enterobacteriaceae, coagulase-positive *Staphylococcus*, both had an initial value at T0 of 1 log CFU/g and after 15 days, the technique with the best values was again modified atmosphere, with 1 log CFU/g for both microorganisms.

Among the preservation methods, modified atmosphere packaging (MAP) exhibited the lowest microbial content, whereas refrigeration and coating were the least effective techniques.

The absence of *Salmonella* spp., *Vibrio parahaemolyticus*, and *Listeria monocytogenes* suggests that the applied preservation methods effectively inhibited the growth of these pathogens, ensuring the microbiological safety of the fillets throughout the storage period. The detection of sulphite-reducing Clostridia spores, although unquantifiable, indicates the potential for anaerobic bacterial growth under specific conditions, warranting further investigation into its impact on product safety. These findings underscore the importance of interpreting microbial dynamics in light of packaging mechanisms and their implications for both shelf life and food safety. The superior performance of MAP is likely attributable to its ability to limit oxygen availability, thereby restricting the growth of many spoilage microorganisms. Conversely, refrigeration and coating were less effective, possibly due to their limited capacity to suppress microbial proliferation under the given storage conditions.

3.5. *Sensory analysis*

Sensory analysis of the crude Atlantic bonito fillets stored under refrigeration alone, MAC, coating, and VAC at 4 °C for 15 days was performed to assess the impact of different preservation techniques on sensory properties. Principal Component Analysis (PCA) was used to explore the relationships between the samples (Fig. 4). The first principal component (PC1) accounted for 53 % of the variation, while the second principal component (PC2) explained 22 %, together capturing the majority of the dataset's variance.

A clear separation of groups was observed, with the most pronounced differences occurring in fillets stored under refrigeration and coating, particularly at days 7, 10, and 15. These samples showed greater deviations from the initial characteristics, likely due to

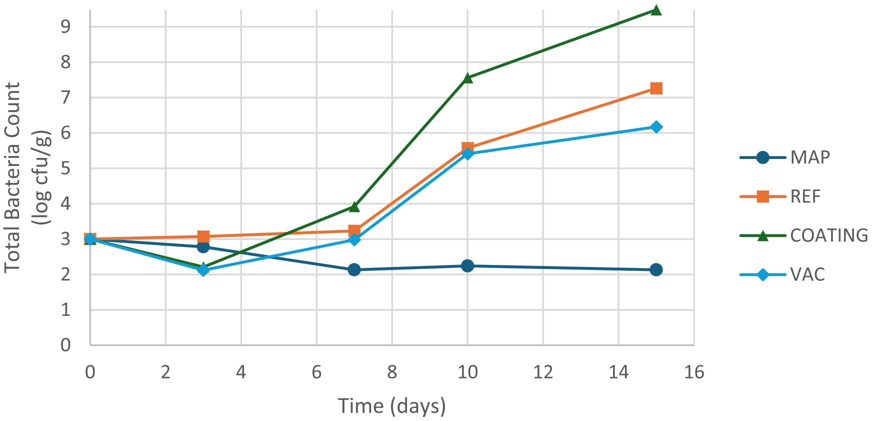

Fig. 3. Results of total bacteria count (log cfu/g) of the Atlantic bonito fillets during 15 days of storage at 4 °C in different preservation techniques (MAP - modified atmosphere, REF – refrigeration alone (control), VAC - vacuum and Coating).

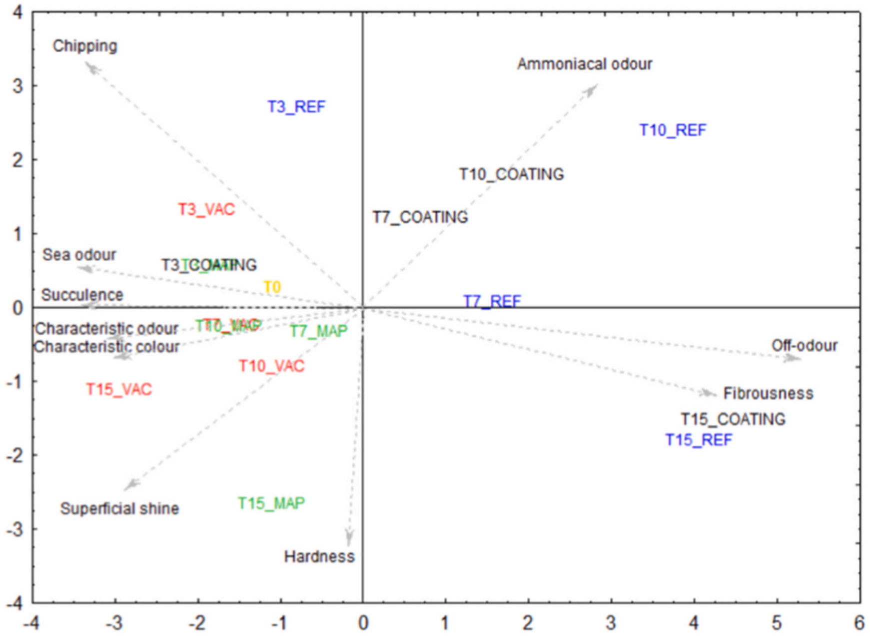

Fig. 4. Principal component analysis of sensory evaluation. Loading plot of different attributes, performed on Atlantic bonito fillets during 15 days of storage: MAP – modified atmosphere; REF- Refrigeration alone (control); VAC – Vacuum.

progressive lipid oxidation, microbial activity, and protein degradation. This resulted in off-flavours, textural softening, and undesirable sensory changes, such as rancidity, excessive dryness, or loss of the fresh fish aroma. These changes suggest that these preservation methods are less effective in maintaining the sensory quality of the fillets over time. According to (Martínez et al., 2018), the alginate coating was associated with 'favouring fish spoilage' during the last sensory evaluation.

