Food and Bioprocess Technology (2026) 19:116
https://doi.org/10.1007/s11947-025-04186-w

RESEARCH

Production and Use of Fish By-Product Protein Hydrolysates in Breadmaking: Effects on Bread's Nutritional, Sensory and Physicochemical Characteristics

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Received: 15 July 2025 / Accepted: 18 December 2025

Abstract
Although there is a precedent for enriching bread with plant-based ingredients or muscle tissue, the use of fish protein hydrolysates (FPH) in baking remains underexplored, especially if the FPH comes from fish by-products. The impact of adding FPH from sea bass and salmon heads in bread-making, analysing its sensory, proximate and physicochemical effects was assessed. The influence of different antioxidants during hydrolysis was also evaluated. Four bread formulations were produced with varying levels of wheat flour replacement by FPH (2.5% and 5%) and a control bread. A comprehensive evaluation of the breads included analyses of proximate composition, texture, colour, sensory attributes and crumb structure at both macro- and microscopic levels. The incorporation of FPH led to increased protein content and moisture, particularly noticeable at the 5% replacement level. Sensory differences emerged between the control and FPH-enriched breads; the 2.5% formulation retained desirable sensory properties, whereas the 5% substitution introduced less favourable traits such as bitterness, a fishy odour and rancid notes. Textural changes included greater bread hardness, which correlated with modifications in crumb microstructure, notably larger alveoli and subtle shifts in crust and crumb colour. Among the tested antioxidants, those based on carnosic acid proved most effective in preserving quality. In conclusion, FPH could serve as a viable option for nutritionally enriching bread, although it is advisable to keep substitution levels low (2.5%). These findings underscore the potential of FPH as an ingredient in baked products and contribute to circular economy in the fishing industry.

Keywords Bread · Fish protein hydrolysate · Bioactive peptides · By-products · Nutritional fortification

Introduction

Bread may be defined as the product resulting from the baking of a dough obtained by mixing flour and water, with or without the addition of salt, leavened with the aid of baker's yeast or sourdough (Spanish Goverment, 2019). It is believed that the first bread was produced around 10,000–12,000 years BCE and may have been developed through deliberate experimentation with water and cereal flour. Some authors speculate that the origins of bread could have been a mixture of semi-milled grains lightly moistened, which could have been baked in the sun, on a hot stone or simply left abandoned next to a fire or various heat sources (Dupaigne, 1999). After that, the Egyptians were the pioneers who popularized the art of bread-making worldwide. The importance of this product led to its control, production and distribution, and has been used as a mean of exerting political influence over the population for at least the last 2000 years. Even today, bread scarceness is synonymous of difficult times, while the promise of its provision is used as a lure for a better life (Mondal & Datta, 2008).

Bread consumption has seen a marked increase globally, with significant increases in countries such as India, Philippines and Egypt, where 38%, 27% and 27% of consumers, respectively, have increased their intake in the last year. This increase highlights the popularity of bread and its derivatives as a basic, convenient and affordable food. In fact, baked goods show a higher average global penetration (81%) than other foods such as meat and poultry (76%), dairy products (74%), desserts and ice cream (64%) and sweet and savoury snacks (64%) (Glanbia nutritionals, 2024). A similar trend is

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Published online: 16 January 2026

observed in European countries like Spain, where the industrial production of fresh bread reached 3.3 million tonnes in 2022, 65% more than the previous year. In addition to fresh bread, there is also significant production of long-life bread (28,397 tonnes of breadsticks in 2022). This led to a consumption of 27.9 kg per person in 2022 (MERCASA, 2023). The reasons for this rise would be its versatility of preparation, relatively low price and high energy contribution. Moreover, in developed countries, the reformulation of bread (whole grain, seeded, low-salt, etc.) has made it a more attractive product (Liu et al., 2019; Sørensen et al., 2022).

Bread is traditionally produced using refined wheat flour due to its gluten-forming proteins, gliadin and glutenin, that provide the desired bread texture and volume. However, refining wheat reduces its nutritional quality through significant losses in dietary fibre, vitamins, minerals and phytochemicals (Wandersleben et al., 2018). Furthermore, the natural absence of certain compounds, such as amino acids, some of which are essential in the human diet (lysine) must be also considered (Pomeranz & Shellenberger, 1970). For this reason, and with the aim of making a more complete product from a nutritional point of view, several studies have included oilseeds and other vegetable ingredients (e.g. flaxseed, sesame, sunflower, chia, soybean or lentils, among others), achieving a nutritional fortification in certain macronutrients (especially protein) and bioactive compounds (such as amino acids, polyunsaturated fatty acids, minerals or vitamins) with different properties (Betoret & Rosell, 2020; de Lamo & Gómez, 2018), but also producing sensory changes whose effect has not always been evaluated. However, the use of fish for enriching this product has been less explored and, to date, has mainly been limited to fortification with fish pieces that are commonly consumed by itself (Pomeranz & Shellenberger, 1970).

The addition of protein hydrolysates (whey, casein, collagen, soy) has been shown to significantly alter the rheological properties of dough. For instance, they tend to reduce water absorption and lead to increased dough development and formation times, which can result in the weakening of the gluten network when the substitution level is high (Nogueira et al., 2020; Prieto-Vázquez del Mercado et al., 2022). Regarding the texture of the final bread product, these hydrolysates often increase hardness, reduce elasticity and may negatively impact specific volume, especially when the added protein replaces a substantial portion of the original flour. Animal-derived hydrolysates exhibit more pronounced effects on firmness and texture compared to many plant-based hydrolysates, which, at moderate levels, may induce less drastic changes (Gani et al., 2015). From a sensory perspective, acceptance may decline when hydrolysates impart bitter flavours or undesirable aromas, or when the texture becomes dry, rubbery or overly firm. However, several studies report that sensory changes are not drastic when the concentration is kept low and conditions such as hydration, dough formulation and the degree of hydrolysis of the protein product are optimized (Song et al., 2018).

In this sense, aquatic animal foods (whether captured or farmed) are characterized by their high protein content (with remarkable quantities of essential amino acids), having also the highest content of long-chain polyunsaturated omega-3 fatty acids than any other animal-derived food. Additionally, they generally have higher mineral and vitamin content than most terrestrial meats and processed meat products, including vitamins (vitamin A, vitamin D, vitamin E, vitamin B12, folic acid, choline, coenzyme Q10) and minerals (calcium, magnesium, iron, copper, zinc, iodine, selenium and trivalent chromium) (Tacon et al., 2020). Furthermore, there is considerable scientific evidence on the direct health benefits of consuming fish and fish products, including (but not limited to) the reduction of the risk of death from coronary heart disease and stroke (Forouhi et al., 2018), the reduction of the risk of diabetes (Wallin et al., 2012), the increase in gestation duration and improvement in visual and cognitive development (Hellberg et al., 2012), among others.

For this reason, fish is a widely consumed food all over the world, with an increase in its consumption in the last decades (FAO, 2022), which has led to a raise in fish by product generation. In recent years, FAO's estimation of post-harvest losses in the seafood sector has remained at 20–35% of the catch, occurring at various stages of the value chain. Approximately 17.9 to 39.5 million tonnes of whole fish are discarded annually by commercial fishing operations. Besides quality deterioration along the supply chain, in 2021, 23.8 million tonnes were directed to by-products, although the same report highlights that not all potential processing discards at sea or from small-scale producers were included (World Economic Forum, 2024). As stated previously, fish processing yields significant by-products, involving the head, bones, skin, viscera and other often discarded parts that usually constitute 30–50% of the total fish weight (Al Khawli et al., 2019). For example, in the case of sea bass and salmon, whose global productions in 2023 and 2024 were 286,967 and 2,800,000 tons, respectively, the amount of by-products generated could reach approximately 114,786.8 tons for sea bass and 1,120,000 tons for salmon (Abdel-Rahim et al., 2025; Haq et al., 2025). However, it is well known that the nutritional richness is not limited exclusively to the traditionally consumed parts. Studies have demonstrated that in fish by-products remain a great source of beneficial macro- (protein, aminoacids, polyunsaturated fatty acids) and micronutrients (vitamins, minerals) for humans (Gehring et al., 2011; Smichi & Kharrat, 2016). In that sense, the production of such substantial quantities of by-products requires the development of sustainable and efficient resource utilization techniques. For that reason, there

is increasing interest in identifying innovative approaches to use these by-products effectively because, as some authors claim, seafood must employ the concepts of life cycle thinking and the circular economy to increase production efficiency and mitigate its environmental impact (Cooney et al., 2023). However, the valorisation of fish by-products still faces several obstacles that hinder their full utilisation. Their composition is highly variable and prone to rapid spoilage, which complicates processing and requires cold-chain logistics. Scaling up also demands significant investment in technology and infrastructure, while economic returns are uncertain. In addition, strict regulations and limited consumer acceptance of products derived from by-products hinder wider adoption. These factors explain why valorisation practices are not yet broadly implemented despite their potential (Coppola et al., 2021; Hopkins et al., 2025; Nirmal et al., 2022).

In this regard, the use of enzymatic hydrolysis technologies could be a useful way to reuse these by-products as it is a rapid, safe, scalable and easily controllable method to produce FPHs. Enzymatic hydrolysis of fish by-products is already applied in the food industry, mainly for the production of protein hydrolysates used as flavourings, feed ingredients or dietary supplements. The process not only hydrolyzes the protein phase but also enables the separation of lipid-rich and mineral-rich fractions. Thus, the process itself is not novel. However, depending on the final composition and intended use of the hydrolysates (e.g., as sources of bioactive peptides in functional foods), they may fall under the scope of Novel Foods regulation (Honrado et al., 2024a, 2024b). This would allow the generation of peptides (which are of a higher biological value than intact proteins) and free amino acids. Additionally, it is considered a fast and easily reproducible method; it can separate not only peptide fractions but also oils from insoluble solids; it avoids extreme physical and chemical treatments, compared to chemical hydrolysis; it has the advantage of avoiding the generation of chemical waste, besides being more easily controllable, thus reducing unwanted reactions that destroy high-value components of proteins. The result is the generation of different fractions that could be applied in various fields, such as biotechnology and the cosmetic or nutrition industry (Araujo et al., 2021), as an ingredient that can strengthen certain products that are nutritionally poor in some nutrients. Hence, the protein fraction of these hydrolysates generated from by-products could serve as ingredients in various foods, making them much more nutritionally complete. However, the production process of hydrolysates involves the application of moderate temperatures, which can negatively impact the already typically compromised lipid profile (polyunsaturated fatty acids) of the by-product. Antioxidants such as BHT (Butylated hydroxytoluene) are known to be highly effective in preventing this degradation (Xu et al., 2021). Concerns over their potential health risks have led to their risk evaluation or ban in several countries. Natural origin antioxidants, especially those based on rosemary extracts and tocopherol mixtures, have demonstrated promising effects (Honrado et al., 2022). Therefore, it is of interest to evaluate which naturally derived compounds are most effective in preventing oxidation, thereby preserving both the nutritional value and sensory quality of the product.

Therefore, the objective of this research was to evaluate the effect of different antioxidants on preventing lipid oxidation during fish protein hydrolysates (FPH) making and, from a sensory, nutritional and physicochemical point of view, the effect of adding FPH from different fish species in bread-making.

