International Journal of Food Science +Technology

Original article

Nutritional enrichment of cereal foods: feasibility study and characterisation of biscuits fortified with seabass by-products

Adrián Honrado, Paula Ardila, Paula Leciñena, José A. Beltrán & Juan Calanche*

Instituto Agroalimentario de Aragón-IA2-(Universidad de Zaragoza-CITA), Miguel Servet 177, 50013 Zaragoza, Spain

(Received 20 March 2024; Accepted in revised form 11 May 2024)

Summary Industrial fish processing generates large amounts of fish by-products. Therefore, this research aimed to explore alternatives that allow better exploitation of these by-products and promote sustainability and a circular economy. To achieve this, the possibility of producing and using fish meal (FM) and fish protein hydrolysates (FPH) from seabass was studied for the nutritional fortification of biscuits. The results showed that the heat applied in the production of FPH was a factor that negatively affected the lipidic fraction, reducing the proportion of polyunsaturated fatty acids but also producing a product with darker shades. The incorporation of FM and FPH into the biscuits produced nutritional enrichment, especially in protein and polyunsaturated fatty acids, but also caused colour changes, which were more intense due to the increase of Maillard reaction, and changes in sensory perception, where higher intensities of colour and toasted flavour were perceived (especially when including FPH), but also fish flavours. Scanning electron microscopy made possible the visualisation of differences in the internal structure, which could be related to differences found in instrumental texture measurements. In conclusion, this research demonstrated that using fish by-products to enrich biscuits is possible from a nutritional perspective. However, other techniques such as the use of antioxidant substances to preserve the fish's fatty acid profile or the combination of enzymes to reduce fish flavours should be considered for future research.

Keywords Bioactive compounds, biscuit, by-product, fatty acids, fish hydrolysate, fishmeal.

Introduction

Seabass is a fish with great acceptability and consumption in Mediterranean countries. Its characteristic aroma and flavour at an affordable price increased its demand in the 1990s and early 2000s (Kelley, 1988). To meet the demand, there was an increase in aquaculture production: while in 1990, the European aquaculture production was 3495 tonnes, in 2021 it increased to 96 527 tonnes. This also led to a decrease in seabass catches, due to the higher profitability and lower price of farmed seabass (FAO, 2022).

Nutritionally, it is a fish with high protein content (18%) and a low-fat content (1.3%), with polyunsaturated fatty acids and eicosapentaenoic acid (0.26%) standing out (BEDCA, 2021). These fatty acids have recognised benefits for human health, reducing cardiovascular diseases (EFSA, 2010; Al Khawli *et al.*, 2019). The protein is of high quality, providing all the essential amino acids. However, some differences related to their plant-based diet have been found between caught and farmed seabass, especially in monounsaturated fatty acid proportion and essential amino acids (Fuentes *et al.*, 2010).

On the other hand, the increase in the production of seabass by-products has been partly caused by a growing inclination towards ready-to-cook products. In this sense, seabass by-products constitute up to 50% of the total weight (Valcarcel *et al.*, 2020). The rising volume of these by-products underscores the urgency of prioritising sustainability and efficient resource utilisation within the seafood industry. Consequently, there is a promising interest in exploring innovative strategies for repurposing seabass by-products. Such endeavours hold the potential to yield valuable secondary products such as fishmeal, fish oil, jelly and, more recently, functional ingredients (Fraterrigo Garofalo *et al.*, 2023). This aligns with the principles of circular economy and addresses key sustainable development goals, such as eradicating hunger or promoting good health and welfare (Ainsa *et al.*, 2021; Do *et al.*, 2022).

In this context, the production of fishmeal (FM) and the generation of fish protein hydrolysates (FPH) from seabass by-products emerge as promising alternatives. The production of FPH also allows the

*Correspondent: E-mail: calanche@unizar.es

doi:10.1111/ijfs.17230

separation of protein and lipid fractions, yielding various peptides with documented positive effects on health. These benefits encompass improvements in skin and bone health, modulation of blood lipid profiles and support for weight control, among others (Nirmal *et al.*, 2022; Yuan *et al.*, 2022).

The use of fish by-products has been employed by different authors in the enrichment of various foods such as beverages or ice cream, with satisfactory results (Egerton *et al.*, 2018; Shaviklo *et al.*, 2011). However, due to the amount of high biological value protein and polyunsaturated fatty acids in these products, their inclusion in cereal products could be of interest. For example, Vijaykrishnaraj *et al.* (2016) improved bread quality in terms of loaf volume, texture and crumb appearance by adding green mussel hydrolysate. Food fortification is a quite common and appreciated activity in the industry to reach vulnerable populations such as children and the elderly. Choosing an attractive and easy food is one of the first barriers to overcome when trying to reach this audience to achieve that beneficial effect on health. Furthermore, the incorporation in bakery products of ingredients derived from fish by-products, not only for their nutritional characteristics but also due to their functional properties, can be of great interest as the young population represents one of the main consumers, being at the same time one of the sectors of the population that consumes the least number of fish and seafood products (Egerton *et al.*, 2018). In that sense, biscuits could be an interesting foodstuff to fortify.

Biscuits boast a rich history spanning 10 000 years, tracing their origins to the discovery by our nomadic ancestors that a cereal-based dough, when subjected to heat, transforms into a form of unleavened bread – creating an easily transportable and ideal sustenance for their extensive journeys (Instituto de la Galleta, 2023). The advent of the Industrial Revolution marked the genesis of the first sea biscuit factories, spearheaded by the United Kingdom and France. However, around 1830, the demand for these biscuits began to decline with the introduction of steam-powered shipping, reducing journey lengths and diminishing the necessity for copious quantities of biscuits on board. In response, British manufacturers adapted by elevating the dough's composition with ingredients such as cocoa, sugar, butter, and milk, rendering the product more appealing to consumers (Moreno, 2001). Nowadays, biscuits are savoured primarily for their hedonistic flavours rather than serving as a dietary staple, captivating the palates of both young and old alike.

The nutritional composition of biscuits is contingent on the type and quantity of ingredients used. Generally, biscuits are abundant in carbohydrates, saturated fat and calories, while they are deficient in protein, fibre, vitamins and minerals (Farzana *et al.*, 2022). Wheat flour, the primary constituent, contains approximately 7–14% protein, with certain essential amino acids, such as lysine, in limited supply (Baljeet *et al.*, 2010). This nutritional profile has spurred research into the correlation between biscuit consumption and issues related to overweight conditions. Notably, a study by Gibson *et al.* (2004) revealed that for children aged 7–18 years, every additional megajoule of energy obtained from biscuits, cakes and confectionery increased the likelihood of being overweight by 24%. However, it is crucial to acknowledge that combatting obesity necessitates a multifaceted strategy that includes both physical activity and dietary habits.