In contrast, fillets stored under MAP and VAC exhibited more stable sensory profiles over time. The samples from days 0, 3, 7, and 15 in these conditions clustered more closely together, suggesting that these preservation techniques were more effective in maintaining the original sensory attributes of the fish, such as colour, texture, and aroma.

These findings highlight the effectiveness of MAP and VAC storage in extending freshness and delaying sensory deterioration. PCA revealed a clear distinction between samples stored under refrigeration alone and coating, particularly at days 7, 10, and 15, indicating that these fillets experienced the most pronounced sensory changes over time. This aligns with the elevated peroxide and thiobarbituric acid reactive substances (TBARs) values observed in these storage conditions, which suggest accelerated lipid oxidation.

The high degree of lipid peroxidation in refrigerated alone and coated samples likely contributed to the development of rancid flavours and off-odours, key factors in sensory rejection. Additionally, microbiological analysis indicated greater microbial growth in these samples, which may explain the undesirable textural changes, increased slime formation, and unpleasant odours detected during sensory evaluation.

In contrast, fillets stored under MAP and VAC exhibited greater consistency in sensory attributes across different time points (0, 3, 7, and 15 days), suggesting improved preservation. This finding correlates with lower peroxide values, reduced lipid oxidation rates, and enhanced microbial stability in these treatments. The limited oxygen exposure in VAC and the CO_2-enriched atmosphere in MAP likely contributed to the preservation of polyunsaturated fatty acids, thereby mitigating the formation of rancid odours and maintaining a fresher sensory profile. Furthermore, these storage methods effectively slowed microbial spoilage, preventing the development of off-odours and textural degradation typically associated with bacterial activity.

4. Conclusions

The results of this study confirm that modified atmosphere packaging (MAP) combined with refrigeration is an effective preservation method for Atlantic bonito fillets, maintaining their quality over 15 days of refrigerated storage at 4 °C. Although MAP is already widely used and well-established, these findings further reinforce its significant advantages in extending shelf life and preserving key quality attributes. According to the combined results of oxidative stability, microbiological analysis, and sensory evaluation, the estimated shelf life of Atlantic bonito fillets under each preservation treatment was established. Fillets stored under refrigeration alone (REF) demonstrated rapid deterioration, with TBARs exceeding 2 mg MDA/kg and significant sensory decline observed by day 3, while microbial growth approached critical limits between day 10 and 15. The coated samples also exhibited lipid oxidation above the acceptable threshold by day 3, and although microbial counts remained within acceptable limits until day 10, sensory properties began to decline by day 7.

In contrast, vacuum-packaged (VAC) samples showed minimal lipid oxidation (0.78 ± 0.06 mg MDA/kg at day 15), stable microbial counts, and preserved sensory quality throughout the 15-day period, supporting a shelf life of 15 days. The best performance was observed in modified atmosphere packaging, where TBARs and peroxide values remained low (1.29 ± 0.06 mg MDA/kg and 1.01 ± 0.05 mEq/kg, respectively), microbial loads stayed well below spoilage thresholds, and sensory attributes remained stable throughout storage. Therefore, MAP-treated fillets maintained high quality for at least 15 days, indicating this method as the most effective in extending shelf life under refrigerated conditions.

The use of a controlled gas mixture effectively limited oxidative and microbial degradation, resulting in lower peroxide values and reduced lipid oxidation compared to refrigeration alone and coating. This slower lipid peroxidation contributed to better colour stability, preventing excessive darkening and undesirable yellowing over time.

From a sensory perspective, fillets stored under MAP retained their freshness, texture, and aroma, with tasters noting that the product preserved the typical characteristics of a fresh fillet. These observations align with the lower sensory deterioration detected in the PCA analysis. MAP also demonstrated superior microbial control, significantly reducing bacterial growth throughout the storage period. The CO_2-rich atmosphere in MAP likely inhibited the proliferation of spoilage microorganisms, thereby preserving texture and preventing the rapid softening and moisture loss observed in other storage methods.

Overall, these findings confirm that MAP remains one of the most effective techniques for prolonging the shelf life of fresh fish products while preserving sensory, microbiological, and physicochemical properties. This study highlights the importance of oxygen-restrictive packaging in delaying spoilage processes, making MAP a valuable approach for enhancing the commercial viability and quality retention of Atlantic bonito fillets during refrigerated storage.

CRediT authorship contribution statement

Joana Solinho: Writing – original draft, Investigation. **Vazquez Manuel:** Writing – review & editing, Methodology. **Joana Santos:** Writing – review & editing, Conceptualization. **Rita Pinheiro:** Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank: To Blue Project, Bioeconomy, People, Sustainability, Health (PT-INNOVATION-0105). This research was funded by Iceland Liechtenstein Norway EEA grants. Blue Growth Programme. Call2 – Business, Development, Innovation and SMEs; to UIDB/05937/2020 and UIDP/05937/2020 – CISAS funded by national funds, through FCT – Fundação para a Ciencia e a Tecnologia.

Data availability

Data will be made available on request.

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