Materials and Methodology

FPH Development

The main raw materials used to produce FPH were sea bass (*Dicentrarchus labrax*) and salmon (*Salmo salar*) heads, as examples of semi-fat farmed fish and fatty farmed fish, respectively. The selection of fish heads was based on their high nutritional value, as well as on the fact that they represent a large proportion of the by-products generated from these species. These were supplied by Barna SA (Mundaka, Spain). The manufacturing procedure followed for the hydrolysates was as described in the research by Honrado et al. (2023) with some modifications and can be visualized in Fig. 1. Briefly, fish heads (2 kg), previously thawed, were ground in a cutter (Sammic mod. SK-3, Azkoitia, Spain), and the resulting paste was mixed with distilled water in equal parts in a 5-L container. The mixture was then placed in a hot water bath at 40 °C. Different commercial products were added as antioxidants separately (0.25 and 0.5% of fish weight) to check the effectiveness for each one. These products were carnosic acid-based oil-soluble Bordantix® (OSB) and water soluble Bordantix® (WSB)—Evesa, La Línea de la Concepción, Spain—and tocopherol based Nutrabiol® (NUT) and Tocobiol® (TOC)—BTSA, Madrid, Spain. A control treatment without antioxidant was also carried out. After that, the pH was adjusted to 7.00 using a pH metre (Crison mod. pHmeter basiC 20, Alella, Spain) with 2 M NaOH (Panreac Química SLU, Castellar del Vallés, Spain). After pH adjustment, a mixture of enzymes was added at a proportion of 0.5% of the weight of the ground fish. Three enzymes were used at the same time: Protamex®, Protana® Prime and Protana® UBoost (Novozymes, Bagsvaerd, Denmark). These enzymes were chosen as previous research demonstrated that this combination provided few undesirable sensory attributes (bitterness or fish flavour, among others). The pH decrease produced by the enzymatic

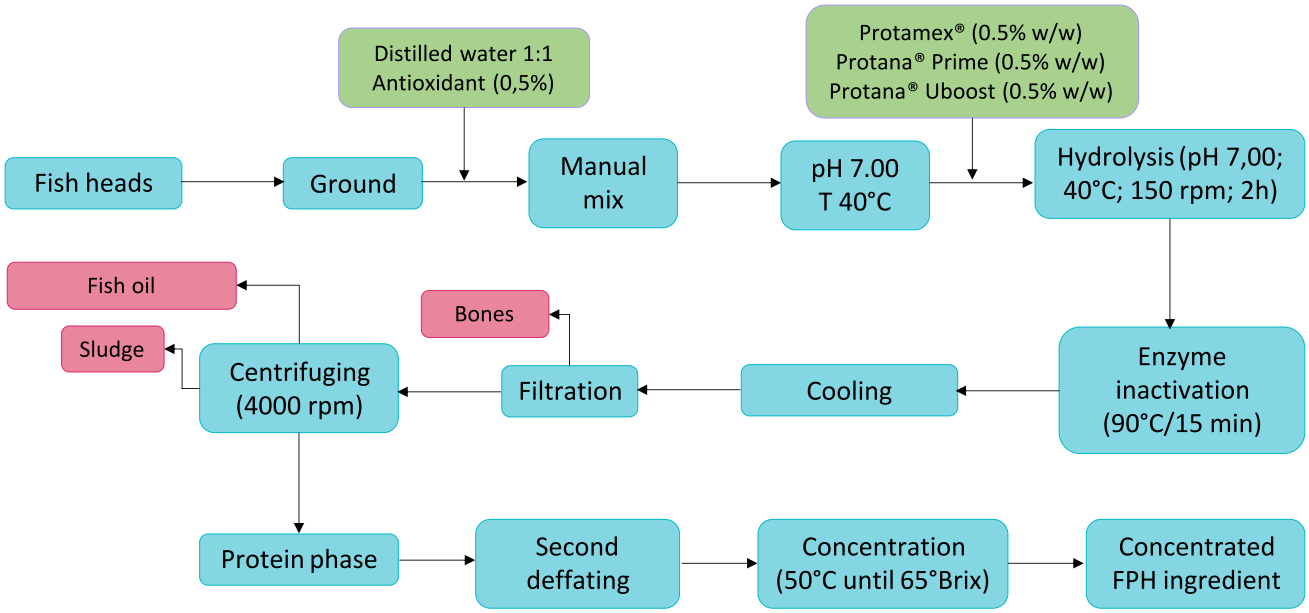

Fig. 1 Functional ingredient (FPH) production process

reaction was compensated by the addition of 2 M NaOH. This process was repeated until there were no pH changes (approximately after 2 h). The enzyme was then inactivated by heating at 90 °C for 15 min. The mixture was filtered through a sieve (Ø 1.2 mm) to remove solid residues, and the liquid part was centrifuged at 4000 rpm for 10 min. Three phases were obtained: the bottom phase consisted of sludge, which was discarded; the aqueous phase, the most abundant, was the FPH; the upper phase, containing fish oil and phospholipids, was also discarded. The liquid FPH was subjected to a second defatting process in a milk skimming machine (Suministros Químicos Arroyo, mod. ARR-DES 125). Finally, it was concentrated using a rotary evaporator (Laborota mod. 4000, Schwabach, Germany) at 50 °C until reaching 65° Brix (Kern mod. ORA 3SA, Albstadt, Germany). The purpose of this stage was to adjust the hydrolysate for incorporation as an ingredient in bread-making while achieving a reduction in water activity to improve its preservation.

Characterization and Stability of the FPH

Oxidative Stability of the FPH

The peroxide value was determined as a measure of the compounds generated during the initial stage of oxidation. It was assessed for each sample using titration with 0.01 N sodium thiosulfate (Panreac Química SLU, Castellar del Vallés, Spain) following the ISO 3960:2017 (International Organization for Standardization, 2017) standard, with some modifications. Between 0.5 and 1.5 g of the oil obtained after centrifugation of each sample were weighed into an Erlenmeyer flask. Subsequently, 7.5 mL of acetic acid (Panreac Química SLU, Castellar del Vallés, Spain), 5 mL of hexane (Carlo Erba, Val de Reuil, France), and 200 µL of a saturated potassium iodide solution (Carlo Erba, Val de Reuil, France) were added. The mixture was stirred for two minutes. Afterward, 25 mL of distilled water and 400 µL of 1% starch solution (Panreac Química SLU, Castellar del Vallés, Spain) were added. Finally, the titration was performed using the 0.01 N sodium thiosulfate solution, and the peroxide index was calculated using the following formula (1).

$$PV\left(meq\frac{O_2}{kg}\right) = \frac{(V - V_0)\cdot C\cdot 1000}{m} \quad (1)$$

where V is the volume of the standard sodium thiosulfate solution used in the determination, in millilitre; V_0 is the volume of the standard sodium thiosulfate solution used in the blank determination, in millilitre; C is the concentration of the sodium thiosulfate solution, in moles per litter and m is the mass of the sample portion analysed, in grammes.

The determination of the malondialdehyde (MDA) content according to the cd 19–90 method (AOCS, 1990) was performed to determine secondary lipid oxidation products. Initially, sample distillation was performed using a distillation apparatus (Velp Sicentidica mod. UDK 129, Usmate Velate, Italy) in the presence of trichloroacetic acid (Merck, San Luis, USA). The distillate was then reacted with 0.28% w/v thiobarbituric acid (Merck, San Luis, USA). Simultaneously, a standard curve was generated using 1,1,3,3-tetramethoxypropane

(TMP) (Merck, San Luis, USA). The standard curve and samples were incubated in a 100 °C water bath for 40 min (J. P. Selecta mod. 3,000,544, Abrera, Spain). Finally, after cooling, absorbance was measured at 532 nm using a spectrophotometer (ONDA mod. UV-20, Beijing, China). Measurements were conducted in triplicate.

Anisidine assay was also conducted following ISO 6885:2016 (International Organization for Standardization, 2016). First, 0.2 to 2 g of the sample was weighed into 25-mL volumetric flasks, which were then filled to volume with isooctane (VWR, Rosny-sous-Bois, France). Three tubes were prepared as follows: A1 contained 5 mL of the sample solution and 1 mL of a p-anisidine solution (Sigma Aldrich, Steinheim, Germany) in acetic acid at a concentration of 0.02 M; A2 contained 5 mL of isooctane and 1 mL of the aforementioned p-anisidine solution; A3 contained 5 mL of the sample solution and 1 mL of acetic acid. After preparation, the tubes were incubated in the dark for 10 min, and the absorbances were measured at a wavelength of 350 nm using a spectrophotometer (ONDA model UV-20, Beijing, China). Finally, the anisidine index was calculated using the following formula (2):

$$AV = \frac{100 \cdot Q \cdot V}{m}((1{,}2 \cdot \left(A_1 - A_2 - A_0\right)) \quad (2)$$

where V is the volume in which the sample for analysis is dissolved, in millilitre; m is the mass of the portion for analysis, in grammes;Q is the sample content of the measured solution, based on which the anisidine index is expressed, in grammes per millilitre (Q = 0.01 g/mL); 1.2 is the correction factor for the dilution of the analysis solution with 1 mL of glacial acetic acid reagent, and A0, A1 and A2 are the absorbance of the contents of tubes A0, A1 and A2 after the reaction time, respectively.

The TOTOX value was determined as a comprehensive indicator of oil deterioration to assess the stability of the raw material. It serves as a metric for evaluating the oxidation level of oils and fats, particularly in products rich in omega-3 fatty acids, such as fish, krill or algal oils, by integrating the peroxide value (primary oxidation) and the anisidine value (secondary oxidation). The following formula was applied (3):

$$totox = (2 \cdot PV) + AV \quad (3)$$

Values below 10 are considered ideal, while values above 20 indicate a rancid oil with reduced health benefits. Intermediate values suggest an acceptable oil but with signs of oxidation (Argalys, 2020; Özdemir et al., 2021).

Proximate Composition of the FPH and Water Activity (a_w)

The determination of the moisture content was performed in triplicate using gravimetry and oven drying (J. P. Selecta mod. 2,005,167, Abrera, Spain) at a temperature of 105 °C according to method 950.46 (AOCS, 1990).

The determination of the fat content was carried out according to the method proposed by Bligh and Dyer (1959). Firstly, the samples were placed in centrifuge tubes, and then fat extraction was performed using chloroform and methanol (CARLO ERBA Reagents, Sabadell, Barcelona), KCl (Panreac Química SLU, Castellar del Vallés, Spain), and distilled water. Once the mixture was homogenized, it was centrifuged at 4000 rpm, 4 °C for 10 min (Hettich, mod 320 R, Tuttingen, Germany), and the lower phase was extracted and transferred to a pre-weighed capsule. The chloroform was allowed to evaporate, and the fat content was determined by weight difference. Measurements were conducted in triplicate.

The total protein content was determined in triplicate using the Kjeldahl method 992.15 (AOCS, 1990), based on the determination of organic nitrogen. One gramme of hydrolysate was weighed and placed in a distillation tube along with a catalyst tablet (VWR, Llinars del Vallès, Spain) and 12 mL of 98% sulfuric acid (Panreac Química SLU, Castellar del Vallés, Spain). It was then placed in a digester (VELP Scientifica, DK 6 heating digester Kjeldahl, Usmate Velate, Italy) at 420 °C for 1 h. Subsequently, distillation of the previously alkalized digest with 50 mL of 35% w/v NaOH (Panreac Química SLU, Castellar del Vallés, Spain) was carried out using a Kjeldahl apparatus (Velp Sicentidica mod. UDK 129, Usmate Velate, Italy). The distillate was collected in a 3% w/v boric acid solution (VWR, Llinars del Vallès, Spain). Finally, titration was performed with 0.1 N HCl (Carlo Erba, Sabadell, Spain) in the presence of the Tashiro indicator. Protein content was calculated using a protein-nitrogen conversion factor of 6.25.

For ash content, 3 g of the different samples were placed in a pre-weighed porcelain crucible and ignited in an ashing furnace maintained at 550 °C by 24 h. The ash content was determined as soon as white ash was obtained, and a constant weight was maintained. The total carbohydrate content was determined by weight difference.

Bread-Making Process

Bread loaves (*baguette* type) were prepared. All bread loaves were made in the Pilot Plant of the Medicine Veterinary Faculty. Four types of bread loaves enriched with different proportions of the prepared FPH from sea bass and salmon heads were prepared and compared against a control bread. Two treatments were enriched with 2.5% and 5% sea bass FPH, and the other two with 2.5% and 5% salmon FPH (Table 1). For the bread preparation, the first step was to mix the flour with salt, and then, warm water mixed with fresh yeast and the hydrolysate was added. After that, a kneading machine was used for 5 min (Sammic Mod. BM-11,

Table 1 Bread formulations developed in this research. *SB* seabass; *S* salmon. 2.5 and 5 refer to the percentage of flour substitution with seabass or salmon FPH

Formulation	Refined wheat flour (g)	Leavening (g)	Salt (g)	Seabass FPH (g)	Salmon FPH (g)	Water (g)
Control	1000	24.00	18.00	0.00	0.00	630.00
SB-2.5	985.00	24.00	18.00	15.00	0.00	620.00
SB-5	970.00	24.00	18.00	30.00	0.00	610.00
S-2.5	985.00	24.00	18.00	00.00	15.00	620.00
S-5	970.00	24.00	18.00	00.00	30.00	610.00

Azkoitia, Spain) to promote gluten development and obtain an elastic and lump-free dough. It was allowed to rest for 1 h in the fermentation oven at 35 °C and 80% relative moisture (Salva mod. FC-05, Guipúzcoa, Spain). Once the dough had doubled in volume, it was divided in 250-g portions and then shaped into baguettes. These rested for an additional 30 min in the fermentation oven. After that, they were baked in the oven with steam injection in the first seconds at 240 °C for 10 min. The loaves measured 38 cm in length, 6 cm in width and 4 cm in height. Once at room temperature, the bread was finally stored in in airtight bags and frozen (− 20 °C) for a period not exceeding 5 days. For all the subsequent analysis bread were baked at 180 °C for 5 min.

FPH Fortified Bread Characterization

Bread Proximate Composition Analysis and a_w

A food mincer (Moulinex model Moulinette A320R1, Alençon, France) was used to homogenize the sample. The moisture, protein fat and ash contents were determined following the same procedures as those described in the "Proximate Composition of the FPH and Water Activity (a_w)" section. Measurements were conducted in triplicate.

The a_w of the samples was measured with the LabMASTER-a_w (Novasina, Switzerland) at 25 °C. Three measurements were performed on each of the pre-crushed breads.

