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Strategies for the valorization of fish by-products: Fish balls formulated with mechanically separated amberjack flesh and mullet hydrolysate

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ABSTRACT

The aim of the present study was to valorise fish by-products by developing fish balls obtained using mechanically separated amberjack (*Seriola dumerili*) flesh and the optimized fish protein hydrolysate prepared from mullet (*Mugil cephalus*) by-products. Shelf life of the developed products was also evaluated during refrigerated storage. The fish by-products hydrolysate (FBH) was added to the basic formulation at a concentration of 1.5%. All fish balls were packed under modified atmosphere (80% N_2/20% CO_2) and stored at 4 °C for 20 days. Physico-chemical and microbiological analyzes were performed. The addition of FBH significantly extended the shelf life to more than 12 days, compared to less than 8 days for conventional products. FBH also effectively prevented the accumulation of histamine and ensured that the EU limits were met throughout the 12-day (137.84 mg/kg). The addition of 1.5% of the FBH promoted a reduction of lipid oxidation, observed by volatile analysis, however, this difference was not perceived by sensorial analysis. The innovative amberjack fish balls can be considered an interesting new fish product obtained by the valorization of fish by-products (mechanically separated meat and FBH).

1. Introduction

In 2020, total fish production reached 214 million tonnes (MT), among that 157 MT were used directly for human consumption (FAO, 2022). Generally, 27% of the total fish captured is unutilized owing to low-value discards, storage problems, short shelf life, and spoilage (FAO, 2022). The remaining harvested fish go through the processing plant where only 20%–50% of the fish is used as an edible product, while the remaining parts (80%–50%) are discarded as by-products or leftovers (Suresh et al., 2018).

In the fish processing sector, the further utilization of side streams and processing waste can contribute to the sustainability of raw materials, access to added value and higher profitability, and environmental protection by reducing the amount of by-products (Silovs, 2018). Despite the considerable potential for the reuse and recycling of fish by-products, their use to produce high-value products is still largely underdeveloped, representing a missed opportunity in the current fisheries and aquaculture sector (Cooney et al., 2023).

Mechanical separation is a technology that has been successfully applied in the fish sector, even though this process often results in the loss or alteration of the normal structure of the muscle fiber (Secci et al., 2016). Mechanical separation of fish flesh could represent an opportunity for recovering the flesh that remains attached to the bones after filleting, and that would otherwise be discarded.

In addition, fish by-products are an excellent source of bioactive compounds such as amino acids, proteins, peptides, enzymes, gelatin, collagen, long-chain omega-3 polyunsaturated fatty acids, chitin, and vitamins (Ozogul et al., 2021; Vázquez et al., 2019) which can be used both as raw materials and as functional ingredients for the production of value-added products (Suresh et al., 2018). In particular, fish proteins have a well-balanced amino acid composition compared to other animal protein sources, and their hydrolysates possess interesting functional properties, that can have several applications in cosmetic, pharmaceutical and food sector.

Fish protein hydrolysates have great potential for use in food formulations as they also possess several technological properties,

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including solubility, water-holding capacity, emulsifying, and foam-forming ability (Kristinsson et al., 2010). Fish hydrolysates can be obtained by application of enzymes such as proteases and lipases. However, the *in-situ* application of microorganisms, due to their complex metabolisms, represents a more cost-effective approach to producing functional hydrolysates with enhanced antioxidant activity and flavor characteristics (Gottardi et al., 2022).

Fish by-products are regulated by Commission Regulations (EU) 1069/2009 and 142/2011, which categorize animal by-products based on their risk to human and animal health and the environment. Fish by-product hydrolysate (FBH) is not a by-product, but is produced from category 3 of by-products (e.g., heads, bones, skins), which are considered low-risk. If they are intended for human consumption, they must comply with strict hygiene regulations that require immediate processing or preservation by freezing, chilling or the addition of substances to maintain quality (Kaushik et al., 2024).

Demand in the global market is shifting toward higher value-added processed seafood that features convenience, ease of preparation, high nutritional value, and that can also satisfy the sensory-hedonic range (Vazhiyil Venugopal, 2005). Fish balls could open new market opportunities for ready-to-cook, nutrient-rich fish products. They can be prepared using mechanically separated flesh with a variety of seasonings that mask fish taste that dissuades many consumers, and therefore be consumed also by the consumers that are used to eating convenience food like fish balls, promoting a healthier lifestyle. Since processing can strongly influence the nutritional value of fish products, the most vulnerable nutrients in fish are the fatty acids, which can easily be subjected to oxidation reactions with a consequent reduction of quality. For the development of innovative, ready-to-cook fish products with high added value, it is therefore important to assess possible changes during processing and storage and to carefully check whether the innovative processing affects the nutritional value of the final product.

The greater amberjack is an important commercial fish and a popular game species in Europe and North America. The farming of amberjack has attracted great interest in the Mediterranean region and is now considered one of the most important species for the diversification of commercial fish production in Mediterranean countries (Divanach, 2002). The greater amberjack is known for its high growth rates in the wild and in captivity, its excellent flesh quality, and its high demand worldwide (Parisi et al., 2014). Given the market size (3–5 kg) and a typical amberjack meat yield of around 60 % after fileting (Öksüz, 2012), a significant proportion of the fish, around 40 %, remains as by-product. Exploring alternative uses for the by-products of amberjack could improve the sustainability of aquaculture for this species, which shows promise for marine aquaculture.

Grey mullet has a good market in southern and eastern Mediterranean region but it is also consumed in many Asian countries. Most, if not all, of the farmed mullet is consumed in the producing countries where increasing demand exists (Crosetti, 2016). According to FAO aquaculture statistics, a total of 11,938.6 t of *M. cephalus* were produced in 2021, with Indonesia (46.3%), Israel (16.8%), and China (11.8%) being the main producers of this species in extensive and semi-intensive aquaculture conditions (FAO, 2023). Furthermore, *M. cephalus* is recognized as an ideal species for addressing food needs in developing countries, showcasing its versatility; moreover, it is highly valued in developed countries for its ability to yield both flesh and valuable processed by-products (Ben Khemis et al., 2019). The importance of grey mullet in aquaculture is increasing also in the Mediterranean and Adriatic Sea. With this in mind, a valorization of its by-products is increasingly important.

In previous research, the optimization of a fermentation process was carried out to obtain a hydrolysate starting from by-products of grey mullet filleting operations (skins, scales, bones and residual meat). The use of a wild-type strain of *Yarrowia lipolitica* allowed to obtain a final hydrolysate rich in antioxidant peptides. Therefore, the objective of this study was to develop fish balls obtained using mechanically separated amberjack (*Seriola dumerili*) flesh and the optimized fish protein hydrolysate prepared from mullet (*Mugil cephalus*) by-products by yeast Yarrowia lipolytica. It was also objetive the evaluation of fish balls quality during refrigerated storage.