To address this concern, the exploration of novel, health-conscious food alternatives and the incorporation of ingredients with potential health benefits emerge as a promising solution. Numerous research endeavours have delved into the formulation of enhanced biscuits by integrating various ingredients such as buckwheat, grapes, pumpkins or copra, among others, aiming to augment their nutritional value (Singh & Kumar, 2018, 2019; Ajay & Pradyuman, 2019). Intriguingly, biscuits fortified with compounds derived from fish, though less common, present a promising avenue for bolstering their nutritional profile.

Hence, the principal objective of this study was to generate and characterise FM and FPH from European seabass (*Dicentrarchus labrax*) by-products. Furthermore, the study aimed to evaluate its use as an ingredient in the nutritional fortification of biscuits studying the physicochemical and sensory changes produced.

Materials and methods

FM and FPH obtention

For the manufacturing process of FM and FPH, *Dicentrarchus labrax* by-products (heads and fishbones) sourced from BARNA SA (Mundaka, Spain) were used. The procedural steps for FPH and FM are elucidated in Fig. 1 and followed the methodology of Honrado *et al.* (2023). For FPH, an enzymatic reaction using Alcalase® was carried out. Then, the protein phase was concentrated by spray drying. To generate the FM, the thawed by-products were sliced into smaller sections, steam-boiled, pressed and subsequently dried in an oven at 60 °C with forced convection for 30 h (Verinox model Junior 1100, Altopiano della Vigolana, Italy). The last step involved grinding them until they transformed into a powder (Moulinex model Moulinette A320R1, Alençon, France).

FPH and FM characterisation

Colour

A colourimeter (Minolta mod. CM-2002, Japan) previously calibrated with black and white standards was

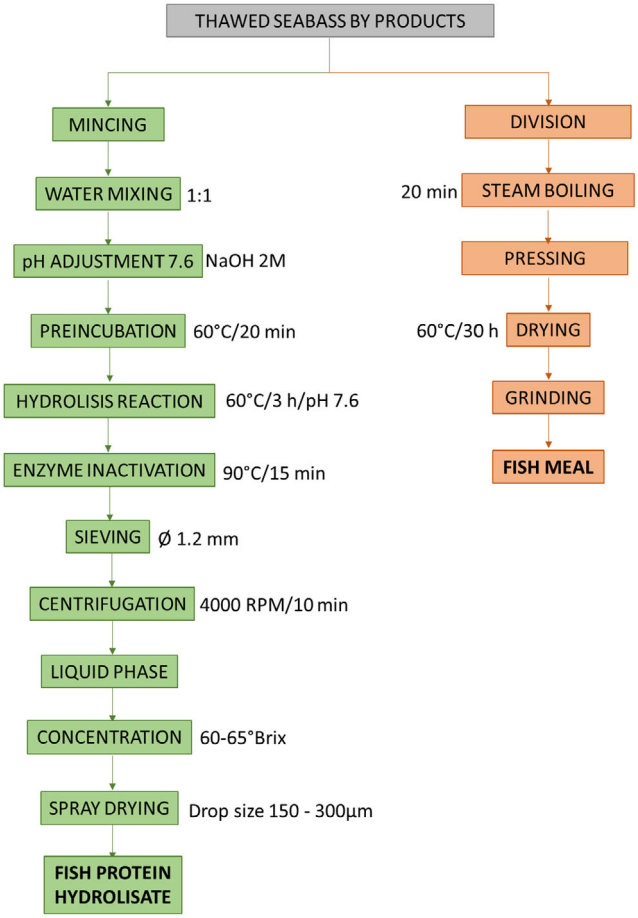

Figure 1 Step by step to produce FPH (fish protein hydrolysate) and FM (fishmeal).

used for colour measurements. Results were expressed in CIE $L^*a^*b^*$ coordinates (Abraha *et al.*, 2018). ΔE was calculated according to a study by Ainsa *et al.* (2021), using wheat flour as a control.

Fat content, lipid profile and lipid oxidation

Methodology AOCS 920.39C (AOCS, 1990) was used to determine the lipid content of seabass by-products, FPH and FM. Fatty acid profiles of raw material, FPH and FM were determined according to Bligh & 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, Spain), KCl (Panreac Química SLU, Castellar del Vallés, Spain) and distilled water. Once the mixture was homogenised, it was centrifuged at 2,147 *g*, 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. Then, methylation was carried out by using potassium hydroxide (Merck, Darmstadt, Germany). C23:0 (TCI, Tokyo, Japan) was used as intern pattern. A gas chromatograph (Hewlett-Packard mod. 6890 II, Palo Alto, EEUU) with a SP-2380 (100 m × 0.25 mm × 0.20 µm) column was employed. The temperature program started at 140 and increased until 165 °C at 3 °C min^{-1} and then 165–220 °C at 5 °C min^{-1}. Fatty acid content was quantified as the percentage of fatty acid methyl esters (FAMEs).

TBARS (thiobarbituric acid reactive substances) were determined in raw material, FM and FPH according to cd 19-9029 (AOCS, 1990). Briefly, the previously crushed sample was distilled in an acid medium and the distillate was reacted with thiobarbituric acid (Merck). Absorbances were measured at 532 nm and concentration was determined by interpolation on a calibration curve made with thiobarbituric acid and increasing concentrations of tetramethoxypropane (Merck). The results were expressed as mg MDA kg^{-1}.

Manufacturing process of biscuits

Table 1 shows the ingredients used for biscuit making. Ingredients were mixed at the same time with a spiral beater (Silvercrest, Neckarsulm, Germany). After mixing, a rolling pin and a circular mould were employed to obtain the biscuit shape. Then, they were baked at 170 °C and 14 min in an oven (Salva mod. LT-4+H 00, Lezo, Spain). When they reached room temperature, biscuits were packaged in plastic trays. Three batches following the same procedure were produced on subsequent days. Each batch and treatment contained 15 biscuits.