Bread Colour Characterization

The colour measurement was conducted in triplicate using a colorimeter (Minolta mod. CM-2002, Osaka, Japan) previously calibrated against a standard black and white reference. The CIELAB system was employed, wherein the L* coordinate signifies brightness on a scale ranging from 0 (black) to 100 (white). The a* coordinate denotes the red-green index, with negative values indicating green hues and positive values indicating red hues. The b* coordinate signifies the yellow-blue index, with negative values representing blue hues and positive values representing yellow hues (Luo, 2015). Colour measurements were taken on both the crust and center of the crumb of 2-cm-thick slices from each type of bread. The variation in colour induced by the addition of the hydrolysate was assessed by calculating the total colour difference (ΔE, Eq. 4), following the methodology outlined in the research of (Honrado et al., 2023).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where $\Delta L = L^*_{\text{Bread containing FPH}} - L^*_{\text{Control bread}}$; $\Delta a = a^*_{\text{Bread containing FPH}} - a^*_{\text{Control bread}}$; $\Delta b = b^*_{\text{Bread containing FPH}} - b^*_{\text{Control bread}}$

Bread Instrumental Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was conducted for the 5 types of bread using a texture analyser (Stable Micro Systems Mod. TA-XT2i, Godalming, UK) equipped with a flat aluminium cylindrical probe (Ø 5 cm). The analysis comprised two compression cycles with a 20-s separation time between them (decompression). Texture parameters determined included hardness, adhesiveness, elasticity, cohesiveness, gumminess, chewiness, resilience and fracturability. Test settings were as follows: pre-test, test and post-test speed: 1 mm/s; sample deformation: 65% of the distance; force threshold: 10 g (Mamat et al., 2014). The texture profile analysis was conducted once the breads had reached room temperature, using 2-cm cube shaped crumb pieces. These cubes were obtained through meticulous cutting with a scalpel to achieve clean cuts that would not alter the crumb structure. Measurements were repeated 8 times for each type of bread.

Evaluation of the Penetration Characteristics of the Bread Crust

A puncture test was also performed to evaluate the penetration characteristics of the crust. The test parameters were as follows: load cell 5 kg, pre-test, test and post-test speed of 15 mm/s and a penetration distance of 15 mm. The puncture was performed using the P2N flat probe. 8 punctures were made on six crust slices.

Digital Image Analysis: Bread Alveolate

To analyze the crumb structure, three slices (2.5 cm thick) were taken from the central region of each bread, and each slice was photographed using a camera (Canon, mod. Powershot G10, Tokyo, Japan) under the same conditions. The image, in TIFF file format, was analysed with the image analysis Image-J software (Rasband, 1997–2012) (. A manually selected area of equal size from the central part of the slice, representative of the total crumb of each slice, was chosen. The colour image was converted to an 8-bit image and analysed in grayscale (0 black, 255 white). Image segmentation (conversion to binary, black-and-white image) was performed by the software. An appropriate grey threshold level was found iteratively, varying the grey level until the image retained the fundamental features of the monochromatic counterpart. This threshold value selection method best represented the fundamental features of the images. With this approach, and using the same program, measuring the surface of the black colour (alveoli), it was possible to determine the average area of the alveoli, the number of alveoli per unit area and the area fraction (the percentage of the area occupied by alveoli).

Bread Scanning Electron Microscopy (SEM)

For SEM visualisation, a cross-section of the crumb was freeze-dried and then Au/Pd coated and then observed at 25×, 100×, 500× and 1000× magnification and 15 kV in a scanning electron microscope (JEOL mod. JSM 6360-LV, Tokyo, Japan). This allowed us to visualise differences in the internal structure of the bread that can explain the behaviour found in the instrumental texture hardness and sensory analysis.

Bread Sensory Characterization

The bread sensory analysis was carried out in the tasting room of the Pilot Plant of the Faculty of Veterinary Medicine, complying with ISO 8589:2007 (International Organization for Standardization, 2007): Individual booths, climate-controlled at 25 °C and equipped with lighting at a colour temperature of 6500 K. A panel of 10 trained sensory assessors was employed following ISO 8586:2023 (International Organization for Standardization, 2023). These sensory assessors had previously demonstrated sensory sensitivity in preliminary tests, received considerable training and were able to make consistent and repeatable assessments of various commercial bread samples (ISO 8586:2023). This also allowed the assessors to acquaint themselves with the attribute terms and the scoring system.

For the sensory characterization of the developed breads, a modified version of the flash profile technique proposed by Dairou & Sieffermann (2002) was employed to delineate the organoleptic properties of treatment. The advantage of this technique is that the sensory assessor receives all the samples at once and evaluates them on the same scale, allowing direct comparison between them. Prior to this characterization, each assessor received a sample of each treatment to develop a lexicon for characterizing the samples. Subsequently, the lexicon generated by each assessor was pooled and analysed through the differential semantic analysis, leading to a consensus that facilitated the compilation of the list of attributes to be evaluated (Aínsa, 2023). Finally, 15 attributes were selected: crust toasting intensity, crumb brown colour, alveoli size, crunchiness intensity, crumb elasticity, crust toasted aroma, crumb typical aroma, crumb fishy smell, crust fishy flavour, crumb rancid smell, bitter taste, umami taste, salty taste and aftertaste.

The analysis involved the utilization of an unstructured lineal scale measuring 10 cm for each descriptor considered wherein sensory evaluators were asked to assess each product. The loaves were heated in the baking oven at 240 °C for 5 min and then cut into 4 cm thick slices. The samples were presented simultaneously on ceramic plates, in a different and randomized sequence for each sensory evaluator. Two sessions were held on consecutive days to check the homogeneity of the evaluators' measurements. Each panelist had access to water and apple ad libitum to cleanse the palate between samples.

Statistical Analysis

All results obtained from the different analyses were subjected to statistical analysis using the Office software Microsoft Excel and the statistical software XLSTAT (Addinsoft®, 2016). The first step was to check the homogeneity of the values using box plot graphs. Once checked and outliers removed (after performing Grubb's test), the normality of the values was verified with Shapiro–Wilk's test. For physicochemical results, an ANOVA (two ways with interaction) was carried out. This model included treatments as fixed variables and the three replicates as a random effect, also the interaction between them was studied too. Approximate *F*-ratio tests for each fixed effect were conducted, and the critical value for a statistical effect was taken at $p < 0.05$. A pairwise comparison between means was carried out using Fisher's multiple comparisons test. However, for the ΔE, a comparison against a control test (bilateral Dunnett) was performed instead of Fisher's test. Regarding sensory analysis, a generalized procrustean analysis (GPA) was conducted to analyse the proximity of sensory attributes to each bread.

For the sensory evaluations results, a flash profiling analysis was conducted, which included a multifactorial analysis (MFA). This test provided the coordinates of the attributes, which were subsequently used to determine the coordinates

of the products. Using these coordinates, an HAC (hierarchical agglomerative clustering) was performed, employing Euclidean distance, Ward's method for agglomeration, and the adapted Hartigan index for truncation. This yielded a dendrogram, allowing for the identification of which treatments differed from each other, and which were similar. However, this method did not reveal significant differences among attributes, nor did it explain the reasons for the observed differences between products.

To address this, a rank table was obtained from the flash profiling analysis: the product with the lowest intensity for a given attribute was assigned a rank of 1 for that attribute. In cases where several products tied for a specific attribute, the assigned rank corresponded to the average of the ranks of the tied products. Based on the mean ranks obtained from this table, the Kruskal–Wallis test was applied at a significance level of 5%, followed by a post hoc multiple comparison test using the Steel–Dwass-Critchlow-Fligner test. This approach allowed for the identification of attributes that significantly differed among the samples.

To obtain a comprehensive overview and identify the variables characterizing each treatment, as well as the potential relationships among these variables, a principal component analysis was conducted. This analysis included proximate composition, colour characterization of both crumb and crust, texture characteristics of crust and crumb and digital image analysis.

Results and Discussion

Characterization and Stability of the FPH

Oxidative Stability of the FPH

Table 2 shows the oxidation indices obtained with the incorporation of different antioxidants for the two species studied.

In relation to the peroxide index, it was observed that sea bass FPH samples treated with Bordantix®, in both its water-soluble (WSB) and lipid-soluble (OSB) forms, exhibited the lowest peroxide indices. However, no significant differences were detected compared to the control treatment. This suggests that the effect of Bordantix on peroxide formation is negligible for this species. In the case of salmon FPH, a similar behaviour was noted. Nevertheless, a significant ($p < 0.05$) reduction in the peroxide index compared to the control was observed when using Bordantix or Tocobiol. This effect might be more noticeable in salmon due to its higher content of polyunsaturated fats (Honrado et al., 2024a, 2024b).

Regarding secondary lipid oxidation indexes, it can be observed that sea bass had significantly higher TBARS and anisidine indices compared to salmon, which could be related to the freshness of the by-products used, as according to the theory of lipid oxidation, all the peroxides would have turned into secondary oxidation compounds (Badui, 2006). Nonetheless, in both species, the effect exerted by the different antioxidants can be observed, and the results are similar for both. Overall, Bordantix®, a product based on rosemary extract, provided good protection against oxidation in both species, especially its water-soluble version (WSB). In both cases, a reduction in oxidation indices was observed when the concentration used was doubled ($p < 0.05$). Tocobiol (TOC) also showed good effectiveness in the case of salmon, although in this situation, no significant differences were observed when the dose was doubled. Nutrabiol (NUT) clearly had no protective effect against oxidation, and a counterproductive effect was observed when the dose was increased.

Considering the peroxide and anisidine indicators, the Totox index was calculated, providing an overall measure of the oxidation of an oil or fat. In the case of sea bass FPH, the lowest indices were obtained using Bordantix, showing statistically significant differences from the rest of treatments ($p < 0.05$). For salmon, although a protective effect

Table 2 Peroxide, TBARS, anisidine and Totox indexes for seabass and salmon FPH. *WSB* water-soluble Bordantix®; *OSB* oil-soluble Bordantix ®; *NUT* Nutrabiol®; *TOC* Tocobiol®. 0.2 and 0.5 refers to the concentration of antioxidants in relation to fish mass. Different letters denote statistically significant differences for the same physicochemical parameter among the various treatments (*** = $p < 0.001$)

		WSB 0.2	WSB 0.5	OSB 0.2	OSB 0.5	NUT 0.2	NUT 0.5	TOC 0.2	TOC 0.5	Control
Seabass	Peroxide value (meq O_2/kg)	4.60^{AB}	4.34^{A}	4.89^{AB}	4.55^{AB}	10.30^{E}	9.38^{D}	5.86^{C}	5.36^{BC}	4.61^{AB}
	TBARS (mg MDA/kg)***	1.25^{C}	0.71^{A}	1.71^{F}	1.42^{CD}	1.66^{EF}	1.00^{B}	1.49^{DE}	1.37^{CD}	1.80^{F}
	Anisidin***	13.48^{A}	12.75^{A}	15.82^{B}	12.68^{A}	23.43^{E}	18.89^{D}	16.15^{BC}	16.18^{C}	20.07^{D}
	Totox***	22.67^{A}	21.42^{A}	25.60^{B}	21.78^{A}	44.03^{F}	37.64^{E}	27.86^{CD}	26.90^{BC}	29.29^{D}
Salmon	Peroxide value (meq O_2/kg)	4.17^{BC}	6.64^{E}	5.58^{DE}	4.45^{BCD}	10.64^{F}	11.65^{F}	3.63^{AB}	2.81^{A}	6.29^{E}
	TBARS (mg MDA/kg)***	0.05^{B}	0.05^{C}	0.05^{B}	0.05^{C}	0.11^{E}	0.19^{F}	0.05^{B}	0.04^{A}	0.10^{D}
	Anisidin***	0.81^{B}	0.62^{AB}	0.86^{B}	0.34^{A}	2.06^{D}	3.07^{G}	0.44^{A}	0.33^{A}	1.45^{C}
	Totox***	9.15^{B}	13.90^{C}	12.02^{C}	9.25^{B}	23.34^{D}	26.37^{E}	7.50^{AB}	5.94^{A}	14.03^{C}

was observed when using Bordantix, the use of Tocobiol significantly reduced this index. Once again, the composition of the fat appears to influence the effectiveness of one antioxidant over another. Moreover, in these polyunsaturated fats, the use of antioxidants composed of mixtures of tocopherols, sterols and squalene appears to have a significantly greater effect compared to other antioxidants, particularly those consisting solely of tocopherol mixtures (Honrado et al., 2022).

These results are consistent with the study conducted by Sajib et al. (2022), in which the use of WSB and OSB was shown to reduce TBARS values during Herring storage. It is worth noting that the use of antioxidants based on rosemary extracts (carnosic acid) proved to be the most effective in preventing the formation of peroxides. This could be related to the structure of this acid and its derivatives, as they possess two hydroxyl groups in the ortho position on carbons C11 and C12, which confer strong antioxidant properties. Moreover, it can act as a "cascade" antioxidant, as the subsequent products of its oxidation reaction still retain some antioxidant function (Hrebień-Filisińska, 2021).