2. Materials and methods

2.1. Fish raw materials

The meat of greater amberjack (*Seriola dumerili*), was obtained by mechanical separation from filleting by-products. Farmed amberjack with an average weight of about 3.2 kg from Croatia were transported under the ice to the fish processing company ECOPESCE srl (Cesenatico, FC, Italy), where they were manually gutted, filleted and skinned in the company's industrial processing facilities. The final yield from manual filleting was 58.40%.

After filleting process, the remained off-cuts were placed in the belt-drum separator (BAADER mod. 600, Baader, Lübeck, Germany), also located in ECOPESCE's fish processing facilities, for mechanical separation. The flesh was forced by a rubber conveyor belt through a perforated drum (holes diameter - 3 mm) and collected from the inside of the drum, while bones were discarded on the outside.

The yield of fish meat after mechanical separation of the amberjack backbone was 28.42%. The mechanically separated amberjack flesh was frozen in an industrial cooling system until reaching - 40 °C at the sample core and stored at the same temperature for no more than 2 weeks.

2.2. Fish by-product hydrolysate

The fish by-product hydrolysate (FBH) used in the formulation of amberjack fish balls was obtained from the side streams of mullet (*Mugil cephalus*) fish (skin, scales, bones, and residual meat, provided by ECOPESCE) autoclaved and then incubated with the yeast *Yarrowia lipolytica* YL2 according to (Gottardi et al., 2022) with some modifications. The biotechnological process was performed for 9 days and then the product was lyophilised and characterized for the volatile molecule profile. The volatile molecule fingerprinting consisted of 139.8 ppm acids (mainly short chain fatty acids), 30.5 ppm alcohols, 5.3 ppm ketones, 3.7 ppm hydrocarbons, 3.1 ppm esters, 0.2 ppm aldehydes, and 9.6 ppm others. The detailed composition is reported in Table S1 (supplementary material). In particular, the acids detected have been reported to be able to extend the shelf life of fish preparations and, together with other volatile compounds, are usually found in Thai fish sauces and fermented fish (Lapsongphon et al., 2015; Li et al., 2013; Wichaphon et al., 2012).

2.3. Preparation fo fish balls

A total of 328 fish balls were prepared, each fish ball weighing approximately 15 g. The amberjack flesh obtained by mechanical separation was mixed with all the ingredients of the basic formulation. In the innovative fish balls the functional ingredient (FBH) was added to the basic formulation at a concentration of 1.5% by replacing the same amount of amberjack meat, while all other ingredients remained constant. This amount was chosen considering available literature on the addition of fish hydrolysate to fish formulation (Balandina et al., 2024; Pezeshk et al., 2017)and during preliminary trials aimed at defining the maximum amount that did not impact negatively on the product sensorial properties. The traditional product (control) was prepared with the basic formulation without FBH. The complete formulation of traditional and innovative products is reported in Table 1.

2.4. Packaging and storage

The fish balls were placed in polypropylene trays (PP) sealed with a

Table 1
Formulation of traditional and innovative fish balls.

Ingredient (%)	Traditional	Innovative
Mechanically separated amberjack flesh	80.00	78.50
Breadcrumbs	6.25	6.25
Olive oil	5.63	5.63
Potato starch	6.88	6.88
Sodium chloride	0.50	0.50
Potassium chloride	0.31	0.31
Black pepper	0.03	0.03
Garlic powder	0.03	0.03
Onion powder	0.06	0.06
Lemon juice	0.13	0.13
Parsley powder	0.13	0.13
Nutmeg	0.06	0.06
FBH[a]	0.00	1.50

[a] FBH: Fish by-product hydrolysate.

high barrier film and packed in a modified atmosphere (MA) with a gas mixture of 80% N_2 and 20% CO_2. The MA was prepared using a quaternary gas mixer (Mod. KM100-4, Witt-Gasetechnik, Witten, Germany) connected to gas cylinders and a gas flow welder (Mod. Multiple 315, Orved Srl, Venice, Italy). Five fish balls were placed in each package. After sealing, the control and innovative products were stored at 4 ± 1 °C for a total of 12 days.

2.5. Analytical determination

The evaluation of fish quality comprises the combined use of different methodologies, due to the complexity of the decomposition process to which it is subjected. The most common are microbiological (main pathogens and spoilage microorganisms), biochemical (biogenic amines, volatile molecule profile, peroxide value), physicochemical (pH, water activity, color, and texture), and sensory methods.

2.5.1. Microbiological analyses

At each sampling time, two trays of amberjack fish balls stored in the refrigerator were opened and sampled under sterile conditions. 10 g of the sample was placed in sterile bags and diluted with 90 mL of sterile saline solution (9 g NaCl/L). Samples were homogenised using a stomacher (Seward, UK) for 2 min at 200 rpm. Decimal serial dilutions were then prepared with sterile saline solution. The individual decimal serial dilutions were seeded onto different culture media depending on the group of microorganisms to be quantified. The types of culture medium and their respective incubation times and temperatures are listed in Table 2.

Table 2
Microbial classes, culture media, incubation temperature and incubation time.

Microbial classes	Culture media	Incubation temperature (°C)	Incubation time
Total viable counts (TVC)	Plate Count Agar (PCA)	30	48 h
Psychrophilic bacteria	Plate Count Agar (PCA)	4	10 days
***Pseudomonas* spp.**	*Pseudomonas* agar base (PAB) with Cetrimide, Fucidin, and Cephalosporin	30	48 h
Enterobacteriaceae	Violet Red Bile Glucose Agar (VRBGA)	37	24 h
Escherichia. coli	Brilliance E. *coli*/ Coliform selective Agar	37	24 h
Coagulase-positive staphylococci	Baird-Parker Agar	35	24/48 h
Lactic acid bacteria (LAB)	De Man, Rogosa, Sharpe (MRS) with Cycloheximide	37	48 h
Yeasts	Yeast extract, peptone and dextrose (YPD) with Chloramphenicol	30	48 h

The mean of two biological replicates and two technical replicates (four replicates in total) was used to calculate the microbial load expressed as $\log_{10}$ CFU/g ± standard deviation (CFU: Colony Forming Unit). The presence of pathogens (*Listeria monocytogenes* and *Salmonella* spp.) was also assessed according to ISO 11290-1 and ISO 6579-1, respectively, only on the first day of sampling since no pathogens were detected.