Characterisation of biscuits

Biscuits' proximate composition

The determination of the moisture content was performed in triplicate using gravimetry and a drying

Table 1 Formulations of biscuits enriched with FPH (fish protein hydrolysate) and FM (fishmeal)

	CONTROL	FPH-2.5	FPH-5	FM-2.5	FM-5
Wheat flour	52.15	49.3	46.8	49.3	46.8
Seabass FPH	0.0	2.5	5.0	0.0	0.0
Seabass FM	0.0	0.0	0.0	2.5	5.0
Sugar	15.6	15.6	15.6	15.6	15.6
Sunflower oil	17.3	17.3	17.3	17.3	17.3
Milk	13.8	13.8	13.8	13.8	13.8
Salt	0.5	0.5	0.5	0.5	0.5
Baking powder	0.5	0.5	0.5	0.5	0.5
Vanilla flavouring	0.5	0.5	0.5	0.5	0.5

oven (J. P. Selecta mod. 2 005 167, Abrera, Spain) at a temperature of 105 °C until constant weight, according to method 950.46 (AOCS, 1990). The determination of the fat content was conducted with Soxhlet extraction according to AOCS 954.02 (1990). For this purpose, about 3 g of crushed sample was weighed in a cellulose cartridge and, after dehydration at 105 °C and 1 h, then the fat was extracted using petroleum ether at 70 °C in a Soxhlet extractor (J. P. Selecta, Mod. Det-Gras, Abrera, Spain). The total protein content was determined in triplicate using the Kjeldahl method 992.15 (AOCS, 1990), based on the determination of organic nitrogen. One gram of crushed sample 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). It was then placed in a digester (VELP Scientifica, DK 6 heating digester Kjeldahl, Usmate Velate, Italy). Distillation of the previously alkalised was carried out using a Kjeldahl apparatus (Velp Scientifica mod. UDK 129, Usmate Velate, Italy). The distillate was collected in a 3% w/v boric acid solution (VWR). Finally, titration was performed with 0.1 N HCl (Carlo Erba, Sabadell, Spain). Protein content was calculated using a protein–nitrogen conversion factor of 5.95. For ash content, 3 g of the different samples was placed in a pre-weighed porcelain crucible and ignited in an ashing furnace maintained at 600 °C. The ash content was determined as soon as white ash was obtained and a constant weight was maintained.

Colour, lipid oxidation and a_w

The procedure followed was the same as the one followed in 2.2.1. Measurements were taken in the upper biscuit face. Control biscuits were used for ΔE. For the acidity index, ISO 660:2020 standard was used (ISO, 2020). Results were expressed as a percentage of oleic acid. For lipid oxidation methodology cd 19–90 was employed (AOCS, 1990). Regarding water activity (a_w), it was instrumentally determined (Decagon Devices mod. Aqualab CX-1, Pullman, WA, USA).

Textural characterisation

A texturometer (Stable Micro Systems mod. TA XT2i, Godalming, UK) was used to run a penetration test. Before, a stainless steel cylindrical P2 probe was installed. The texturometer was set to a return cycle at start-up, with a speed of 10 mm s^{-1} (Abraha *et al.*, 2018). Hardness (g) and fracturability (cm) were obtained (Szczesniak, 2002).

Sensory characterisation

A sensory room according to ISO 8589:2007 standard (ISO, 2007) was employed. A 10 trained sensory assessor panel was used according to ISO 8586:2023 (ISO, 2023). Sensory evaluators were assessed, demonstrating sensory sensitivity in preliminary tests as well as consistence and reproducibility in their evaluations.

Previously, the sensory panel generated a consensus profile. For this, commercial samples and existing literature were used (San José *et al.*, 2018; Farzana *et al.*, 2022). Finally, 14 attributes were selected: These included, porosity, aspect, homogeneity, typical biscuit flavour, typical biscuit colour, rancid odour, oil smell, fracturability, hardness, salty taste, fish flavour, rancidity flavour and off flavour.

The "Comparison with a Reference" method was employed (Larson-Powers & Pangborn, 1978), assessing the samples against a reference according to ISO 13299:2016 (ISO, 2016). In this context, the control biscuit served as the reference. Three sessions were executed, employing a non-structured and end-anchored scale. The reference sample (R) was placed in the centre of the scale, maintaining a ± 5 cm distance to the ends. Samples were consistently presented in pairs to each trained sensory evaluator, adhering to a balanced and equilibrated design.

An overview of biscuits (global analysis)

A PCA (principal component analysis) was carried out with all descriptors that were significant ($P < 0.05$). This allowed us to visualise better the relationships between treatments and sensory and physicochemical variables.

Microstructure characterisation

For SEM visualisation, the intern part of the biscuit was Au/Pd coated and then observed at 500× 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 biscuit that can explain the behaviour found in the instrumental texture hardness and sensory analysis.

Statistical analysis

The XLSTAT Statistical Software ("XLSTAT", 2016) was used. The calculation of quartiles, medians, means, variances, box plots, scatterplots and normality tests were performed. For the physicochemical parameters, an ANOVA was conducted including treatments and replicates as fixed variables and random effect respectively. *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 Dunnett's multiple comparison test. For the sensory data, a panel analysis was performed. Then, an analysis of variance was conducted using Fisher's test (LSD) as a posteriori test. To visualise which biscuits were the most and the least like the control, a ranking table was made employing the significant descriptors. Then, a dissimilarity matrix

based on the Euclidean distance was made, applying the Ward method as an agglomeration technique. The proximity matrix obtained was used to perform multidimensional scaling (MDS) with the following parameters: absolute model, Kruskal stress, two dimensions, random initial configuration, five repetitions, convergence 0.00001 and 500 iterations.

Results

FPH and FM characterisation

Colour

The colour characterisation is shown in Fig. 2. In the L^* coordinate, significant differences ($P < 0.05$) were established among the three products, with wheat flour showing the greatest luminosity, followed by FM and, finally, FPH. The differences between these two would be related to the drying method intensity applied to the FPH as well as to a higher fat content of the FM. Concerning the a^* coordinate, no significant differences were established between FM and FPH, although differences between them and wheat flour were found. The reason could lie in the fact that seabass has low haemoglobin content in comparison to other species such as tuna (Thiansilakul *et al.*, 2011). In the b^* coordinate, significant differences were established, being the FM more yellow, due to the higher fat content (Iñarra *et al.*, 2018). Thus, for ΔE significant differences ($P < 0.05$) were established among the three products analysed, with FM being the most like wheat flour. Replicates and interaction between fixed effects were not significant.

Fat content, lipid profile and lipid oxidation

The FPH contained a lower fat due to the defatting process. Therefore, the seabass FPH obtained an average value of 5.88% while the FM showed a higher fat content: 22.23% ($P < 0.05$). The TBARS oxidation index determined for FM and FPH was 0.90 and 0.60 mg MDA kg^{-1} product respectively ($P < 0.05$). Although it seems that the FM reached higher oxidation levels, the FM indeed contains a greater amount of fat. If the TBARS index is expressed on a fat basis, 2.55 times higher oxidation is observed in the FPH than in the FM.