The different behaviour observed for Bordantix and Tocobiol among species could be explained by the interaction between the chemical structure of the antioxidants and the lipid profile of the raw materials studied. Bordantix is a rosemary extract enriched in phenolic diterpenes (e.g., carnosol, carnosic acid), which act as chelators and hydrogen donors in lipid matrices. In contrast, Tocobiol is a formulation based on tocopherol mixtures, whose main mechanism involves scavenging peroxyl radicals in lipid phases. Salmon is known for its high content of long-chain n-3 fatty acids, which are particularly susceptible to lipid peroxidation, whereas the lipid composition of sea bass contains comparatively more monounsaturated fatty acids (Nechev et al., 2021). Consequently, it is plausible that the mixture of phenolic diterpenes in Bordantix is more effective in a matrix with a lower relative proportion of highly unsaturated PUFAs (sea bass), while the tocopherols in Tocobiol, due to their ability to scavenge radicals in highly unsaturated lipid phases, perform better against the highly polyunsaturated profile of salmon (Nogala-Kalucka et al., 2005). These results are consistent with those obtained by Mira Sánchez (2019), who found that in monounsaturated oils rosemary extracts were effective in reducing both primary and secondary oxidation products, whereas in fish oil (highly polyunsaturated) they were able to reduce hydroperoxide formation but not that of secondary products. A synergistic effect between diterpenes has also been demonstrated: effectiveness increases when the carnosic acid/carnosol ratio is between 5 and 6.

The behaviour of Nutrabiol® has also been observed in other studies in which tocopherols have been used, such as those by Zuta et al. (2007) and Drusch et al. (2008), concluding that the best effect was obtained at concentrations between 50 and 100 ppm. Later, it was discovered that α-tocopherols could participate in free radical reactions and contribute to the accumulation of hydroperoxides, especially in polyunsaturated fats like that of salmon and when oils are exposed to heat (Caño-Ochoa et al., 2022). Additionally, tocopherols can also reduce $Fe^{+3}$and Cu^{+2} ions to generate hydroperoxides, making the addition of chelating agents an option to avoid this undesirable effect (Drusch et al., 2008). For all these reasons, the hydrolysates used for bread production included 0.5% WSB as an antioxidant.

Proximate Composition Analysis and a_w

Table 3 shows the proximate composition of salmon and seabass FPH once they were concentrated until moisture was around 40%. It was observed that an FPH with a high protein content was obtained. It is worth noting that this protein is mostly hydrolyzed, so the possible presence of peptides with bioactive functions (antioxidant, antimicrobial, anticancer and antihypertensive, among others) could be of interest when enriching other food matrices (Honrado et al., 2024a, 2024b). The produced hydrolysates represented a significant increase in protein content compared to the raw material: 3.02 times more in the case of seabass heads (Munekata et al., 2020) and 3.52 in the case of salmon (Pires et al., 2024). However, these FPH were below the protein levels found in other studies, as water removal was not so intense. For example, in the study by Gbogouri et al. (2004), where FPH from salmon heads were obtained, the protein content was 82.3%, but the sample was freeze-dried to remove the water.

Regarding the fat content, minimal values were obtained because of the defatting process carried out. The present fat, mostly polyunsaturated, may have beneficial effects on consumers. Several experimental investigations on in vivo and in vitro models have proven that fish oil possesses several health benefits, including anti-obesity, anticancer, cardioprotective, anti-inflammatory, neuroprotective, hepatoprotective and immunostimulatory properties (Das et al., 2024).

The ash values were quite similar for both FPH and, surprisingly, relatively high. Various studies have reported average ash values ranging between 10 and 15%, although in some cases they have reached up to 27%. The reasons for these elevated levels lie in the hydrolysate production

Table 3 Proximate composition for seabass and salmon FPH. Different letters show statistically differences between FPH for each parameter (*** = $p < 0.001$)

	Moisture (%)***	Protein (%)	Fat (%)***	Ash (%)	a_w
Seabass	41.67^{B}	48.33	1.05^{B}	8.96	0.845
Salmon	40.16^{A}	48.66	0.37^{A}	10.81	0.835

process. Specifically, the mincing step, in which the skeleton is also ground, and the addition of NaOH during hydrolysis to maintain a stable pH (Chalamaiah et al., 2012; Henriques et al., 2021).

FPH Fortified Bread Characterization

Proximate Composition and a_w

Table 4 shows the proximate composition of the breads developed in this study. Regarding moisture content, it can be observed that it increased as the percentage of hydrolysate substitution rose, being more pronounced in the case of salmon FPH and statistically significant ($p < 0.05$) at the 5% substitution level.

In terms of protein content, there was an increase of approximately 1.5%, which was significantly different from the control ($p < 0.05$). In this context, these prototypes could potentially be labelled as a source of protein. Furthermore, the increase in biologically valuable amino acids would likely be evident, and with a high probability, this increase in protein content may include peptides with bioactive effects, thus providing health benefits to consumers (Gbogouri et al., 2004; Pires et al., 2024) and also better digestibility. Additionally, the presence of these peptides could be related to the observed increase in moisture content, as several studies have determined that peptides generated during hydrolysis, containing polar groups (NH2 and COOH), could enhance water retention capacity (Alahmad et al., 2022; Dinakarkumar et al., 2022).

Fat content remained similar across all samples, with only SB-5, S-2.5 and S-5 showing significant differences from the control ($p < 0.05$), although the differences were lower than 0.2%. This could be because the FPHs exhibited lower lipid content compared to wheat flour. However, as demonstrated by previous studies, this fat is of excellent quality, with a predominance of polyunsaturated fatty acids such as ALA, EPA and DHA (Honrado et al., 2024a, 2024b).

Ash content also increased as the substitution percentage rose, contributing various minerals. For instance, the study by de la Fuente et al. (2023) found 6832 µg/g of P, 822 µg/g of Mg, 789 µg/g of Ca, 74.5 µg/g of Zn and 0.98 µg/g of Se in hydrolysates obtained from salmon heads. Similarly, values of 2507 µg/g of Ca and 1277 µg/g of P have been reported for sea bass heads (Munekata et al., 2020).

The water activity (a_w) values increased as the substitution percentage did, being more evident in the samples containing salmon FPH. Similar behaviour was found in the study of Oprea et al. (2024), although in a slighter manner. This could be associated with the fact that replacing part of the wheat flour with fish hydrolysate reduces the amount of available gluten, which could alter the dough's ability to retain water in a structured manner. If the hydrolysate cannot compensate for this structural function of gluten, there may be more free water that is not bound to the protein network, and this could potentially increase a_w (Arendt & Dal Bello, 2011).

Bread Colour Characterization

Figure 2 shows the CIELAB coordinates for both the crust and crumb of the control bread and the breads with wheat flour replaced by FPH.

Regarding the crust, significant differences ($p < 0.05$) were found in the a* and b* coordinates. In terms of the a* coordinate (green–red), the breads with FPH were characterized by having a less reddish colour compared to the control. Concerning the b* coordinate (blue-yellow), only S-2.5 and S-5 were differentiated by having less yellowish tones, which could be related to the inherent colour of the FPH. However, this behaviour may be explained by the fact that the breads with FPH have a higher moisture content, affecting water evaporation during baking. A moister crust tends to caramelize less and form a less intense colour (Huang et al., 2022). Another possible reason could be that the peptides present in the FPH have an inhibitory effect on the formation of advanced glycation end-products, as proposed in the study by Arasteh et al. (2023), where inhibitions of up to 99% were achieved using FPH at a concentration of 0.16% in model systems.

As for the crumb, significant differences were only found in the a* coordinate, where the SB-2.5 sample was

Table 4 Proximate composition and a_w of breads developed. *SB* seabass; *S* salmon. 2.5 and 5 refers to percentage of wheat flour substitution with FPH. Different letters denote statistically significant differences between treatments for the same physicochemical parameter (* = $p < 0.05$; *** = $p < 0.001$)

	Moisture (%)***	Protein (%)*	Fat (%)***	Ash (%)*	Carbohydrates (%)*	a_w***
Control	24.96^{A}	10.23^{A}	0.86^{C}	2.45^{A}	60.82^{B}	0.875^{A}
SB-2.5	25.03^{A}	11.70^{B}	0.85^{BC}	2.56^{AB}	59.45^{B}	0.899^{B}
SB-5	26.69^{B}	11.76^{B}	0.82^{B}	2.68^{BC}	58.34^{AB}	0.912^{C}
S-2.5	25.00^{A}	11.45^{B}	0.75^{A}	2.59^{ABC}	60.16^{B}	0.899^{B}
S-5	28.86^{C}	12.16^{B}	0.74^{A}	2.72^{C}	56.66^{A}	0.923^{D}

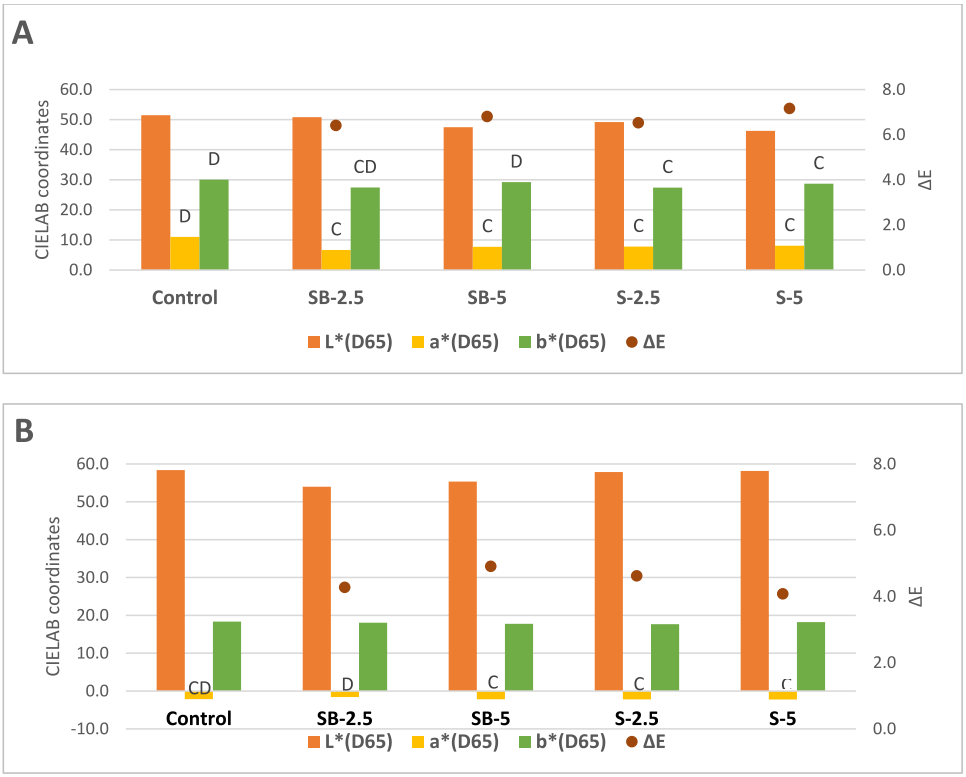

Fig. 2 Colour characterization for crust (**A**) and crumb (**B**) of different breads assayed. SB, seabass; S, salmon; 2.5 and 5 refer to the susbtitution percentaje with FPH. Different capital letters denote statistically significant differences between treatments for the same colour coordinate ($p < 0.05$). ΔE was also significant againts control for both crust and crumb ($p < 0.05$)

significantly different from the other FPH samples, but none of them differed from the control. The small colour differences observed in the crumb could be related to the inherent colour of the hydrolysate used. A similar behaviour was found in the study by Desai et al. (2018), which employed a protein concentrate from salmon fish (*Oncorhynchus tschawytscha*). The authors stated that bread colour is the result of complex reactions depending on the physicochemical characteristics of the dough (water, starch and lysine content) and the temperature used during the baking process. A similar effect was observed in bread enriched with protein of plant origin, as in the study by Sanz-Penella et al. (2013) using *Amaranthus cruentus* flour. Regarding ΔE, all treatments were significantly different from the control, although none of them were significantly different from each other. According to (Ozgoren & Yapar, 2019), the crumb values would be considered detectable by untrained individuals, whereas the crust values would be regarded as different colors within the same color group.

Bread Instrumental Texture Profile Analysis (TPA)

The Table 5 presents the results of the TPA conducted on the various treatments. The mechanical characteristics of the TPA are categorized into primary attributes: hardness, cohesiveness and springiness in bread. Secondary attributes in bread include chewiness and gumminess, which are derived from the calculations of the primary attributes (Setser, 1993).