2.5.2. Biochemical analyses

2.5.2.1. Biogenic amine content. For the detection and quantification of histamine, biogenic amines were extracted with trichloroacetic acid and then derivatized with dansyl chloride, as described by Montanari et al. (2021). Samples were analyzed using an HPLC Agilent 1260 Infinity (Agilent Technologies Inc., Santa Clara, CA, USA) with a UV detector (G1314F VWD 1260) at 254 nm. The amount of histamine was expressed as mg/kg using a calibration curve prepared with aqueous biogenic amine standards derivatized as described for the samples.

2.5.2.2. Peroxide value. Lipids were extracted from the fish balls using a method previously described by Bligh and Dyer (1959), and the peroxide value (PV) was used to determine primary lipid oxidation.

The value of PV was determined by the ferrothiocyanate method (Chapman & Mackay, 1949). The results were expressed in milliequivalents of O_2 per kg^{-1} of lipid ($meqO_2$/kg).

2.5.2.3. Volatile molecule profile. The volatile molecule profiles were detected with SPME/GC-MS technique. A DVB/CAR/PDMS fiber (SUPELCO, Bellafonte, PA, USA) was used to perform solid phase microextraction (SPME) according to Gottardi et al. (2022). The samples (3 g) were placed in vials, together with the internal standard (4-methyl-2-pentanol, final concentration of 100 ppm, used to estimate the relative concentrations) (Sigma, Milan, Italy), and incubated for 10 min at 45 °C. Then, the fiber was exposed to the vial headspace for 30 min at 45 °C. The volatile molecules adsorbed were desorbed in the gas chromatograph (GC) injector port in splitless mode at 250 °C for 10 min. The headspace of the volatile compounds was analyzed using Gas-Chromatography (GC) 7890A, Network GC System with mass spectrometry (MS) 5975C (Agilent Hewlett–Packard, Geneva, Switzerland). The column used was J&W CP-Wax 52 CB (50 m × 320 μm × 1.2 μm). The initial temperature was 40 °C for 1 min and then increased by 4.5 °C/min up to 65 °C. After that, the temperature increased by 10 °C/min up to 230 °C and remained at this temperature for 17 min. The compounds were identified by comparison based on NIST 11 (National Institute of Standards and Technology) database. The gas carrier was helium with a flow rate of 1.0 mL/min.

2.5.3. Physico-chemical analyses

2.5.3.1. pH and water activity. The pH of the fish ball samples was measured using a pH meter (Crison, Barcelona, Spain). The water activity (a_w) of the samples was measured using an AquaLab dew point hygrometer (Series 3 TE, Decagon Device, Nelson Court, NE, USA). For both analyzes, three replicates were performed for each sample.

2.5.3.2. Color. The color parameters lightness (L*), redness (a*) and yellowness (b*) were measured with a spectrophotocolorimeter ColorFlex™ (Hunterlab, Reston, VA, USA). The tristimulus L*, a*, b* measurement mode (C.I.E., 1987) was used. The average of 5 measurements was calculated for each sample.

2.5.3.3. Texture. Texture measurements were performed using a texture analyser (TA -HDi500 from Stable Micro Systems, Surrey,

England) with a force of 25 kg and using a rectangular compression plate (70 × 70 mm). Force vs distance curves were evaluated for hardness (N), springiness and gumminess as mean value ± standard deviation. Test speed was set at 1.0 mm/s with a total strain of 40% and a relaxation time of 5 s. Moreover, a shear test was performed using a 2-mm thick steel blade probe with a load cell of 5 kg and a test speed of 2 mm/s. The analysis was performed on 5 replicates for each sample.

2.5.4. Sensory analysis

Sensory analysis was performed by a panel of 6 trained panellists (aged 25–45 years) from the staff of the Department of Agricultural and Food Sciences, University of Bologna. The test included visual, olfactory, textural and overall liking evaluation of the raw fish balls samples. The evaluation of the different fish ball formulations was conducted by the panel using the following attributes: i) color uniformity; ii) color liking; iii) odour intensity; iv) odour liking; v) consistency; vi) overall liking. Attributes were rated based on a hedonic scale of 1–9, where 1 corresponded to "extremely low" and 9 to "extremely high" and 5 was the threshold for acceptability.

2.6. Statistical analysis

Data were analyzed by Statistica 8.0 software (StatSoft, Tulsa, USA) through the factorial Analysis of Variance (ANOVA) or one-way-ANOVA. Statistical significance of the experimental data was verified using Tukey as post-hoc ($p \leq 0.05$). The volatile molecule profiles were analyzed using a principal component analysis (PCA) performed by Statistica software (v 8.0; StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Microbiological analysis

The microbial counts of the microorganisms present in the traditional or innovative fish balls are reported in Table 3.

Total viable count (TVC) is often used to assess the fish freshness. At the beginning of the trial, TVC was approximately 5.6 log CFU/g of product in both innovative and traditional formulations. The high initial microbial contamination was probably due to a lack of proper management of the by-products that were collected in a real industrial scenario. Although, this represent a limitation of the present research, it is possibly a very realistic scenario that allows to properly evaluate the effect of formulation and of the addition of FBH on the product shelf life.