Regarding the fatty acid profile, certain changes can be observed in Table 2. The greatest changes in saturated fatty acids occurred in the FPH, with an increase of 15% concerning the raw material ($P < 0.05$). This increase is especially noticeable in palmitic acid, with an increase of 7.28%. However, these increases may be related to the decrease in polyunsaturated fatty acids. In the sum of monounsaturated fatty acids, there were also significant differences, especially pronounced in the FPH.

The most notable change was in oleic acid. This fatty acid, present in a high proportion of the diet used in aquaculture seabass (Montero *et al.*, 2005), decreased 29.16% in the FPH in comparison to the raw material. At the same time, there was an increase in vaccenic acid (VA), which is a positional and geometric isomer of oleic acid (Routray *et al.*, 2018). Furthermore, studies conducted in animals suggest an antitumoral effect associated with the intake of this acid, while epidemiological studies propose that VA intake may reduce the risk of coronary heart disease. It is noteworthy that VA is the sole dietary precursor of cis-9, trans-11 CLA, which is also associated with a potential positive health effect (Rainer & Heiss, 2004; Field *et al.*, 2009). However, the group of polyunsaturated fatty acids was one of the most affected, especially in the case of FPH, in which the total sum decreased by 9.03% in comparison to the raw material

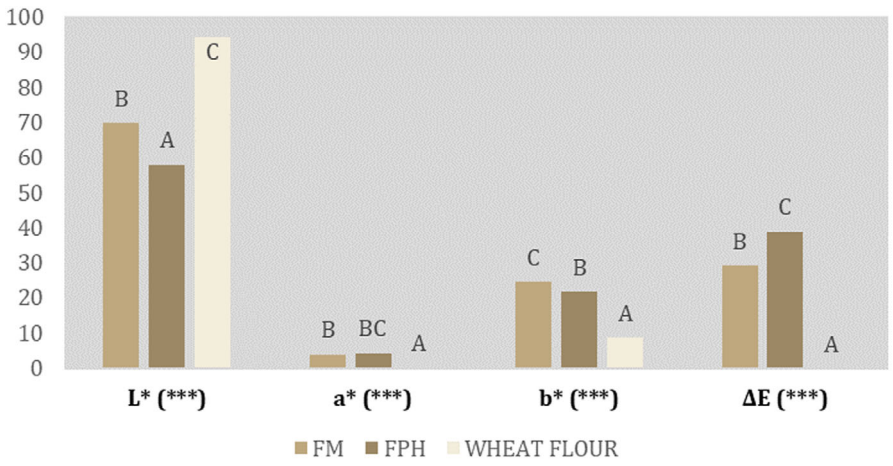

Figure 2 Colour characterisation for fish protein hydrolysates (FPH) and fishmeal (FM). ***$P < 0.001$.

Table 2 Fatty acid profile of raw material, seabass FPH (fish protein hydrolysate) and seabass FM (fishmeal)

Fatty acid (% FAMEs)	Raw material	Seabass FPH	Seabass FM
C14***	3.126 b	4.500 c	1.461 a
C15***	0.303 b	0.817 c	0.226 a
C16*** (PALMITIC)	15.814 b	23.107 c	14.956 a
C17*** (MARGARIC)	0.287 a	1.881 b	0.276 a
C18***	3.113 a	7.216 c	3.541 b
C19**	0.089 b	0.070 b	0.011 a
C20**	0.318 b	0.524 c	0.226 a
C21*	0.029 a	0.097 b	0.011 a
C22	0.124 a	0.093 a	0.131 a
C23**	0.017 a	0.099 b	0.011 a
C24 (LIGNOCERIC)	0.013 a	0.000 a	0.011 a
SFA***	23.249 b	38.497 c	20.898 a
C14:1*	0.017 a	0.000 a	0.056 b
C15:1	0.013 a	0.026 a	0.011 a
C16:1***	4.179 b	5.895 c	3.346 a
C17:1***	0.213 a	0.667 b	0.231 a
C20:1***	7.693 c	5.830 b	2.036 a
C24:1***	0.449 b	1.069 c	0.010 a
tC18:1 n-7**	0.000 a	0.031 b	0.101 c
C18:1 n-9*** (OLEIC)	32.729 b	3.573 a	37.791 c
tC18:1 n-9***	0.163 a	0.572 c	0.256 b
C18:1 n-11*** (VACCENIC)	2.963 b	25.397 c	2.741 a
C22:1 n-9***	0.776 c	0.100 a	0.231 b
MUFA***	49.196 c	43.162 a	46.806 b
C18:2 n-6*** (LINOLEIC)	13.385 b	3.455 a	17.031 c
tC18:2 n-6***	0.235 b	0.029 a	0.301 c
C18:3 n-3*** (ALA)	0.198 a	0.307 b	3.036 c
C18:3 n-6***	0.178 a	0.956 b	1.201 c
C20:2 n-3**	0.014 a	0.241 b	0.011 a
C20:2 n-6***	0.997 c	0.392 a	0.696 b
C20:3 n-3***	0.066 b	0.564 c	0.010 a
C20:3 n-6***	3.000 b	5.284 c	0.630 a
C20:4 n-6***	0.342 b	0.027 a	0.531 c
C20:5 n-3*** (EPA)	3.109 c	2.205 b	1.806 a
C22:2 n-6**	0.121 b	0.243 c	0.010 a
C22:5 n-3***	1.189 c	0.603 b	0.010 a
C22:6 n-3*** (DHA)	4.819 b	4.318 a	4.341 a
PUFA***	27.652 b	18.626 a	29.612 c
∑Ω3**	4.576 b	3.920 a	4.873 c
∑Ω6***	18.257 b	10.388 a	20.399 c
∑Ω6/∑Ω3***	3.990 b	2.650 a	4.187 c
P/S RATIO***	1.189 b	0.484 a	1.417 c

Different letters show significant differences between columns.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

($P < 0.05$). Linoleic acid and EPA stood out, with decreases of 9.93% and 0.91%.

Moreover, maintaining a Ω-6/Ω-3 fatty acid ratio within the range of 4:1 aligns with findings from research on evolutionary dietary patterns, neurodevelopment and genetic influences (Gómez *et al.*, 2011).

This ratio is deemed to be more favourable, contributing to the mitigation of various prevalent chronic diseases such as coronary heart disease in Western societies and developing countries (Simopoulos, 2010). Notably, seabass FM demonstrated the closest adherence to the recommended ratio. Furthermore, studies emphasise that the polyunsaturated to saturated (p/s) ratio should ideally approach 1 as it is suggested that this ratio would reduce the risk of atherosclerosis and coronary heart disease (Bender, 2009). Again, seabass FM exhibited values remarkably close to this optimal ratio. In this sense, an index of at least 0.4, a nutritional requirement for the proportion between PUFA and saturated fatty acids is suitable for human health and good values have been achieved in extruded food based on cereal and fish by-products (Calanche *et al.*, 2019). The interaction between fixed effects or replicates was not significant.