Hardness increases or decreases depending on the protein source, addition level or flour treatment (Prieto-Vázquez del Mercado et al., 2022). In our study, hardness increased with

Table 5 Results of parameters assessed in the instrumental texture profile analysis (TPA). *SB* seabass; *S* salmon; 2.5 and 5 refers to the susbtitution percentaje with FPH. Different letters denote statistically significant differences between treatments for the same TPA parameter (*** = $p < 0.001$)

	Hardness (g)***	Cohesiveness***	Springiness***	Chewiness***	Gumminess***
CONTROL	920.42^{A}	0.69^{C}	0.96^{C}	611.74^{A}	635.43^{A}
SB-2.5	2515.14^{D}	0.67^{BC}	0.87^{A}	1447.91^{D}	1676.47^{D}
SB-5	1498.31^{B}	0.65^{B}	0.91^{B}	870.68^{BC}	965.05^{B}
S-2.5	2071.32^{C}	0.59^{A}	0.85^{A}	1040.02^{C}	1227.61^{C}
S-5	1155.66^{AB}	0.67^{BC}	0.91^{B}	704.66^{AB}	772.88^{AB}

the incorporation of FPH, and several factors may explain this behaviour. Firstly, there may be an interaction between FPH and gluten proteins, hindering the development of the gluten structure and leading to greater rigidity in the bread structure. Additionally, water absorption by the FPH reduces the amount of free water available for proper gluten development. The contribution of minerals could also affect dough hardness. This behaviour has also been observed in other studies, such as the research by Cho et al. (2019) where an increase in the substitution percentage with anchovy (*Engraulis japonicus*) hydrolysate led to an increase in hardness. However, in our study, a 5% substitution resulted in a decrease in hardness compared to the 2.5% level, which may be related to the higher moisture content of these samples. This behaviour was also observed in the study of Desai et al. (2018) with breads supplemented with Tilapia (*Oncorhynchus tschawytscha*) powder.

Bread cohesiveness refers to how the dough holds together during chewing. The existing literature states that this parameter is barely modified when extra protein is incorporated (Setser, 1993). This is what occurred in this study, where statistically significant differences compared to the control were only shown in SB-5 and S-2.5. The same behaviour has been observed in other studies conducted where protein concentrates or hydrolysates have been incorporated (Desai et al., 2018). This suggests that cohesiveness may be influenced by additional factors beyond gluten network integrity, such as water distribution or interactions between non-gluten proteins and starch. Further analysis would be needed to clarify the specific mechanisms involved. Springiness as resilience indicates the ability of bread to recover after deformation due to compression. The behaviour is similar to cohesiveness, decreasing when making higher substitution percentages. This behaviour could be related to the gas trapped in the crumb. Some authors claim that the more gas is trapped in the alveoli, the greater the recovery is (Haber et al., 2019). Chewiness represents the energy needed to disintegrate bread structure ready to swallow (Setser, 1993). As a secondary attribute, gumminess is related to hardness and areas during compression, so the behaviour is quite similar. In our study chewiness increased when adding FPH, but this increment was higher at 2.5% substitution level. Finally, gumminess is also used to evaluate bread textural characteristics as it becomes semisolid during mastication, having the same behaviour than the previous one (Prieto-Vázquez del Mercado et al., 2022). Similar behaviour was found in bread fortified with animal protein, such as the one of grasshopper (*Schistocerca gregaria*) of Haber et al. (2019).

Evaluation of the Penetration Characteristics of the Bread Crust

The hardness and fracturability measurements obtained for the different bread samples is shown in Table 6. An increase in both properties was observed with the incorporation of FPH. However, as with the crumb, the hardness and crust fracturability values at 5% FPH were lower than at 2.5%. The observed behavior could be related to the increase in moisture content and the a_w of the samples. The differences between the FPHs could be due to slight variations in fat content or differences in the size and length of the peptides present. Authors such as Pico et al. (2019) have found correlations of $r = -0.81$ between hardness and moisture, and $r = -0.86$ between hardness and water activity. Various studies have shown that the presence of water induces the plasticization and softening of the starch-protein structure, and that the increase in water activity causes a transition from a crystalline state to a rubbery state (Castro-Prada et al., 2009; Pico et al., 2019).

Table 6 Results of fracturability and hardness for the penetration test carried out on bread's crust. *SB* seabass; *S* salmon; 2.5 and 5 refers to the susbtitution percentaje with FPH. Different letters denote statistically significant differences for the same parameter and between treatments (*** = $p < 0.001$)

	Fracturability (kg)***	Hardness (kg)***
CONTROL	0.213^{A}	0.246^{A}
SB-2.5	0.519^{C}	0.548^{C}
SB-5	0.370^{B}	0.421^{BC}
S-2.5	0.160^{A}	0.275^{A}
S-5	0.187^{A}	0.307^{AB}

Digital Image Analysis: Bread Alveolate

Table 7 shows the characterization performed on the bread crumb. Specifically, the average size of the alveoli, the number of alveoli per unit area, as well as the percentage of the total area occupied by an alveolus were studied.

Regarding the average size of the alveoli, it was observed that the addition of FPH from both sea bass and salmon led to an increase in their size ($p < 0.05$). This increase was more

Table 7 Results of digital image analysis regarding the characterization of the breads crumb: average alveoli area, alveoli density per surface unit and total area belonging to alveoli. *SB* seabass; *S* salmon; 2.5 and 5 refers to the susbtitution percentaje with FPH. Different letters denote statistically significant differences for the same parameter and between treatments (*** = $p < 0.001$)

	Average alveoli area***	Alveoli/cm^2 ***	Total area fraction (%)***
CONTROL	0.29^{A}	172.18^{E}	50.68^{A}
SB-2,5	0.61^{C}	99.31^{B}	60.14^{B}
SB-5	0.81^{D}	83.12^{A}	67.63^{D}
S-2,5	0.44^{B}	148.85^{D}	65.33^{C}
S-5	0.63^{C}	106.92^{C}	67.81^{D}

pronounced in the case of sea bass (106% and 176% larger for SB-2.5 and SB-5, respectively) compared to salmon (49% and 115% for S-2.5 and S-5, respectively). Because of this increase in alveoli size, there was a decrease in the number of alveoli per unit area ($p < 0.05$). Finally, regarding the area of the crumb occupied by an alveolus, an increase was observed upon incorporating FPH.

This behaviour has been observed in other studies. For example, the study by Ozón et al. (2023), in which bread was fortified with bioactive peptides derived from chia, showed that alveolar size was proportional to the increase in the percentage of fortification (3, 5 and 10 mg/g of flour). The most probable reason for this behaviour would be the higher moisture content of the dough. This can lead to a more flexible and extensible dough, with more plastic behaviour, allowing greater alveolar expansion (Rathnayake et al., 2018). However, it could also be due to an interaction between the peptides and gluten, weakening the bonds between gluten molecules, allowing the alveoli to expand further before breaking, or to increased yeast activity due to the additional amino acids provided by the FPH.

Bread Scanning Electron Microscopy (SEM)

The dough, and later the crumb, forms an intricate matrix composed of starch and gluten proteins, whose structure and interactions can reveal important macroscopic changes. Figure 3 displays the microstructure of the crumb across different bread samples. Distinct differences are observed between the control sample and those containing FPH.

In the control sample, a well-developed gluten network is evident, characterized by smaller and fewer cavities. This structural integrity aligns with findings by Gallo et al. (2022), who observed similar effects in breads enriched with lentil flour. The control crumb also contains spherical particles of varying sizes, which are identifiable as starch granules (Ikram et al., 2021).

Conversely, samples with FPH show a different structural pattern. Here, starch granules appear partially embedded in the protein matrix, which may result from incomplete gluten network development or reduced water availability for gelatinization due to the binding of water to peptides and proteins in the FPH (Pomeranz et al., 1984).

Notably, the FPH-enriched samples exhibit a denser, more compact gluten network, with this effect being increasingly pronounced as FPH concentration rises. Some studies suggest that this enhancement in network density may stem from charged glutamate residues present in FPH, which contribute to the strengthening of the gluten matrix. These residues also likely increase the elastic (G′) and viscous (G″) moduli of the starch gel (Gałkowska & Juszczak, 2019; Zhang et al., 2024).

However, it is important to note that there appears to be a threshold for FPH addition, beyond which the gluten structure weakens, leading to larger cavities and thinner walls, as shown in the SEM images. This threshold effect may result from the emulsifying properties of FPH, which encourage starch granule aggregation and disrupt the orderly gluten structure (Zhu et al., 2024). This structural shift correlates with the trend observed in texture profile analysis (TPA), where increased FPH levels (from 2.5 to 5%) are associated with a reduction in bread hardness.

In summary, the SEM analysis indicates that moderate FPH incorporation promotes a cohesive and dense crumb structure, but higher FPH levels may compromise gluten integrity, affecting texture.

Bread Sensory Characterization

Figure 4 illustrates the hierarchical agglomerative clustering derived from the coordinates of the different products. As observed, this technique allowed for the differentiation of two clusters: a first group comprising the control treatment and a second cluster consisting of all treatments that incorporated some type of FPH. Within this second cluster, treatments with a 5% substitution of flour by FPH were grouped in the same subgroup, whereas those with a 2.5% substitution were in different subgroups.

To understand the reasons behind this behaviour, a radar chart was created based on the rank table obtained from the flash profiling analysis, as shown in Fig. 5. The results of the Kruskal–Wallis test were also applied to this chart. Differences were identified in 10 of the 14 evaluated attributes.

Specifically, significant differences were observed in crust toast intensity and crumb brown colour, with FPH-containing breads showing significantly higher values than the control. This correlates with the decrease in L*, a* and b* coordinates in the crust and the decrease in L* in the crumb. This may be attributed to the inherent colour of the FPH, as well as a greater degree of surface toasting due to the addition of protein and fat. Correspondingly, a higher crust toast aroma was also observed. Furthermore, FPH breads displayed a significantly larger alveolar size compared to the control, which, as indicated by the digital image analysis, could be related to different dough behaviour due to the higher water content.

The remaining attributes in which differences were found could be considered undesirable. The addition of FPH caused a fishy odour in the crumb and a rancid odour, particularly in the sea bass FPH and at the 5% substitution level, correlating with the TBARS and anisidine analyses. In the crust, the fishy flavour was significantly different from the control for all treatments except SB-5. Possibly, the protein contribution and the slight fat content (higher in comparison to salmon FPH) helped to create a toasted flavour that masks

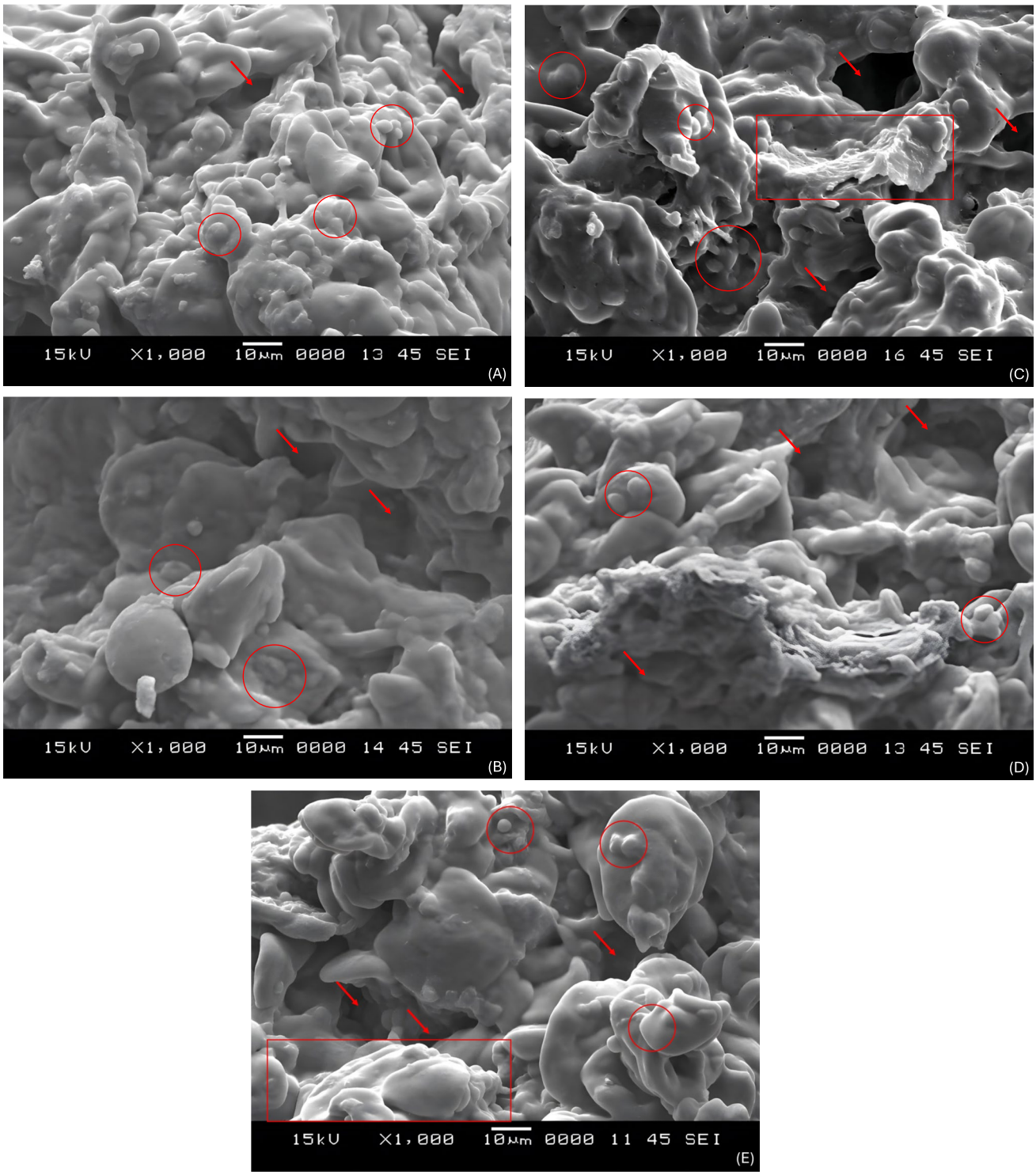

Fig. 3 Scanning electron micrographs of control bread and FPH-enriched breads. **A** Control bread: dense gluten network with distinct, smaller cavities. **B** Bread with 2.5% seabass FPH: moderately compact gluten network. **C** Bread with 5% seabass FPH: further gluten densification, increased starch embedding. **D** Bread with 2.5% salmon FPH: signs of compact structure similar to seabass. **E** Bread with 5% salmon FPH: reduced structural integrity, showing more cavities. Circles denote starch granules, arrows indicate cavities and rectangles mark areas likely weakened by increased FPH levels