The initial count increased over time, reaching about 7.9 log CFU/g in the traditional sample after 8 days of storage at 4 °C. According to the International Commission on Microbiological Specifications for Food (ICMSF), the maximum acceptable level of mesophilic microorganisms for this type of product is 7 log CFU/g (Balikçi et al., 2022). In fact, while a range between 2 and 6 log CFU/g is usually recommended for whole and cut fish fillets, 7–8 CFU/g is typically associated with sensory rejection (Duarte et al., 2020). Therefore, the traditional fish balls became unacceptable as early as day 8. Conversely, the addition of the functional hydrolysate in the innovative product extended the acceptability of the fish balls for up to 12 days, as only at that point, total mesophilic bacteria exceeded 7 log CFU/g. Due to the low temperatures of storage (4 °C), psychrotolerant microorganisms are favored and so their counting is also suggested as a marker of fish quality (Boziaris & Parlapani, 2017). In the traditional product, an increase in psychrophilic microorganisms was observed from day 8 onward, reaching 8.0 log CFU/g after 12 days of storage. Conversely, the innovative sample exhibited a delay on their growth, with 7.7 log CFU/g being reached only after 12 days at 4 °C. Common psychrotrophic bacteria naturally present in fish are *Pseudomonas* spp. These bacteria are associated with the development of fruity and cloying odors and the production of different volatile sulfides, ketones, esters and aldehydes (Duarte et al., 2020). Both samples presented an initial concentration of *Pseudomonas* spp. of approximately 6 log CFU/g. While in the traditional product this genus reached 7.2 log CFU/g after 8 days, in the innovative fish balls *Pseudomonas* spp. counts remained at 6.4 log CFU/g for up to 12 days of storage. Another microbial marker of product quality is the concentration of *Enterobacteriaceae*. In the traditional product, an increase in these microorganisms was observed over time, passing the acceptable limits (>4 log CFU/g) as early as day 8 day of storage (4.6 log CFU/g). Similarly, in the innovative product, *Enterobacteriaceae* levels increased with time, but it took 12 days of storage to reach 4.8 log CFU/g. Within *Enterobacteriaceae* is important to evaluate the presence and quantity of β-glucuronidase-positive *Escherichia coli*. To obtain an acceptable product, the concentration of *E. coli* must be less than 2 log CFU/g. This value was maintained in the traditional product from day 0 up to day 8, while the addition of the functional hydrolysate extended innovative product's acceptability for at least a couple of days, as *E. coli* counts only reached 2.5 log CFU/g after 12 days of storage. Lactic acid bacteria (LAB) were also quantified in the samples. Although LAB are not of much concern in fish or fish preparations, they may acquire a central role in the final product especially when packed in MAP (Françoise, 2010). Although the two types of fish ball started with a comparable concentration of LAB (2.8 and 3.3 log CFU/g, in control and innovative product, respectively), the growth rate in the traditional product was faster during the first 8 days, while no significant differences were observed between the two samples from day 8 onwards. Eventually, yeasts were quantified. No significant differences on yeast counts were observed over time; the values remained constant at around 4 log CFU/g for all samples throughout the entire storage period under consideration.

Marine antimicrobial peptides are believed to be structurally different from their counterparts produced by terrestrial species and display a broad-spectrum antimicrobial activity (Cheung et al., 2015). Hydrolysis may generate hydrophobic and aromatic amino acids (AAAs) and in a decrease in molecular weight (MW) protein fraction. Lower molecular weight is generally associated with higher antimicrobial activity (Pezeshk et al., 2017).

The antimicrobial mechanism that has been proposed is related to an effect on bacterial cell membranes, that involves the initial binding to

Table 3
Microbiological analyses (log CFU/g) of fish balls during 12 days of storage at 4 °C. Traditional: control fish balls; Innovative: fish balls containing functional hydrolysate.

	Traditional		Innovative		Traditional		Innovative		Traditional		Innovative		Traditional		Innovative	
	Day 0				Day 5				Day 8				Day 12			
Total viable counts	5.5	±0.1^{a}	5.7	±0.1^{a}	6.1	±0.2^{a}	6.2	±0.2^{a}	7.9	±0.1^{a}	6.0	±0.2^{b}	7.9	±0.3^{a}	7.0	±0.4^{a}
Psychrophilic bacteria	6.0	±0.2^{a}	5.6	±0.4^{a}	6.0	±0.3^{a}	5.8	±0.3^{a}	6.7	±0.5^{a}	5.7	±0.3^{b}	8.0	±0.6^{a}	6.9	±0.1^{b}
***Pseudomonas* spp.**	6.2	±0.5^{a}	5.9	±0.3^{a}	6.5	±0.4^{a}	6.1	±0.4^{a}	7.2	±0.3^{a}	6.1	±0.3^{a}	7.4	±0.3^{a}	6.4	±0.2^{a}
Enterobacteriaceae	2.7	±0.1^{a}	2.9	±0.1^{a}	3.4	±0.4^{a}	3.3	±0.2^{a}	4.6	±0.1^{a}	3.5	±0.1^{b}	5.3	±0.4^{a}	4.8	±0.3^{a}
Escherichia coli	1.9	±0.2^{a}	1.6	±0.1^{a}	–		–		2.0	±0.1	–		3.5	±0.2^{a}	2.5	±0.1^{b}
Lactic acid bacteria (LAB)	2.8	±0.4^{a}	3.3	±0.1^{a}	4.3	±0.4^{a}	3.6	±0.2^{a}	6.6	±0.2^{a}	5.4	±0.3^{b}	6.8	±0.3^{a}	6.5	±0.3^{a}
Yeasts	4.0	±0.4^{a}	3.5	±0.1^{a}	3.9	±0.2^{a}	3.9	±0.1^{a}	3.9	±0.1^{a}	4.2	±0.4^{a}	5.0	±0.3^{a}	4.5	±0.1^{a}

- below the sensitivity method threshold (<1 log CFU/g).
Different letters indicate significant differences ($p \leq 0.05$) within a timepoint for a specific microbial group.

the membrane surface followed by the formation of pores. The disruption of the integrity and permeability of the membrane that follow, result in impairment of cell respiration, interference with the electrochemical gradient, and influx of water and ions, that is finally leading to cell swelling and lysis (Perez Espitia et al., 2012).

In Da Rocha et al. (2018), hydrolysates obtained from a fish protein isolate were evaluated against various microorganisms, only seven were inhibited, most being Gram-positive. However, in the majority of the cases reported by (Nemati et al., 2024), the effect was towards both Gram + and Gram -. In our case, mainly enterobacteriaceae (Gram -) were inhibited.

Taken all together, the microbiological data showed that the fish balls already on day 0 were a low quality product maybe due to the fact that they were made with fish by-products not correctly handled. However, the shelf life of the traditional fish ball formulation (<8 days) was extended of at least a couple of days by applying fish by-product hydrolysates.

3.2. Biochemical analyses

3.2.1. Histamine content

Since the shelf life of the tested fish balls ranged between 8 and 12 days, the histamine content was evaluated on day 0, 8 and 12. No histamine was below the detection limit (3 mg/kg) on day 0, immediately after production. However, during storage, histamine accumulated, particularly in the traditional samples, reaching 76.57 mg/kg after 8 days and increasing to 266.88 mg/kg after 12 days. On the other hand, histamine was only detected on day 12 (137.84 mg/kg) in the innovative sample. Histamine is a biogenic amine (BA) produced by Enterobacteriaceae, *Pseudomonas* spp. and LAB, after decarboxylation of free amino acids or amination of carbonyl-containing organic compounds. It is the most toxic BA acting as neurotransmitter and vasodilator, causing headache, hypotension, heart palpitations, asthma attacks, and cutaneous or gastrointestinal effects (Visciano et al., 2020). Its accumulation in food can be considered both as an indicator of possible spoilage (if above 10 mg/kg) but also it can be seen as a safety risk. In fact, The Commission Regulation (EC) No 2073/2005 fixed maximum levels of 200 mg/kg for histamine in raw fish. Since we worked with fish by product that, as reported by microbiological data, presented already a high level of spoilage bacteria, finding histamine level above 10 mg/kg was not surprising and it confirmed that we were not working with fresh fish. On the other hand, since the value, in some conditions were below 200 mg/kg, this means that the product was still safe from a regulatory point of view. Therefore, the histamine content in the traditional fish balls exceeded safety limits after 12 days of storage. This can be related to the faster increase of *Enterobacteriaceae*, *Pseudomonas* and LAB. Nevertheless, the innovative products, where these bacteria grew slower, presented levels of histamine that remained within the acceptable limits up to 12 days.