These results also correlated with the TBARS index. This suggests that some stage of the FPH manufacturing process could be affecting these changes. This stage could be spray drying, in which temperatures of 220 °C were reached. In this sense, some parameters such as inlet and outlet temperature could affect the fatty acid content and composition in samples processed by spray-drying technology (Javed *et al.*, 2018). For that reason, the use of antioxidants seems to be essential when working with products with high levels of polyunsaturated fatty acids (Gómez-Guillén *et al.*, 2022). Several antioxidants have been studied and their effectiveness has been confirmed (Honrado *et al.*, 2022).

Characterisation of biscuits

Biscuits' proximate composition

Significant differences were found in all analysed parameters, regarding the proximate composition (Table 3).

Regarding moisture, control biscuits showed a low value, while those containing FPH increased their moisture value. Studies incorporating FPH in biscuits have shown similar behaviour, which could be related to water-soluble hydrophilic peptides (Mieszkowska & Marzec, 2016). Concerning fat content, the addition of FM and FPH increased lipid content in the biscuits, which was more noticeable with the addition of FM. In both cases, the contribution of ALA and DHA fatty acids was significant and of great interest. Moreover, the greatest differences were observed in protein content, with biscuits containing 5% FPH having the highest content: 4.71% higher, with the consequent fortification of high biological value protein, which together with the polyunsaturated fatty acids could have bioactive effects. As a result of the increases in fat and protein, there was a reduction in carbohydrate content, which was predominant in the control biscuit.

Table 3 Proximal composition of the enriched biscuits developed

	MOISTURE**	FAT***	PROTEIN***	CARBOHYDRATES***	ASHES*
CONTROL	4.60 b	20.89 a	6.64 a	67.78 e	0.45 a
FM-2.5	4.26 a	21.84 d	7.92 b	66.14 d	0.51 a
FM-5	4.31 a	22.29 e	9.23 d	64.16 b	0.65 ab
FPH-2.5	4.73 c	21.16 b	8.98 c	65.19 c	0.81 b
FPH-5	4.62 c	21.34 c	11.35 e	62.63 a	0.99 c

FM, fishmeal; FPH, fish protein hydrolysate.
2.5 and 5 refer to the replacement percentage. Different letters show significant differences between rows. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Colour, lipid oxidation and a_w

Table 4 shows the physicochemical results of the biscuits developed. About colour, a tendency was established for the L^* coordinate to decrease as FM or FPH is incorporated, establishing significant differences for the L^* coordinate between the control and the biscuits with FPH ($P < 0.05$). The same behaviour was observed for the a^* and b^* coordinates, although in this case, a higher percentage of inclusion produced an increase in the values associated with these coordinates, being significant in the case of biscuits with FPH ($P < 0.05$). This translated into significant differences for ΔE, especially in the case of biscuits with FPH. In general, the biscuits exhibited distinguishable characteristics different from the control group, featuring a reduced white tone and an elevated red-yellow colouration. A similar trend was noted in biscuits produced by Mohamed *et al.* (2014) using carp and shark FPH. These differences may be attributed to two factors: the inherent colour of FM and FPH used, and the Maillard reaction (Martins *et al.*, 2000). The Maillard reaction, particularly pronounced in FPH biscuits due to its heightened protein content, involves a reaction between proteins and reducing sugars, leading to the observed colour variations (Gani *et al.*, 2015).

Regarding lipid stability, the acidity index increased, especially when FPH was included ($P < 0.05$). This could be attributed to the FM and FPH production process. However, TBARS was lower in FPH biscuits when compared to the FM biscuits due to differences in fat content (Table 3). These values might not be sensory detectable, as some researchers, like Connell (1995), suggest that the threshold levels range between 1 and 2 mg MDA kg^{-1}. No significant differences were observed for any parameter between replicates, and the interaction between fixed effects was not significant.

Textural characterisation

The addition of both FM and FPH from seabass allowed us to observe a decrease in hardness as well as a tendency to increase fracturability (Table 4). This tendency was more pronounced in the case of FPH, so it is possible that the inclusion of a protein source other than gluten as well as its interaction with gluten proteins could cause this behaviour. Similar behaviour was found in the study of Sinthusamran *et al.* (2019).

Sensory characterisation

Figure 3 shows the general sensory profile based on the QDA. Significant differences were established only in four of the 14 descriptors evaluated. Porosity was one of them and biscuits with FM and especially FPH-5 were significantly more porous, which could be related to the addition of protein different than gluten. Regarding typical biscuit colour, the same

Table 4 Physicochemical characterisation of the developed products

	CONTROL	FM-2.5	FM-5	FPH-2.5	FPH-5
Fracturability (cm)	1.50	1.63	1.52	1.77	1.57
Hardness (g)	2318.04	2012.91	2020.98	1572.24*	1518.29**
L^*	68.76	65.21	63.34	59.43***	53.11***
a^*	2.74	5.21	6.27	7.11**	8.63**
b^*	23.89	26.47	28.29	28.57*	28.74**
ΔE	0	5.75***	4.57***	11.67***	17.50***
a_w	0.454	0.440	0.440	0.479	0.479
TBARS (mg/kg MDA)	0.09	0.16**	0.25**	0.14	0.15
Acidity index (oleic acid %)	0.07	0.09**	0.12	0.13	0.15

FM, fishmeal; FPH, fish protein hydrolysate.
2.5 and 5 refer to the percentage of flour substitution. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