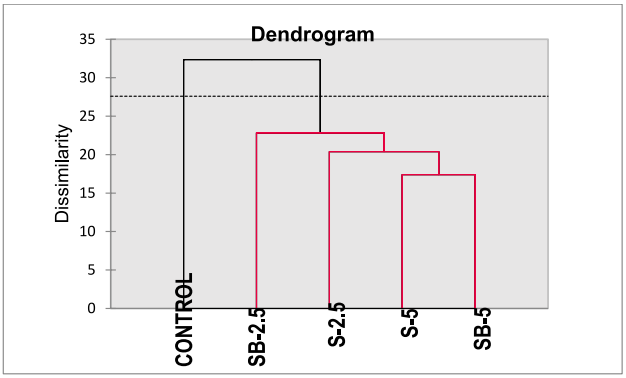

Fig. 4 Dendrogram representing the hierarchical agglomerative clustering for the different studied products. SB, seabass; S, salmon; 2.5 and 5 refer to the substitution percentage with FPH

the fishy flavour. Umami and bitter flavours also intensified proportionally with the increase in FPH, likely due to the use of enzymes that promote the generation of peptides with these flavours. Lastly, samples with a 5% substitution were the only ones that presented an aftertaste significantly different from the control.

The organoleptic properties associated with the fortification of bread using FPH or fish by-product flours show notable variation depending on the type of fish used and the level of substitution. For instance, Oprea et al. (2024) observed that breads enriched with FPH derived from cod (*Gadus morhua*) by-products exhibited intensified bitter, salty and sour notes, which became more pronounced with higher substitution levels. These sensory traits also persisted after swallowing, suggesting a potential limitation in consumer acceptability at higher concentrations.

In contrast, Bastos et al. (2014) reported more favourable outcomes when using flour made from the head, bones, viscera and skin of Red-tailed Brycon (*Brycon cephalus*). Their study showed that breads containing 4.2% and 8.4% substitution were preferred over the control, with consumers giving higher ratings for texture, appearance, taste and colour. This indicates that moderate inclusion levels, depending on the source, can enhance overall acceptability. Adding further complexity to the discussion, Akusu et al. (2023) found no significant differences in taste, aroma, colour or overall acceptability when 20% of wheat flour was substituted with a composite fish flour made from croaker and tilapia. Unlike Oprea et al. (2024), whose findings pointed to a sensory threshold, and Bastos et al. (2014), who identified an optimal substitution range, Akusu et al. (2023) suggest that a relatively high inclusion level can maintain sensory neutrality, at least under certain formulation conditions. The differences observed compared to other studies could be due to the raw materials

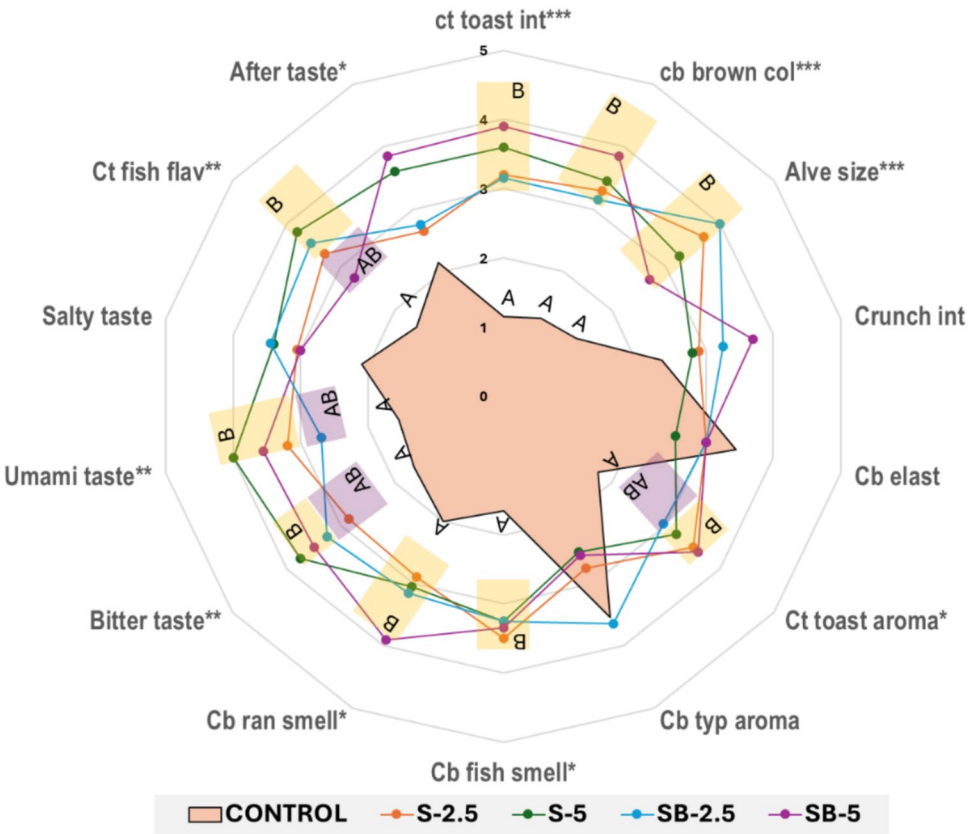

Fig. 5 Sensory characterization for the different breads incorporating FPH. SB, seabass; S, salmon; cb elast: crumb elasticity; cb typ aroma, crumb typical aroma; cb ran smell, crumb rancid smell; aft taste, after taste; cb fish smell, crumb fishy smell; ct fish flav, crust fishy flavor; ct toast int, crust toasting intensity; ct toast aroma, crust toasted aroma; cb brown col, crumb brown color; crunch int, crunchiness intensity; alve size, alveoli size. Different letters denote statistically significant differences among treatments for the same sensory attribute(* = $p < 0.05$;** = $p < 0.01$ *** = $p < 0.001$)

used, as well as the enzymes employed, which may alter the organoleptic properties of the hydrolysate. Another possible reason could be the use of consumers rather than a trained sensory panel.

Taken together, these studies illustrate how the source of fish material, processing method and substitution level can lead to divergent sensory outcomes, emphasizing the importance of tailoring fortification strategies to both the raw material and consumer preferences.

Global Characterization of Breads

From an overall point of view, the principal component analysis -PCA- (Fig. 6) biplot reveals a clear separation between the control bread and those enriched with FPH, particularly along the first principal component (F1), which accounts for 58.34% of the total variance. The control sample clusters on the negative side of F1, associated with higher air pocket density, cohesiveness, springiness and favourable crust colour parameters (a* and b*). In contrast, breads with increasing levels of FPH substitution (S-2.5, SB-2.5, S-5, SB-5) shift progressively toward the positive side of F1 and F2, where they are strongly associated with undesirable sensory traits such as fishy smell and flavour, rancid odour, bitter and umami tastes, higher ash content and increased moisture. Texture-related variables such as chewiness, gumminess, crumb hardness and crust fracturability also increase with FPH fortification, especially at higher substitution levels.

The control bread was associated with higher carbohydrate and fat content, greater red and yellow crust colour and greater elasticity and cohesiveness. The breads containing FPH were associated with higher moisture and ash content, more intense red crumb colour, larger cell size and certain sensory attributes related to a higher degree of toastiness. Likewise, the breads with the highest percentage of substitution were associated with a greater intensity of umami flavour, brown crumb colour, rancid odour and a greater aftertaste. To some extent, it was possible to establish the existence of a certain relationship between moisture and cell size and general textural characteristics.

These findings suggest that while FPH fortification may alter nutritional content (e.g. higher fat and ash), it significantly impacts sensory and texture attributes in a direction likely to reduce consumer acceptability. Future studies should focus on optimizing the level and type of FPH used, possibly combining it with flavour-masking agents or texture-modifying ingredients to improve the sensory profile. Moreover, understanding the threshold at which fortification

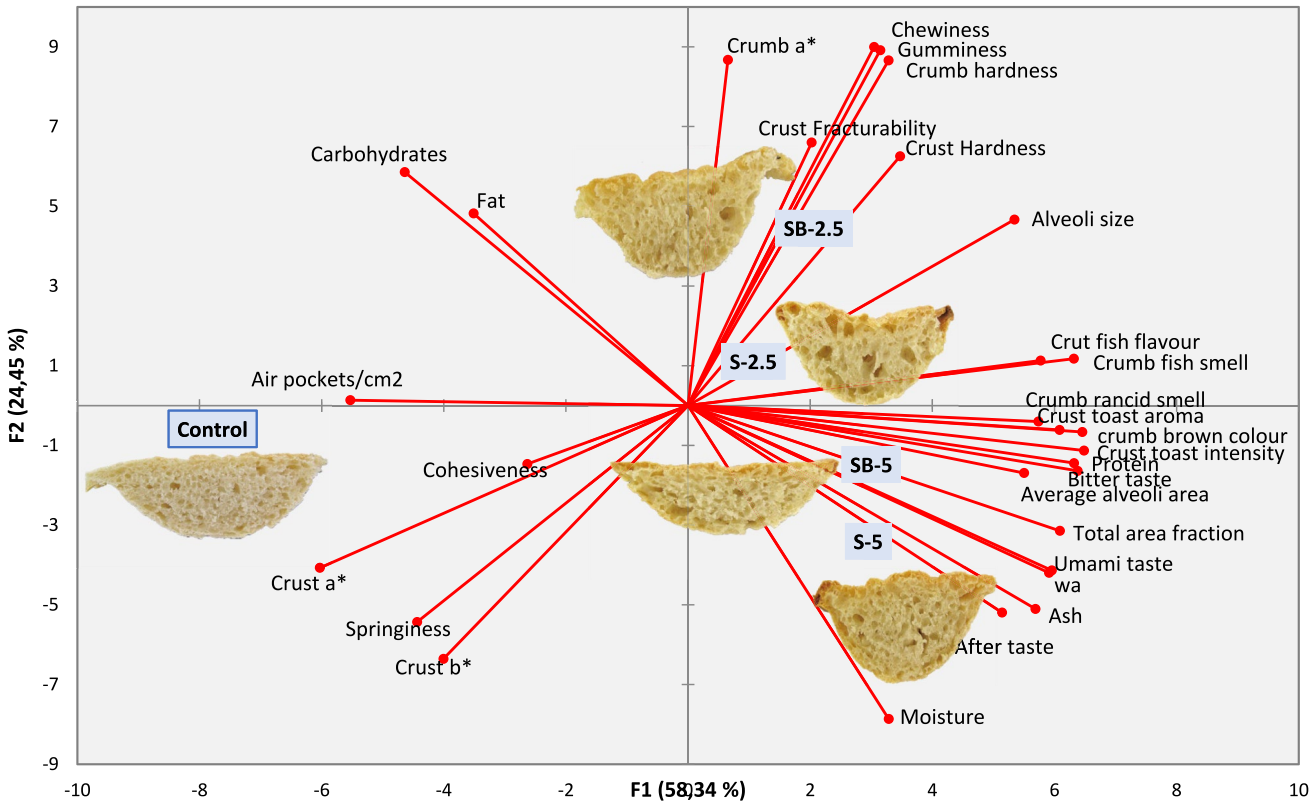

Fig. 6 Principal component analysis showing the global characterization for control and breads containing FPH and possible interactions among these variables

begins to negatively affect consumer-relevant traits could guide more targeted formulation strategies in functional bread development.