3.2.2. Peroxide value

The lipid oxidation is an important index of the nutritional quality of fish products, therefore, the peroxide value (PV) was evaluated for primary oxidation indices and measured immediately after preparation of the fish balls and during 12 days of storage at 4 °C (Fig. 1). From the beginning of storage until day 5, both products had relatively low PV values, never exceeding 5.5 meq O_2/kg of fat. From day 5, the number of peroxides increased for both products, reaching a maximum value of 13.83 meq O_2/kg for the innovative fish balls and 13.69 meq O_2/kg for the traditional fish balls at the end of the storage period. It is important to note that for both products, the PV doubles between the 5th and 8th day of storage, while it increases slightly from the 8th to the 12th day. From the PV results, it appears that the innovative ingredient at the concentration of 1.5% in the new formulation had no effect in preventing primary lipid oxidation. The presence of hydrophobic sequences in peptides could interact with lipid molecules and act as radical scavengers by delivering protons to lipid-derived radicals (Saidi et al., 2018). However, the antioxidant properties of peptides isolated from fish proteins are related to their sequence, composition and hydrophobicity. At the same time, PV alone might not be sufficient to determine the state of lipid oxidation, but other indices must be considered, such as secondary oxidation metabolites and/or volatile compounds.

3.2.3. Volatile molecule profiles

During fish storage, microbial metabolisms, enzymatic and non-enzymatic reactions may cause the development of volatile molecules which impact the final acceptability of the product. Therefore, volatile molecule fingerprinting was determined on the different fish balls collected during the 12 days of storage at 4 °C (microbiological shelf life). Around 67 molecules were identified namely 9 alcohols, 13 hydrocarbon volatile compounds, 10 terpenoids, 7 acids, 11 aldehydes, 7 ketones, 5 phenylpropenes, and 3 sulphur compounds (Table S2, supplementary material). Similar proportions were also reported in fish balls by You et al. (2024). Innovative formulation was richer in volatile compounds which increased over time with respect to the traditional formulation. However, the increase in innovative formulated fish ball was mainly related to hydrocarbon volatile compounds (96 vs 45.9 ppm), acids (37.9 vs 20.9 ppm), and phenylpropenes (56 vs 11.6 ppm) while the traditional one showed a faster increase in alcohols, aldehydes (30 vs 15.9 ppm), ketones (16 vs 2.8 ppm), and sulphur compounds (6.7

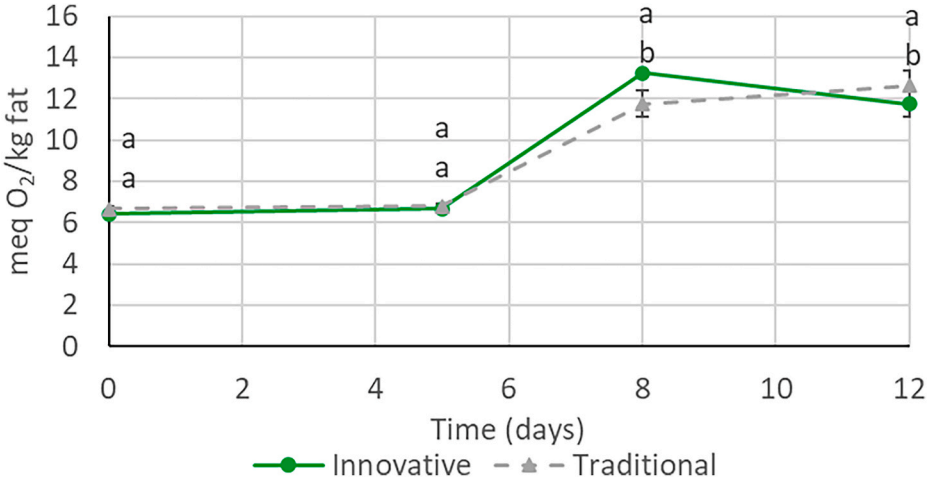

Fig. 1. Peroxide values (mEq 0_2/kg fat) measured in traditional and innovative fish balls during storage. Different letters indicate significant differences among samples at the same storage time ($p < 0.05$).

vs 0.1 ppm). To better explain the volatile molecule results, a PCA was performed. PCA graphical results are shown in Fig. 2. The projection of the samples is reported in Fig. 2a where PC1 and PC2 can explain the 40.78 and 20.16 % of the total variance of the data. Three main clusters were observed: cluster 1 (traditional and innovative fish ball of day 0 and 5), cluster 2 (innovative and traditional fish ball of day 8 and innovative fish ball of day 12), cluster 3 (traditional fish ball of day 12). Fig. 2b shows the molecules responsible for the cluster of the samples. Cluster 1 was mainly characterized by terpenes deriving from spices freshly added into the fish balls, while cluster 2 was characterized by alcohols (terpinene-4-ol, cymen-8-ol), aldehydes (hexanal, benzaldehyde, 2,4-heptadienal, 2,4-decadienal), hydrocarbon volatile compounds (pentadecane, hexadecane, heptadecane), ketones (methyl isobutyl ketone, acetophenone) and acids (propanoic and pentanoic acid). Cluster 3, instead, was mainly associated with ethanol, acetoin, dimethyl disulphide (DMDS), pentanal, and 1-octen-3-ol. The impact of the variables in PC1 and PC2 is reported in supplementary material.

Lipid oxidation is the main pathways for the formation of several volatile molecules such as alcohols, aldehydes, ketones, and acids (Ye et al., 2024). For instance, concentration of 3-methyl-1-butanol (balsamic odour, burnt, malt), 1-hexanol, 1-octen-3-ol (notes of mushroom, metallic), pentanal (sour, grass), hexanal (molasses, grass, nut, fat, green), 3-ethyl-benzaldehyde, acetoin (creamy dairy), and acetic acid (sour) developed faster in traditional fish ball compared to the innovative ones, meaning that oxidative processes took place before in the traditional product. Therefore, although no changes were detected in PV, an effect of reduction of secondary oxidation compounds (volatiles) was observed following the addition of the FBH.