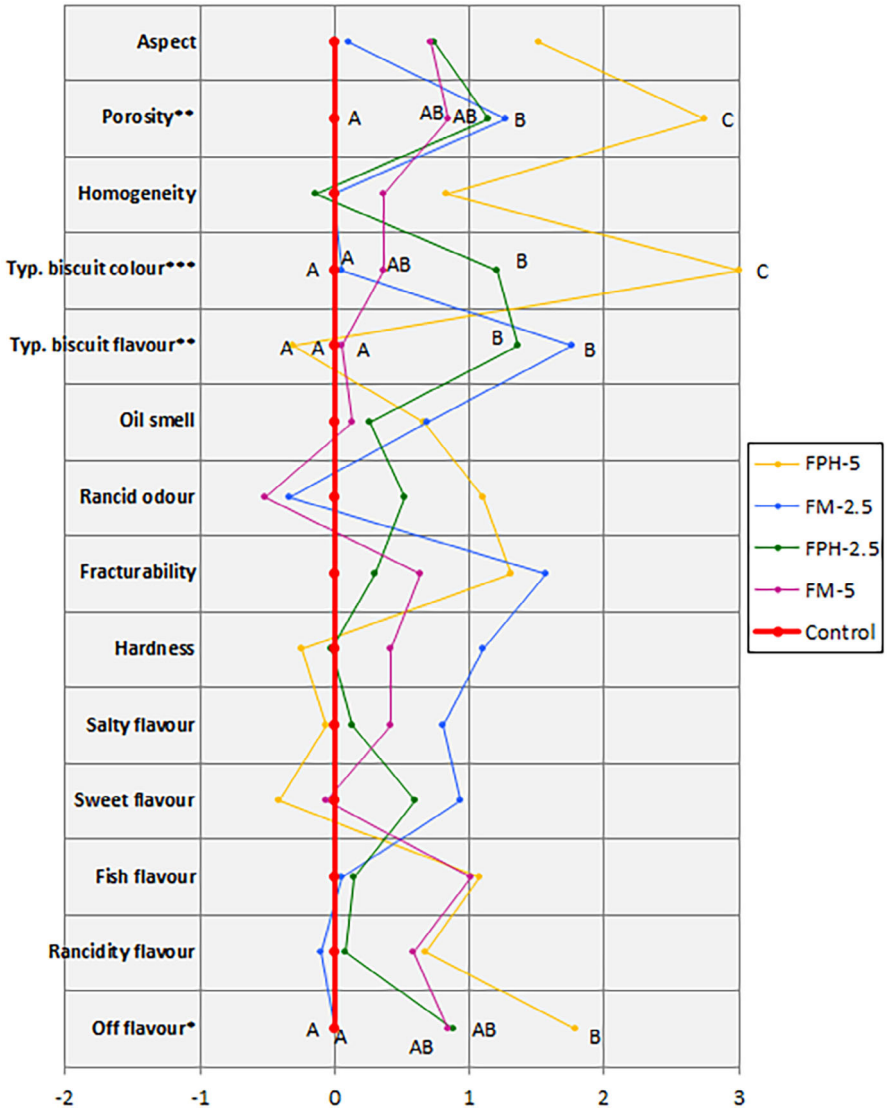

Figure 3 Sensory profiles of FM and FPH-fortified biscuits. FM, fishmeal; FPH, fish protein hydrolysate. 2.5 and 5 refer to the substitution percentage. The scale has been modified to allow a better result visualisation. Different letters show significant differences between treatments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

behaviour was observed, with higher values than the control biscuit due to the Maillard reaction (Wang *et al.*, 2011; Cai *et al.*, 2016). For the typical biscuit flavour, FPH-2.5 and FM-2.5 were significantly different from the rest, while those with a higher substitution percentage of both FM and FPH were not statistically different from the control. This would indicate that the flavours derived from compounds generated in the Maillard reaction can camouflage those typical fish flavours.

It should also be noted that only one endoprotease enzyme (Alcalase®) has been used in the production of FPH. It is known that the mixture of endoprotease and exoprotease enzymes can modify the taste, generating more neutral FPH (Steinsholm, 2021). Finally, the FPH-5 treatment was significantly different from the control and FM-2.5, so that a 5% substitution with FPH might be excessive.

The multidimensional scaling (Fig. 4) allowed a better visualisation of treatments that were globally more like the control. In this case, the most similar treatments were those in which the substitution was carried out with FM-5 and FM-2.5, being the most different from those containing FPH.

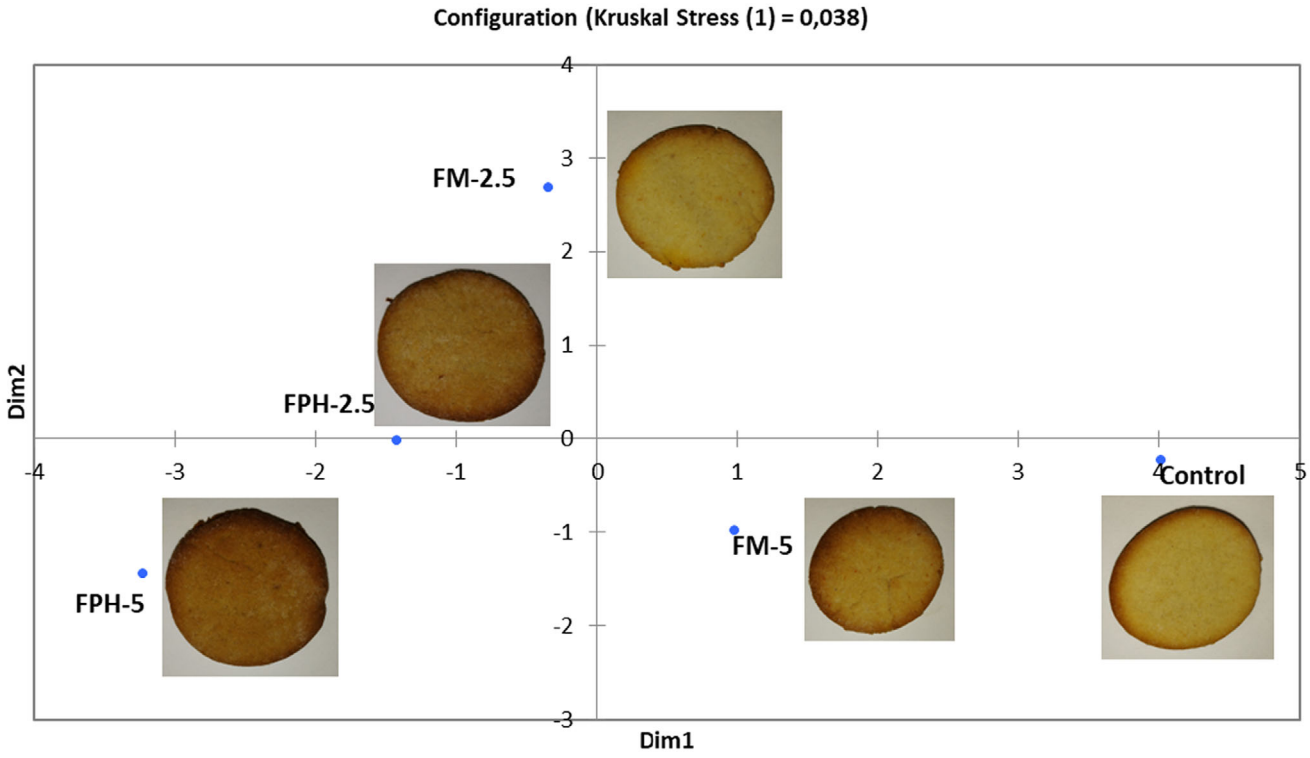

Figure 4 Multidimensional scaling of the different biscuits ($P < 0.05$). FM, fishmeal; FPH, fish protein hydrolysate. 2.5 and 5 refer to the substitution percentage.