Conclusions

This study demonstrates that fish protein hydrolysates (FPH) from sea bass and salmon heads can be successfully incorporated into bread-making as sustainable functional ingredients. Their inclusion enriched the breads with protein and moisture, but also altered dough behaviour and crumb architecture, leading to increased alveolar size and hardness. These structural modifications were directly linked to sensory outcomes: while breads with 2.5% FPH retained desirable qualities such as pleasant aroma, balanced texture and uniform toasting, the 5% substitution intensified off-flavours (bitterness, rancid and fishy odours) and reduced overall acceptability. Visual changes in crust and crumb colour further underscored the interplay between composition and consumer perception.

Overall, FPH addition illustrates the dual potential of nutritional enhancement and circular economy valorisation of fish by-products. However, ensuring an optimal balance between health-promoting benefits and consumer acceptance requires limiting substitution levels to 2.5%. Future work should focus on improving the sensory profile of higher inclusions, for instance through flavour-masking or process optimisation, while exploring the functional bioactivity of the peptides to fully unlock their value in bakery applications.

Acknowledgements The authors would like to express their gratitude to Barna SA for the generous contribution of raw materials and enzymes used in this study. Authors would like to acknowledge the use of Servicio General de Apoyo a la Investigación-SAI, Universidad de Zaragoza

Author Contribution AH: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing—Original Draft, Writing—Review & Editing, Visualization; PA: Methodology, Investigation, Writing—Review & Editing; JAB: Conceptualization, Validation, Resources, Data Curation, Writing—Review & Editing, Visualization, Supervision, Project administration, Funding acquisition; JC: Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data Curation, Writing—Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research was supported by OTRI UNIZAR project 2022/0492.

Data Availability Data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

References

Abdel-Rahim, M. M., Lotfy, A. M., Razek, F. A. A., Ahmed, H. O., Taha, S. M., Shahin, S. A., Mohamed, R. M., Zayed, M. M., Hebalah, M. M., & Elhetawy, A. I. G. (2025). Integrating clams and *Dicentrarchus labrax* in groundwater-based aquaculture: A prototype for bioremediation and enhanced fish performance, health and production. *Egyptian Journal of Aquatic Research, 51*(3), 430–442. https://doi.org/10.1016/j.ejar.2025.06.002

Addinsoft. (2016). *XLSTAT* (Version 2016.02.28451). Addinsoft. https://www.xlstat.com

Aínsa, A. (2023). *Pastas alimenticias enriquecidas con productos marinos: Innovación y desarrollo tecnológico, mejora nutricional, vida útil y aceptación por el consumidor*. Universidad de Zaragoza.

Akusu, O. M., Ramoni, S. E., Wordu, G. O., & Chibor, B. S. (2023). Physiochemical, nutritional and sensory quality of bread produced from wheat, croaker and tilapia fish composite flour. *Research Journal of Food Science and Quality Control (RJFSQC), 9*(1), 38–55. https://doi.org/10.56201/rjfsqc.v9.no1.2023.pg38.55

Al Khawli, F., Pateiro, M., Domínguez, R., Lorenzo, J. M., Gullón, P., Kousoulaki, K., Ferrer, E., Berrada, H., & Barba, F. J. (2019). Innovative green technologies of intensification for valorization of seafood and their by-products. *Marine Drugs, 17*(12), 689. https://doi.org/10.3390/md17120689

Alahmad, K., Xia, W., Jiang, Q., & Xu, Y. (2022). Effect of the degree of hydrolysis on nutritional, functional, and morphological characteristics of protein hydrolysate produced from bighead carp (*Hypophthalmichthys nobilis*) using ficin enzyme. *Foods, 11*(9), 1320. https://doi.org/10.3390/foods11091320

AOCS. (1990). *Offical methods and recommended practices of the AOCS* (AOCS, Ed.; 7th ed.). AOCS Press.

Arasteh, F., Barzegar, M., & Gavlighi, H. A. (2023). Potential inhibitory effect of fish, maize, and whey protein hydrolysates on advanced glycation end-products (<scp>AGEs</scp>). *Food Science & Nutrition, 11*(6), 3075–3082. https://doi.org/10.1002/fsn3.3289

Araujo, J., Sica, P., Costa, C., & Márquez, M. C. (2021). Enzymatic hydrolysis of fish waste as an alternative to produce high value-added products. *Waste and Biomass Valorization, 12*(2), 847–855. https://doi.org/10.1007/s12649-020-01029-x

Arendt, E., & Dal Bello, F. (2011). *Gluten-free cereal products and beverages*. Elsevier.

Argalys. (2020). *The quality of Omega 3: TOTOX index and other criteria*. The Argalys Blog. https://www.argalys.com/en/blogs/advices/the-quality-of-omega-3-totox-index-and-other-criteria?srsltid=

AfmBOopNDltMR7t7AxWNO8pvwj7qG6OhbGHXibVWsG70-hvXuyrbStPW&utm_source=chatgpt.com. Accessed 15 May 2025.
Badui, S. (2006). Química de los Alimentos. In E. Quintanar (Ed.), (4th ed). Pearson Educacion.
Bastos, S. C., Tavares, T., de Sousa Gomes Pimenta, M. E., Leal, R., Fabrício, L. F., Pimenta, C. J., Nunes, C. A., & Pinheiro, A. C. M. (2014). Fish filleting residues for enrichment of wheat bread: chemical and sensory characteristics. *Journal of Food Science and Technology, 51*(9), 2240–2245. https://doi.org/10.1007/s13197-014-1258-1
Betoret, E., & Rosell, C. M. (2020). Enrichment of bread with fruits and vegetables: Trends and strategies to increase functionality. *Cereal Chemistry, 97*(1), 9–19. https://doi.org/10.1002/cche.10204
Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917. https://doi.org/10.1139/o59-099
Castro-Prada, E. M., Primo-Martín, C., Meinders, M. B. J., Hamer, R. J., & Van Vliet, T. (2009). Relationship between water activity, deformation speed, and crispness characterization. *Journal of Texture Studies, 40*(2), 127–156. https://doi.org/10.1111/j.1745-4603.2009.00173.x
Chalamaiah, M., Dinesh kumar, B., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry, 135*(4), 3020–3038. https://doi.org/10.1016/j.foodchem.2012.06.100
Cho, Y., Lee, J., Lee, M.-Y., Cho, H.-Y., & Choi, M.-J. (2019). Effects of hydrolyzed animal protein on the enhancement of saltiness and quality characteristics of white pan bread. *Food and Bioprocess Technology, 12*(11), 1832–1841. https://doi.org/10.1007/s11947-019-02332-9
Cooney, R., de Sousa, D. B., Fernández-Ríos, A., Mellett, S., Rowan, N., Morse, A. P., Hayes, M., Laso, J., Regueiro, L., Wan, A. H. L., & Clifford, E. (2023). A circular economy framework for seafood waste valorisation to meet challenges and opportunities for intensive production and sustainability. *Journal of Cleaner Production, 392*, Article 136283. https://doi.org/10.1016/j.jclepro.2023.136283
Coppola, D., Lauritano, C., Palma Esposito, F., Riccio, G., Rizzo, C., & de Pascale, D. (2021). Fish waste: From problem to valuable resource. *Marine Drugs, 19*(2), 116. https://doi.org/10.3390/md19020116
Dairou, V., & Sieffermann, J. -M. (2002). A comparison of 14 jams characterized by conventional profile and a quick original method, the flash profile. *Journal of Food Science, 67*(2), 826–834. https://doi.org/10.1111/j.1365-2621.2002.tb10685.x
Das, P., Dutta, A., Panchali, T., Khatun, A., Kar, R., Das, T. K., Phoujdar, M., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Advances in therapeutic applications of fish oil: A review. *Measurement: Food, 13*, Article 100142. https://doi.org/10.1016/j.meafoo.2024.100142
de la Fuente, B., Aspevik, T., Barba, F. J., Kousoulaki, K., & Berrada, H. (2023). Mineral bioaccessibility and antioxidant capacity of protein hydrolysates from salmon (*Salmo salar*) and mackerel (*Scomber scombrus*) backbones and heads. *Marine Drugs, 21*(5), 294. https://doi.org/10.3390/md21050294
de Lamo, B., & Gómez, M. (2018). Bread enrichment with oilseeds. A review. *Foods, 7*(11), Article 191. https://doi.org/10.3390/foods7110191
Caño-Ochoa, Sdel, Ruiz-Aracama, A., & Guillén, M. D. (2022). Alpha-tocopherol, a powerful molecule, leads to the formation of oxylipins in polyunsaturated oils differently to the temperature increase: A detailed study by proton nuclear magnetic resonance of walnut oil oxidation. *Antioxidants, 11*(4), Article 604. https://doi.org/10.3390/antiox11040604
Desai, A. S., Beibeia, T., Brennan, M. A., Guo, X., Zeng, X.-A., & Brennan, C. S. (2018). Protein, amino acid, fatty acid composition, and in vitro digestibility of bread fortified with *Oncorhynchus tschawytscha* powder. *Nutrients, 10*(12), 1923. https://doi.org/10.3390/nu10121923
Dinakarkumar, Y., Krishnamoorthy, S., Margavelu, G., Ramakrishnan, G., & Chandran, M. (2022). Production and characterization of fish protein hydrolysate: Effective utilization of trawl by-catch. *Food Chemistry Advances, 1*, Article 100138. https://doi.org/10.1016/j.focha.2022.100138
Drusch, S., Groß, N., & Schwarz, K. (2008). Efficient stabilization of bulk fish oil rich in long-chain polyunsaturated fatty acids. *European Journal of Lipid Science and Technology, 110*(4), 351–359. https://doi.org/10.1002/ejlt.200700195
Dupaigne, B. (1999). The History of Bread. In H. N. Abrams (Ed.), (1st ed). Abrams.
FAO. (2022). El estado mundial de la pesca y la acuicultura 2022. *FAO*. https://doi.org/10.4060/cc0461es
Forouhi, N. G., Misra, A., Mohan, V., Taylor, R., & Yancy, W. (2018). Dietary and nutritional approaches for prevention and management of type 2 diabetes. *BMJ*, k2234. https://doi.org/10.1136/bmj.k2234
Gałkowska, D., & Juszczak, L. (2019). Effects of amino acids on gelatinization, pasting and rheological properties of modified potato starches. *Food Hydrocolloids, 92*, 143–154. https://doi.org/10.1016/j.foodhyd.2019.01.063
Gallo, V., Romano, A., Miralles, B., Ferranti, P., Masi, P., Santos-Hernández, M., & Recio, I. (2022). Physicochemical properties, structure and digestibility in simulated gastrointestinal environment of bread added with green lentil flour. *LWT, 154*, Article 112713. https://doi.org/10.1016/j.lwt.2021.112713
Gani, A., Broadway, A. A., Masoodi, F. A., Wani, A. A., Maqsood, S., Ashwar, B. A., Shah, A., Rather, S. A., & Gani, A. (2015). Enzymatic hydrolysis of whey and casein protein- Effect on functional, rheological, textural and sensory properties of breads. *Journal of Food Science and Technology, 52*(12), 7697–7709. https://doi.org/10.1007/s13197-015-1840-1
Gbogouri, G. A., Linder, M., Fanni, J., & Parmentier, M. (2004). Influence of hydrolysis degree on the functional properties of Salmon byproducts hydrolysates. *Journal of Food Science, 69*(8). https://doi.org/10.1111/j.1365-2621.2004.tb09909.x
Gehring, C. K., Gigliotti, J. C., Moritz, J. S., Tou, J. C., & Jaczynski, J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review. *Food Chemistry, 124*(2), 422–431. https://doi.org/10.1016/j.foodchem.2010.06.078
Glanbia nutritionals. (2024). *Bakery trends for 2024*. Glanbia nutritionals web page. https://www.glanbianutritionals.com/en/nutri-knowledge-center/insights/trends-bakery. Accessed 05 June 2025.
Haber, M., Mishyna, M., Martinez, J. J. I., & Benjamin, O. (2019). The influence of grasshopper (*Schistocerca gregaria*) powder enrichment on bread nutritional and sensorial properties. *LWT, 115*, Article 108395. https://doi.org/10.1016/j.lwt.2019.108395
Haq, M., Ali, M. S., Park, J.-S., Kim, J.-W., Zhang, W., & Chun, B.-S. (2025). Atlantic salmon (*Salmo salar*) waste as a unique source of biofunctional protein hydrolysates: Emerging productions, promising applications, and challenges mitigation. *Food Chemistry, 462*, Article 141017. https://doi.org/10.1016/j.foodchem.2024.141017
Hellberg, R. S., DeWitt, C. A. M., & Morrissey, M. T. (2012). Risk-benefit analysis of seafood consumption: A review. *Comprehensive Reviews in Food Science and Food Safety, 11*(5), 490–517. https://doi.org/10.1111/j.1541-4337.2012.00200.x
Henriques, A., Vázquez, J. A., Valcarcel, J., Mendes, R., Bandarra, N. M., & Pires, C. (2021). Characterization of protein hydrolysates

from fish discards and by-products from the North-West Spain fishing fleet as potential sources of bioactive peptides. *Marine Drugs, 19*(6), 338. https://doi.org/10.3390/md19060338