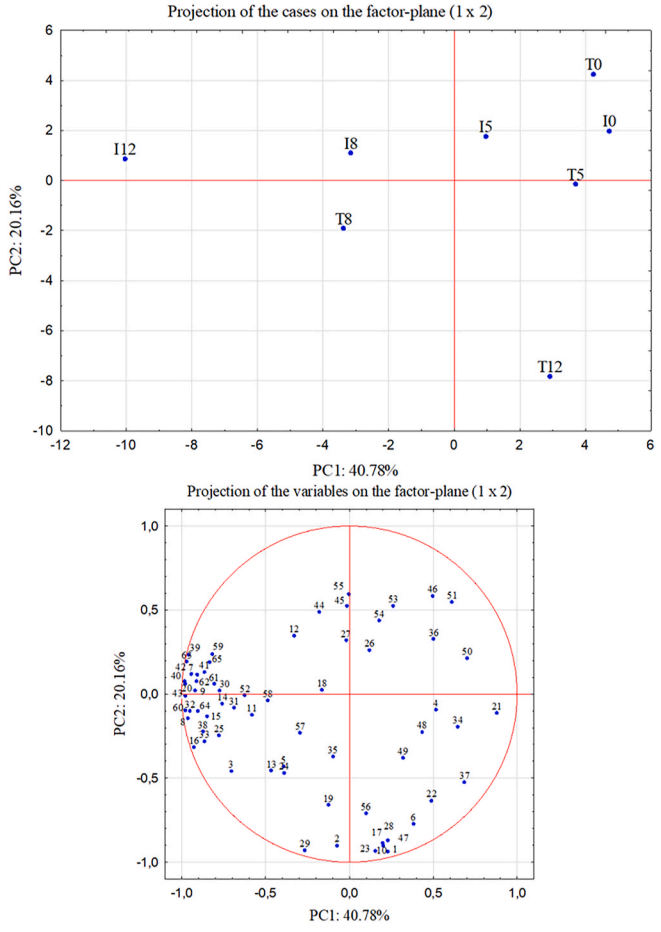

Fig. 2. Projection on the factor plane (1 × 2) of traditional (T) and innovative (I) formulation of fish ball (A) and their variables (B) when stored at 4 °C for 0, 5, 8 and 12 days.

The antioxidant activity of hydrolysed fish protein has been known since the report published by Shahidi et al., in 1995, followed by numerous ones aimed at studying the production, isolation, and identification of antioxidant peptides derived from different fish species.

The mechanisms explaining hydrolysed proteins antioxidant capacity are several, such as the neutralization of free radicals by donating electrons or hydrogen atoms, the chelation of metal ions like iron and copper, inhibiting their ability to catalyse the production of reactive oxygen species (ROS), the deactivatation of molecular oxygen, reducing the formation of ROS, and the ability of donating hydrogen atoms to ROS or other reactive species, interrupting the chain reaction of lipid peroxidation and preventing the propagation of oxidative damage (Nemati et al., 2024). Moreover, peptides are able to physically prevent the penetration of oxidized fat initiators by forming protective layers around oil droplets (Halim et al., 2016).

Da Rocha et al. (2018) found that a higher degree of hydrolysis fish hydrolysates promoted a higher 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity, metal chelation, and ferric-reducing antioxidant power. In our previous work, we tested several microbial strains for the fermentation of mullet by-products and optimized the process to maximise the ability of scavenging free radicals through the ABTS and DPPH assay. However, the effect on a real food system has rarely been studied. Pezeshk et al. (2017) observed a reduction of both PV and TBARS during storage of minced silver carp due to the addition of a protein hydrolysate. In the previous work, the antioxidant activity of the FBH was detected not in the primary phase but in the secondary one.

Some other volatile compounds, such as 3-methyl-1-butanol, ethanol, acetoin, acetic acid, DMDS (notes of sulphurous), have been associated with the growth of *Pseudomonas* and *Enterobacteriaceae* (Parlapani et al., 2023). Indeed, in traditional samples, where *Pseudomonas* spp. grew faster (>7 log UFC/g already after 8 days of storage), higher level of ethanol (up to 50.3 ppm), acetoin (up to 12 ppm), and DMDS (up to 5.6 ppm) were observed on day 12, while these compounds were below the detection limit in the innovative fish balls. Ethanol could be produced also by yeasts, however, in our study, there were no differences in yeast counts between traditional and innovative fish balls. Production of acetic acid and ethanol have been also attributed to the metabolic activity of some LAB under reduced oxygen and under MAP, respectively (Parlapani et al., 2015. For this reason, acetic acid has been proposed as spoilage marker in fresh king salmon (Wierda et al., 2006) and in MAP stored pangasious (Noseda et al., 2012). It is also important to mention that some aldehydes, such as 2,4-decadienal (fatty, fish), 1-octen-3-ol, hexanal, can also contribute to the characteristic and desired aromas of Tilapia head soup (Fu et al., 2023). In our study, their concentrations, either found in traditional or innovative fish balls, were below the levels reported for this type of product.

Hydrocarbon volatile compounds are produced primarily by homolytic cleavage of alkoxy radicals in fatty acids, they typically have a high odour threshold and contribute little to the flavour of seafoods (You et al., 2024). For this class of volatile compounds, innovative formulated fish ball presented higher concentrations compared to the traditional ones, especially at 12 days of storage.

Although this aspect represents a possible drawback since hydrocarbons can react under certain conditions to produce other ketones and aldehydes with bad odors (Lu et al., 2011), in our study this was not observed. To mitigate the modifications of fish aroma, usually spices are added into food formulations. In our case, as shown in Table 1, black pepper, garlic powder, onion powder, lemon juice, parsley powder and nutmeg were added and therefore terpenoids were detected. β-pinene, delta-3-carene, β-phellandrene, γ-terpinene, o-cymene, α-copaene, β-bisabolene, α-farnesene and Cadinene are typical compounds which can derive from spices (Gottardi et al., 2016). During microbial growth, some of these compounds were converted into the respective alcoholic form (e.g., terpinen-4-ol and cymen-8-ol) due to a detoxification process, as already reported by Gottardi et al. (2021) and Siroli et al. (2019).

3.3. Physico-chemical analyses

3.3.1. pH and water activity

The pH and water activity values were measured during the storage period of the amberjack meatballs (data not shown). At the beginning of the storage period, the pH of the amberjack fish balls with the addition of FBH hydrolysate was slightly lower than the samples with the conventional formulation (5.91 and 5.86), but the difference was not significant. These values are consistent with data reported in the literature for similar products such as fish balls, burgers, sausages, and fish fillets (Baygar et al., 2008; Dilucia et al., 2021; Secci et al., 2016).