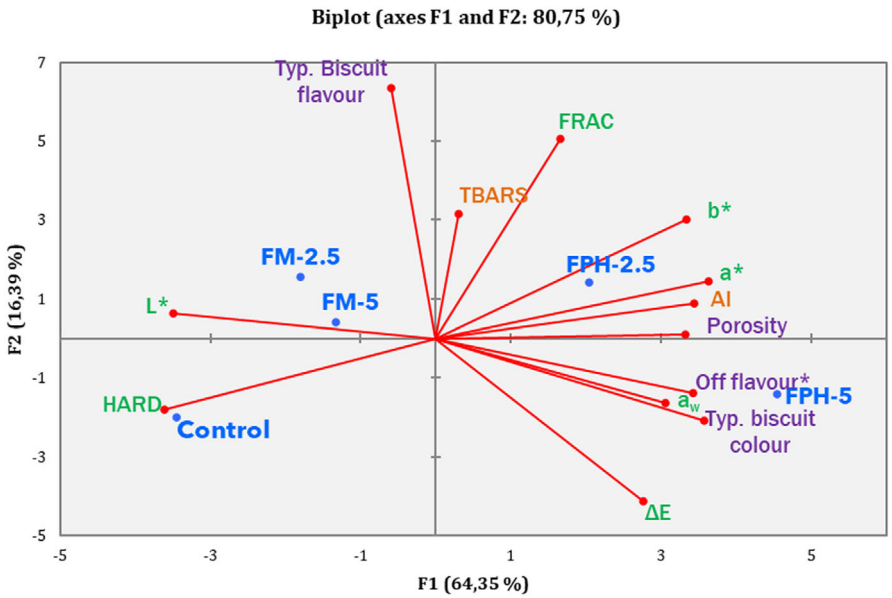

Figure 5 Principal component analysis of biscuits fortified with FPH and FM. FM, fishmeal; FPH, fish protein hydrolysate. 2.5 and 5 refer to the replacement percentage.

An overview of biscuits

Figure 5 shows the principal component analysis carried out with all treatments and the physicochemical and significant sensory variables. This graph was able to describe around 80.75% of the variability of the experiment. The F1 component, with 64.35%, was able to separate the biscuit according to the ingredient included: FM or FPH, associating the biscuit with FM to a higher brightness, hardness and typical biscuit flavour. On the other hand, biscuits with FPH were associated with higher red and yellow shades, as well as with higher lipid oxidation, fracturability, porosity, off-flavour and typical colour. The second component, which only accounted for 16.39% of the variability of the experiment, was only able to separate the control biscuit and FPH-5 from the rest of the tested treatments. However, it would be necessary to investigate whether potential consumers prefer a biscuit with a typical flavour, greater hardness and brightness or whether they prefer biscuits with greater porosity, typical colour and porosity.

Microstructure characterisation

Figure 6 shows SEM images comparing the control biscuits with FM-2.5 and FPH-2.5 biscuits, identified as the most like the control. SEM facilitated an in-depth examination of internal changes in biscuit structure, such as particle size and uniformity, which can influence sensory characteristics. In the control biscuits, circular structures dominate (highlighted with circles in Fig. 6), indicative of partially gelatinised starch granules embedded in a well-developed gluten network (Arp *et al.*, 2018). The presence of sugars in the dough, coupled with limited water content, makes it impossible to complete gelatinisation, resulting in a distinctive linear structure (Kulp *et al.*, 1991). Furthermore, increased porosity is evident, with darker zones suggesting greater depth and air-filled spaces that enhance biscuit porosity (marked with rectangles in Fig. 6).

Contrastingly, the biscuit enriched with 2.5% seabass FPH closely resembled the control, displaying darker zones associated with increased porosity. However, SEM reveals that starch granules are partially embedded in a continuous phase primarily composed of sugars, fat and peptides (Ashwath Kumar & Sudha, 2021). These components may produce interactions between protein and starch, reducing hardness, as has been checked in instrumental hardness measurements (Sudha *et al.*, 2007). The most notable SEM deviations from the control were observed in FM-5 biscuits. These exhibited an intense continuous phase comprising protein and fat, with starch granules subtly protruding and appearing almost submerged (outlined with dashed rectangles in Fig. 6). This unique phase results from FM's higher fat content compared to