Honrado, A., Ardila, P., Leciñena, P., Beltrán, J. A., & Calanche, J. B. (2023). Transforming 'bonito del norte' tuna by-products into functional ingredients for nutritional enhancement of cereal-based foods. *Foods, 12*(24), 4437. https://doi.org/10.3390/foods12244437

Honrado, A., Ardila, P., Leciñena, P., Beltrán, J. A., & Calanche, J. (2024a). Nutritional enrichment of cereal foods: Feasibility study and characterisation of biscuits fortified with seabass by-products. *International Journal of Food Science & Technology, 59*(8), 5376–5388. https://doi.org/10.1111/ijfs.17230

Honrado, A., Miguel, M., Ardila, P., Beltrán, J. A., & Calanche, J. B. (2024b). From waste to value: Fish protein hydrolysates as a technological and functional ingredient in human nutrition. *Foods, 13*(19), 3120. https://doi.org/10.3390/foods13193120

Honrado, A., Rubio, S., Beltrán, J. A., & Calanche, J. (2022). Fish by-product valorization as source of bioactive compounds for food enrichment: Characterization, suitability and shelf life. *Foods, 11*(22), 3656. https://doi.org/10.3390/foods11223656

Hopkins, D. T., Berrué, F., Khiari, Z., & Hawboldt, K. A. (2025). Valorization of fisheries by-products via enzymatic protein hydrolysis: A review of operating conditions, process design, and future trends. *Process Biochemistry, 149*, 306–320. https://doi.org/10.1016/j.procbio.2024.12.024

Hrebień-Filisińska, A. (2021). Application of natural antioxidants in the oxidative stabilization of fish oils: A mini-review. *Journal of Food Processing and Preservation*, *45*(4). https://doi.org/10.1111/jfpp.15342

Huang, C., Cui, H., Hayat, K., Zhang, X., & Ho, C.-T. (2022). Variation of moisture state and taste characteristics during vacuum drying of Maillard reaction intermediates of hydrolyzed soybean protein and characterization of browning precursors via fluorescence spectroscopy. *Food Research International, 162*, Article 112086. https://doi.org/10.1016/j.foodres.2022.112086

Ikram, A., Saeed, F., Arshad, M. U., Afzaal, M., & Anjum, F. M. (2021). Structural and nutritional portrayal of rye-supplemented bread using Fourier transform infrared spectroscopy and scanning electron microscopy. *Food Science & Nutrition, 9*(11), 6314–6321. https://doi.org/10.1002/fsn3.2592

International Organization for Standardization. (2007). *ISO 8589:2007 Sensory analysis—General guidance for the design of test rooms*

International Organization for Standardization. (2016). *ISO 6885:2016 Animal and vegetable fats and oils—Determination of anisidine value.*

International Organization for Standardization. (2017). *ISO 3960:2017 Animal and vegetable fats and oils—Determination of peroxide value—Iodometric (visual) endpoint determination.*

International Organization for Standardization. (2023). *ISO 8586:2023 Sensory analysis—Selection and training of sensory assessors.*

Liu, R., Cheng, J., & Wu, H. (2019). Discovery of food-derived dipeptidyl peptidase IV inhibitory peptides: A review. *International Journal of Molecular Sciences, 20*(3), 463. https://doi.org/10.3390/ijms20030463

Luo, M. R. (2015). CIELAB. In *Encyclopedia of color science and technology* (pp. 1–7). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-27851-8_11-1

Mamat, H., Matanjun, P., Ibrahim, S., Md. Amin, S. F., Abdul Hamid, M., & Rameli, A. S. (2014). The effect of seaweed composite flour on the textural properties of dough and bread. *Journal of Applied Phycology, 26*(2), 1057–1062. https://doi.org/10.1007/s10811-013-0082-8

Mercasa. (2023). *Alimentación En España 2023: Producción, industria, distribución y consumo* (Mercasa – Distribución y Consumo, Ed.; 26th ed., Vol. 1).

Mondal, A., & Datta, A. K. (2008). Bread baking – A review. *Journal of Food Engineering, 86*(4), 465–474. https://doi.org/10.1016/j.jfoodeng.2007.11.014

Munekata, P. E. S., Pateiro, M., Domínguez, R., Zhou, J., Barba, F. J., & Lorenzo, J. M. (2020). Nutritional characterization of sea bass processing by-products. *Biomolecules, 10*(2), 232. https://doi.org/10.3390/biom10020232

Nechev, J. T., Edvinsen, G. K., & Eilertsen, K.-E. (2021). Fatty acid composition of the lipids from Atlantic salmon—Comparison of two extraction methods without halogenated solvents. *Foods, 10*(1), 73. https://doi.org/10.3390/foods10010073

Nirmal, N. P., Santivarangkna, C., Rajput, M. S., Benjakul, S., & Maqsood, S. (2022). Valorization of fish byproducts: Sources to end-product applications of bioactive protein hydrolysate. *Comprehensive Reviews in Food Science and Food Safety, 21*(2), 1803–1842. https://doi.org/10.1111/1541-4337.12917

Nogala-Kalucka, M., Korczak, J., Dratwia, M., Lampart-Szczapa, E., Siger, A., & Buchowski, M. (2005). Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. *Food Chemistry, 93*(2), 227–235. https://doi.org/10.1016/j.foodchem.2004.09.021

Nogueira, AdeC., Oliveira, RAde, & Steel, C. J. (2020). Protein enrichment of wheat flour doughs: Empirical rheology using protein hydrolysates. *Food Science and Technology, 40*(suppl 1), 97–105. https://doi.org/10.1590/fst.06219

Oprea, O. B., Sannan, S., Tolstorebrov, I., Claussen, I. C., & Gaceu, L. (2024). Effects of Fish Protein Hydrolysate on the nutritional, rheological, sensorial, and textural characteristics of bread. *Foods, 13*(5), 698. https://doi.org/10.3390/foods13050698

Özdemir, H., Bakkalbaşı, E., & Javidipour, I. (2021). Effect of seed roasting on oxidative stability and antioxidant content of hemp seed oil. *Journal of Food Science and Technology, 58*(7), 2606–2616. https://doi.org/10.1007/s13197-020-04767-x

Ozgoren, E., & Yapar, E. (2019). Effect of the addition of smoked trout fillet powder to the quality properties of pasta. *Italian Journal of Food Science, 31*, 110–124.

Ozón, B., Cotabarren, J., Geier, F. R., Kise, M. P., García-Pardo, J., Parisi, M. G., & Obregón, W. D. (2023). Development of fortified breads enriched with plant-based bioactive peptides derived from the chia (*Salvia hispanica* L.) expeller. *Foods, 12*(18), Article 3382. https://doi.org/10.3390/foods12183382

Pico, J., Reguilón, M. P., Bernal, J., & Gómez, M. (2019). Effect of rice, pea, egg white and whey proteins on crust quality of rice flour-corn starch based gluten-free breads. *Journal of Cereal Science, 86*, 92–101. https://doi.org/10.1016/j.jcs.2019.01.014

Pires, C., Leitão, M., Sapatinha, M., Gonçalves, A., Oliveira, H., Nunes, M. L., Teixeira, B., Mendes, R., Camacho, C., Machado, M., Pintado, M., Ribeiro, A. R., Vieira, E. F., Delerue-Matos, C., Lourenço, H. M., & Marques, A. (2024). Protein hydrolysates from Salmon heads and cape hake by-products: Comparing enzymatic method with subcritical water extraction on bioactivity properties. *Foods, 13*(15), 2418. https://doi.org/10.3390/foods13152418

Pomeranz, Y., Meyer, D., & Seibel, W. (1984). Wheat, wheat-rye, and rye dough and bread studied by scanning electron microscopy. *Cereal Chemistry, 61*(1), 53–59.

Pomeranz, Y., & Shellenberger, J. A. (1970). Protein-enriched bread. *CRC Critical Reviews in Food Technology, 1*(3), 453–478. https://doi.org/10.1080/10408397009527108

Prieto-Vázquez del Mercado, P., Mojica, L., & Morales-Hernández, N. (2022). Protein ingredients in bread: Technological, textural

and health implications. *Foods, 11*(16), 2399. https://doi.org/10.3390/foods11162399

Rasband, W. S. (1997–2012). *ImageJ* (Versión 1.46r) [Software]. National Institutes of Health. https://imagej.nih.gov/ij/

Rathnayake, H. A., Navaratne, S. B., & Navaratne, C. M. (2018). Porous crumb structure of leavened baked products. *International Journal of Food Science, 2018*, 1–15. https://doi.org/10.1155/2018/8187318

Spanish Goverment. (2019). *Real Decreto 308/2019, de 26 de abril, por el que se aprueba la norma de calidad para el pan*. Boletín Oficial del Estado, núm. 105.

Sajib, M., Langeland, M., & Undeland, I. (2022). Effect of antioxidants on lipid oxidation in herring (*Clupea harengus*) co-product silage during its production, heat-treatment and storage. *Scientific Reports, 12*(1), 3362. https://doi.org/10.1038/s41598-022-07409-8

Sánchez, M., & María, D. (2019). *Uso de extractos de romero y otras fuentes vegetales como antioxidantes tecnológicos en aceites alimentarios. Estudio comparativo de eficacia frente a antioxidantes sintéticos. Relación estructuraactividad.* Universidad Católica de Murcia.

Sanz-Penella, J. M., Wronkowska, M., Soral-Smietana, M., & Haros, M. (2013). Effect of whole amaranth flour on bread properties and nutritive value. *LWT--Food Science and Technology, 50*(2), 679–685. https://doi.org/10.1016/j.lwt.2012.07.031

Setser, C. S. (1993). *Advances in Baking Technology* (B. S. Kamel & C. E. Stauffer, Eds.; 1st ed.). Springer US. https://doi.org/10.1007/978-1-4899-7256-9

Smichi, N., & Kharrat, N. (2016). Physicochemical characterization and nutritional quality of fish by-products: In vitro oils digestibility and synthesis of flavour esters. *Journal of Food Processing & Technology*, *7*(7). https://doi.org/10.4172/2157-7110.1000602

Song, X., Perez-Cueto, F. J. A., & Bredie, W. L. P. (2018). Sensory-driven development of protein-enriched rye bread and cream cheese for the nutritional demands of older adults. *Nutrients, 10*(8), 1006. https://doi.org/10.3390/nu10081006

Sørensen, A.-D.M., Getachew, A. T., & Jacobsen, C. (2022). Starfish (Asterias rubens) as a new source of marine lipids: Effect of season, size and oil extraction methods. *Foods, 11*(19), Article 2998. https://doi.org/10.3390/foods11192998

Tacon, A. G. J., Lemos, D., & Metian, M. (2020). Fish for health: Improved nutritional quality of cultured fish for human consumption. *Reviews in Fisheries Science & Aquaculture, 28*(4), 449–458. https://doi.org/10.1080/23308249.2020.1762163

Wallin, A., Di Giuseppe, D., Orsini, N., Patel, P. S., Forouhi, N. G., & Wolk, A. (2012). Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes. *Diabetes Care, 35*(4), 918–929. https://doi.org/10.2337/dc11-1631

Wandersleben, T., Morales, E., Burgos-Díaz, C., Barahona, T., Labra, E., Rubilar, M., & Salvo-Garrido, H. (2018). Enhancement of functional and nutritional properties of bread using a mix of natural ingredients from novel varieties of flaxseed and lupine. *LWT, 91*, 48–54. https://doi.org/10.1016/j.lwt.2018.01.029

World Economic Forum. (2024). *Investigating Global Aquatic Food Loss and Waste 2024*. World Economic Forum. https://www.weforum.org/publications/investigating-global-aquatic-food-loss-and-waste/?utm_source=chatgpt.com. Accessed 25 May 2025.

Xu, X., Liu, A., Hu, S., Ares, I., Martínez-Larrañaga, M.-R., Wang, X., Martínez, M., Anadón, A., & Martínez, M.-A. (2021). Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. *Food Chemistry, 353*, Article 129488. https://doi.org/10.1016/j.foodchem.2021.129488

Zhang, G., Guo, J., Zhao, F., Bai, Z., & Zhang, L. (2024). Peptide incorporation in frozen dough and steamed bread: Characteristics and structure-function relationship. *LWT, 205*, Article 116551. https://doi.org/10.1016/j.lwt.2024.116551

Zhu, J., Huang, T., Chen, X., & Tian, D. (2024). Effect of vanillin-conjugated chitosan-stabilized emulsions on dough and bread characteristics. *Current Research in Food Science, 8*, Article 100691. https://doi.org/10.1016/j.crfs.2024.100691

Zuta, P. C., Simpson, B. K., Zhao, X., & Leclerc, L. (2007). The effect of α-tocopherol on the oxidation of mackerel oil. *Food Chemistry, 100*(2), 800–807. https://doi.org/10.1016/j.foodchem.2005.11.003