During the storage period, the pH values of both samples remained relatively constant until the 8th day of storage, after which they increased slightly until the 12th day of storage; however, at the end of the storage period, the innovative product had a significantly lower pH value than the traditional product (5.95 and 6.02).

The values of water activity at the beginning of storage ranged from a minimum of 0.972 (innovative sample) to a maximum of 0.985 (traditional sample). During storage, the a_w values of the innovative samples were significantly lower (of about 0.005) than those of the traditional fish balls, this was probably due to the replacement of 1.5 % of meat (which contains water) with free freeze-dried ingredient.

3.3.2. Color

The color of the product is one of the most important criteria by which consumers make their choice when buying food. It has long been known that the color of fish and seafood is much more than an aesthetic effect, and consumers associate the right coloring with a healthy, high-quality food product (Amaya & Nickell, 2015). The color of traditional raw amberjack fish balls (control) was characterised by a pale red appearance. As it can be seen in Fig. 3, at the beginning of the storage (day 0), the innovative fish balls had a slightly deeper red color than the traditional fish balls. At the end of the storage period, however, this difference was no longer noticeable.

After the addition of the functional hydrolysate, a significant decrease in lightness (L*) was observed in the innovative fish balls (Fig. 4). Throughout the storage period, the traditional samples showed higher L* values than the innovative samples. For both samples, the L* values remained almost constant during storage.

At the beginning of the storage period, the traditional fish balls had a lower a* value than the innovative fish balls (Fig. 4B), probably due to the pigments in the fish by-products hydrolysate added to the innovative formulation, which is characterized by an orange color. This difference was maintained until the 12th day of storage.

The results of the b* values of the amberjack fish balls show that the innovative products had a statistically higher value than the traditional products at the beginning of the storage period, but was similar at day 8 and 12 (Fig. 4C).

3.3.3. Texture

The effects of the innovative formulation on the texture of raw amberjack fish balls were investigated during cold storage by TPA analysis and shear tests. Results are reported in Table 4. Texture parameters are commonly used to investigate and evaluate fish quality. This is particularly evident when assessing the influence of handling and processing methods on the shelf life of seafood products and on consumer preferences and satisfaction (Cheng et al., 2014).

Protein hydrolysates impart unique textural, rheological and mechanical properties to foods. Many factors can influence the textural properties of the incorporated hydrolysate, including the degree of hydrolysis, protein source, enzyme type, pH and globular structure (Asaithambi et al., 2022).

In the present research, no differences were observed between the traditional and innovative sample, formulated with FBH for the 4 parameters considered during the 12 days of storage.

3.4. Sensory analysis

The sensory evaluation results for the raw traditional and innovative amberjack fish balls at beginning (day 0) and the end of microbiological shelf life (day 12) are presented in Table 5. The results of the panel showed that for all attributes there were no significant differences between the scores of the traditional and innovative formulations both at the beginning (day 0) and at the end of the shelf life (day 12). The reduction of lipid oxidation detected by a lower development of volatile compounds, was not perceived by the panel. This might be due to the presence of various spice and herbs in the formulation that might have masked the differences.

At the end of the shelf life, both the innovative and traditional fish balls lost scores in all attributes. In particular, in innovative fish balls the most negatively affected attributes were "overall acceptability" and "odour liking", while the traditional fish balls lost the most scores in "color uniformity" and “overall acceptability”.The results obtained show that the innovative fish balls were still appreciated from a sensorial point of view at the end of the shelf life, confirming that the addition of 1.5% FBH to the formulation of the amberjack fish balls did not affect the "overall acceptability" of the final product during the storage at 4 °C.

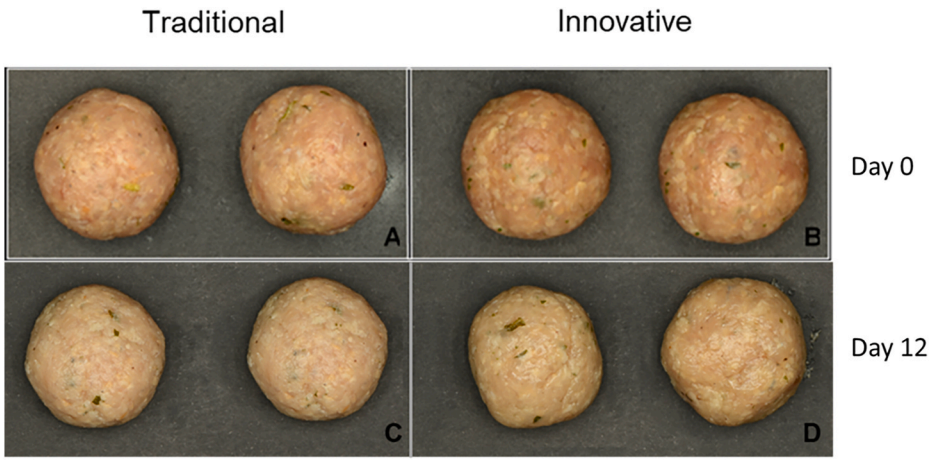

Fig. 3. Appearance of raw traditional fish balls (A) and innovative fish balls (B) at the beginning of the storage period; appearance of raw traditional fish balls (C) and innovative fish balls (D) at the end of the storage period.

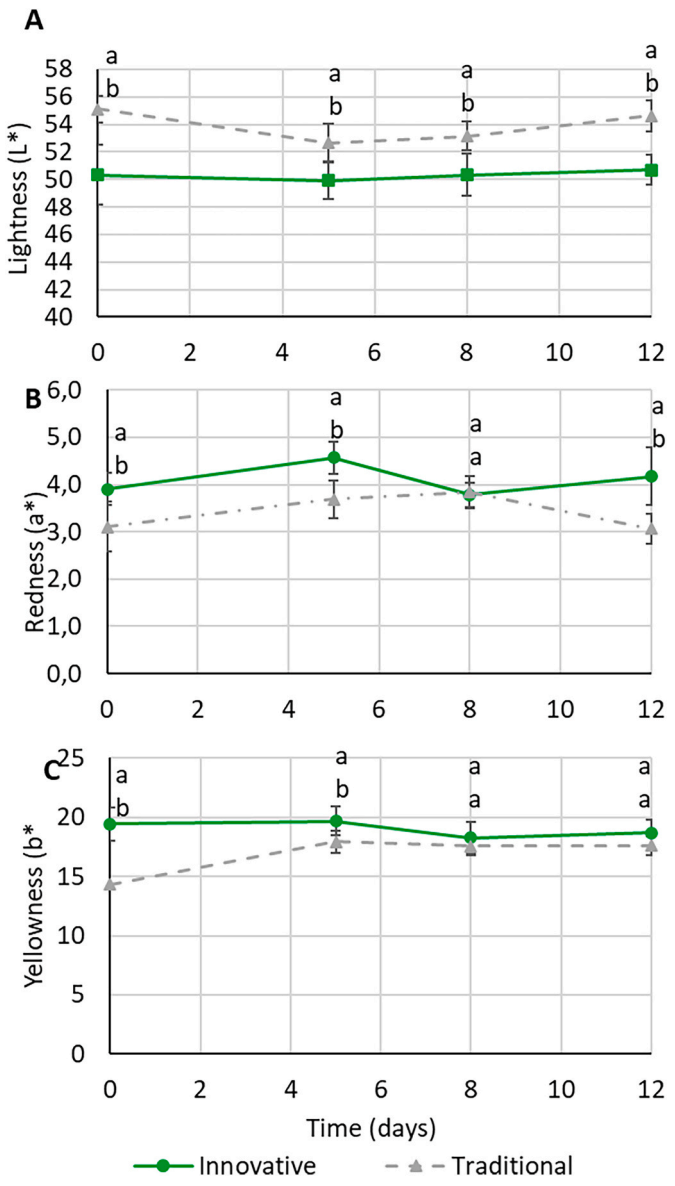

Fig. 4. Changes in color parameter of lightness (L*) (A), red index (a*) (B) and yellow index (b*) (C) of traditional and innovative fish balls during storage at 4 °C. Different letters indicate significant differences among samples at the same storage time ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusions

The innovative formulation of fish balls using mechanically separated amberjack meat and innovative ingredients allowed to obtain new fish-based products with good chemical-physical properties similar to those of commercial fish balls and with a longer shelf life. The initial high level of microbial contamination surely limited the shelf life of the product, but it is a realistic representation of the by-products management in industrial environment and allowed to actually test the effect of the traditional and formulations. The addition of the functional hydrolysate extended the shelf life of the fish balls (less than 12 days) and guaranteed its safety when compared to the traditional product (less than 8 days) with a 50% of extension. During the shelf life, the innovative fish balls showed lower water activity than the conventional fish balls until the 12th day of storage; this may have contributed to improved microbiological stability of the samples. The addition of 1.5% of the fish by-product hydrolysate promoted a reduction of secondary lipid oxidation, observed by volatile analysis, however, this difference was not perceived by sensorial analysis, probably because the presence of spices and herbs masked the perception of these compounds.

We can conclude that the innovative amberjack fish balls can be considered an interesting new fish product obtained through fish by-product (mechanically separated flesh and FBH) valorization. At the same time, to extend fish balls shelf life and make them safer, more stringent handling procedures must be implemented during by-product production and management, to reduce the initial contamination level.

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CRediT authorship contribution statement

Ana Cristina De Aguiar Saldanha Pinheiro: Writing – original draft, Methodology, Formal analysis, Data curation. **Maria Alessia Schouten:** Investigation, Formal analysis, Data curation. **Silvia Tappi:**

Table 5
Sensory properties of amberjack fish balls comparing traditional and innovative formulation at the beginning and end of storage period. Traditional: control fish balls; Innovative: fish balls containing functional hydrolysate.

Attributes	Traditional	Innovative	Traditional	Innovative
	Day 0		Day 12	
Color uniformity	6.83 ± 0.75[a]	7.00 ± 0.63[a]	5.50 ± 1.05[a]	6.00 ± 0.63[a]
Color liking	7.00 ± 1.10[a]	7.17 ± 0.41[a]	5.83 ± 0.75[a]	6.33 ± 1.03[a]
Odour intensity	6.17 ± 0.75[a]	6.50 ± 0.55[a]	5.83 ± 0.41[a]	6.00 ± 0.52[a]
Odour liking	6.67 ± 0.82[a]	6.83 ± 1.17[a]	6.00 ± 0.63[a]	5.67 ± 1.17[a]
Consistency	6.33 ± 0.52[a]	6.50 ± 0.55[a]	6.00 ± 1.10[a]	5.67 ± 0.55[a]
Overall acceptability	7.50 ± 0.84[a]	7.67 ± 0.52[a]	6.17 ± 0.75[a]	6.00 ± 0.55[a]

Different letters indicate significant differences ($p \leq 0.05$) within a timepoint for a specific sensorial attribute.

Table 4
Textural parameters of traditional and innovative fish balls during 12 days of storage at 4 °C. Traditional: control fish balls; Innovative: fish balls containing functional hydrolysate.

Attribute	Traditional		Innovative		Traditional		Innovative		Traditional		Innovative		Traditional		Innovative	
	Day 0				Day 5				Day 8				Day 12			
Hardness (N)	1.53	±0.30[a]	1.55	±0.12[a]	1.51	±0.26[a]	1.37	±0.20[a]	1.38	±0.24[a]	1.45	±0.23[a]	1.54	±0.28[a]	1.32	±0.19[a]
Springiness	3.58	±0.03[a]	3.57	±0.01[a]	3.56	±0.02[a]	3.53	±0.02[a]	3.56	±0.01[a]	3.55	±0.02[a]	3.56	±0.02[a]	3.56	±0.02[a]
Gumminess (N)	0.57	±0.15[a]	0.54	±0.05[a]	0.53	±0.11[a]	0.44	±0.09[a]	0.47	±0.10[a]	0.47	±0.09[a]	0.54	±0.12[a]	0.42	±0.07[a]
Shear force (N)	1.54	±0.15[a]	1.47	±0.06[a]	1.21	±0.20[a]	1.22	±0.17[a]	1.38	±0.12[a]	1.29	±0.10[a]	1.47	±0.02[a]	1.59	±0.14[a]

Different letters indicate significant differences ($p \leq 0.05$) within a timepoint for a specific textural attribute.

Writing – original draft, Visualization, Supervision, Conceptualization. **Davide Gottardi:** Writing – original draft, Methodology, Investigation, Conceptualization. **Federica Barbieri:** Formal analysis, Data curation. **Marianna Ciccone:** Formal analysis, Data curation. **Solidea Amadei:** Formal analysis, Data curation. **Urszula Tylewicz:** Writing – review & editing, Validation, Supervision, Investigation. **Francesca Patrignani:** Writing – review & editing, Resources, Project administration. **Pietro Rocculi:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116724.

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