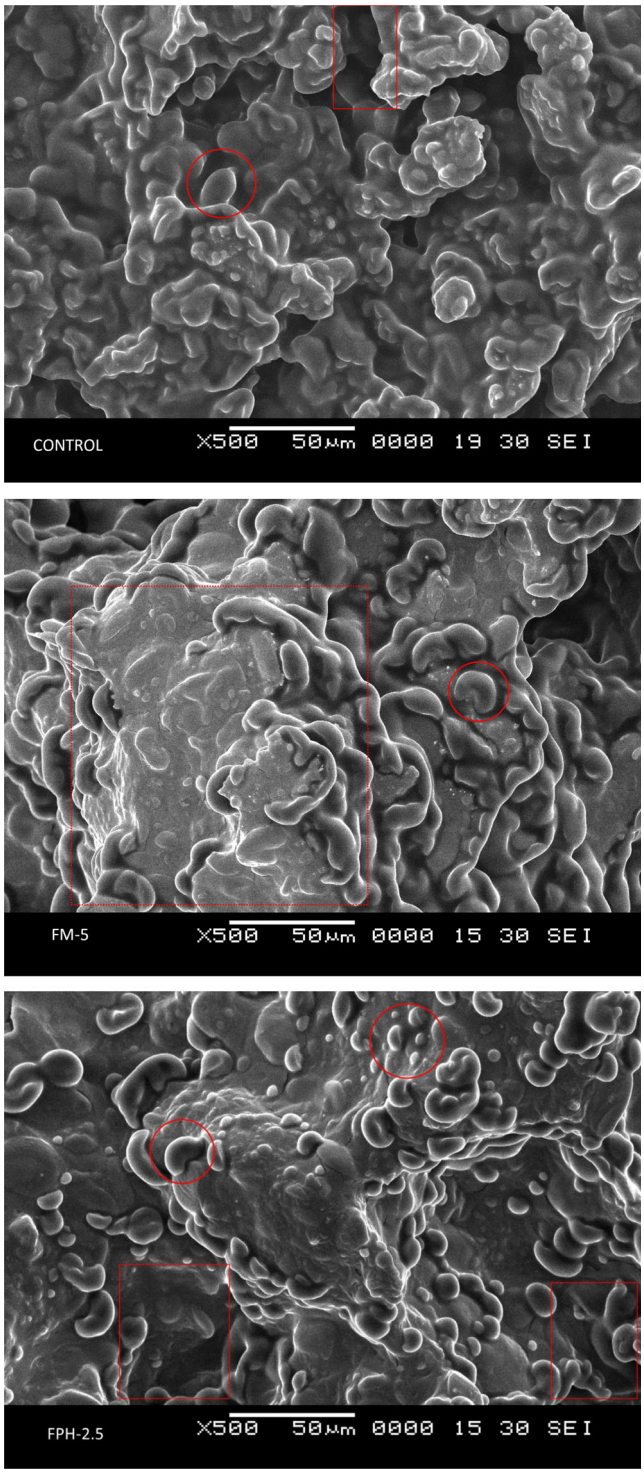

Figure 6 Scanning electron microscopy of control biscuit, FPH-2.5 and FM-2.5. FM, fishmeal; FPH, fish protein hydrolysate. 2.5 and 5 refer to substitution percentage. Circles show partially gelatinised starch granules. Rectangles show hollow areas. The rectangles with dashed lines show areas where the protein/fat matrix is observed.

FPH biscuits and the prevalence of proteins over peptides (Brites *et al*., 2019).

Instrumental texture analysis aligns with scanning electron microscopy (SEM) findings, highlighting variations in biscuit fracturability linked to their rheological properties, as depicted in the images. However, the sensory analysis indicated such subtle differences in fracturability that assessors were unable to discern among them.

Conclusions

It can be concluded that the addition of seabass protein hydrolysate (FPH) and seabass fishmeal (FM) into biscuit formulations is feasible. Furthermore, this study has revealed additional insights. To produce FPH and FM, it was observed that the temperature attained during the spray-drying process, employed in FPH manufacturing, adversely impacts essential fatty acids such as ALA, EPA and DHA, leading to an elevated TBARS index. To mitigate this, the application of antioxidants emerges as a potentially effective strategy. In the context of biscuits, the Maillard reaction was identified as decisive. The inclusion of FM and FPH resulted in a more pronounced toasting effect under similar baking conditions, particularly noticeable in FPH-enriched biscuits due to their higher protein content. Biscuits containing FPH displayed increased moisture content, attributed to the presence of hydrophilic peptides, potentially influencing fracturability and texture and SEM images indicated structural alterations upon the addition of FPH or FM. From a sensory standpoint, biscuits with FPH exhibited a more pronounced flavour and distinctive colours, due to Maillard's reaction. Sensory analysis highlighted the vulnerability of fatty acids to oxidation. In summary, the initial products developed in this study lay the foundation for a new path forward, promising a stable product with enhanced nutritional value, necessitating successful consumer acceptance. Looking forward, future research in this domain should incorporate new FPH produced with other enzymes to check if there is an effect in its flavour and a consumer sensory panel to comprehensively assess acceptance levels.

Acknowledgments

The authors would like to acknowledge BARNA S.A. company in Mundaka, Spain. They would also like to acknowledge the use of Servicio General de Apoyo a la Investigación-SAI, Universidad de Zaragoza.

Author contributions

Adrián Honrado: Conceptualization; investigation; writing – original draft; methodology; validation; writing – review and editing; software; formal analysis; data curation. **Paula Ardila:** Investigation; validation; methodology. **Paula Leciñena:** Investigation; methodology; validation. **José A. Beltrán:** Funding acquisition; validation; writing – review and editing; project administration; supervision; resources. **Juan Calanche:** Resources; supervision; project administration; writing – review and editing; validation; funding acquisition.

Funding

This research was funded by OTRI UNIZAR, project number 2022/0492.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval was not required for this research.

Data availability statement

All data generated or analysed during this study are included in this published article.

References

Abraha, B., Mahmud, A., Admassu, H. *et al*. (2018). Production and quality evaluation of biscuit incorporated with fish fillet protein concentrate. *Journal of Nutrition & Food Sciences*, **8**, 1000744.

This reference provides an overview of fish protein concentrate, which has some similarities with fish protein hydrolysates. It also includes a methodology for the evaluation of quality parameters in biscuits. For that reason, it is an interesting background for our research.

Ainsa, A., Honrado, A., Marquina, P.L., Roncalés, P., Beltrán, J.A. & Calanche M, J.B. (2021). Innovative development of pasta with the addition of fish by-products from two species. *Foods*, **10**, 1889.

Ajay, S. & Pradyuman, K. (2019). Optimization of gluten free biscuit from foxtail, copra meal and amaranth. *Food Science and Technology*, **39**, 43–49.

Al Khawli, F., Pateiro, M., Domínguez, R. *et al*. (2019). Innovative green technologies of intensification for valorization of seafood and their by-products. *Marine Drugs*, **17**, 689.

(edited by AOCS) (1990). *Offical Methods and Recommended Practices of the AOCS*, 7th edn. Champaign, IL: AOCS Press.

Arp, C.G., Correa, M.J. & Ferrero, C. (2018). Rheological and microstructural characterization of wheat dough formulated with high levels of resistant starch. *Food and Bioprocess Technology*, **11**, 1149–1163.

This article, clearly helped to interpret the SEM image of biscuits, identifying partially gelatinised starch, protein and sugars. Also, it has made it possible to identify similar behaviour and structures in the biscuits made in our research.

Ashwath Kumar, K. & Sudha, M.L. (2021). Effect of fat and sugar replacement on rheological, textural and nutritional characteristics of multigrain cookies. *Journal of Food Science and Technology*, **58**, 2630–2640.

Baljeet, S.Y., Ritika, B.Y. & Roshan, L.Y. (2010). Studies on functional properties and incorporation of buckwheat flour for biscuit making. *International Food Research Journal*, **17**, 1067–1076.
BEDCA. (2021). BEDCA, Base de Datos Española de Composición de Alimentos [WWW Document]. https://www.bedca.net/bdpub/ (accessed 9.16.21).
Bender, D.A. (2009). *A Dictionary of Food and Nutrition, Oxford Paperbacks*. Oxford: Oxford University Press.
Bligh, E.G. & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.
Brites, L.T., Ortolan, F., Silva, D.W. *et al.* (2019). Gluten-free cookies elaborated with buckwheat flour, millet flour and chia seeds. *Food Science and Technology*, **39**, 458–466.
Cai, L., Li, D., Dong, Z., Cao, A., Lin, H. & Li, J. (2016). Change regularity of the characteristics of Maillard reaction products derived from xylose and Chinese shrimp waste hydrolysates. *LWT – Food Science and Technology*, **65**, 908–916.
Calanche, J., Beltrán, H., Marquina, P., Roncalés, P. & Beltrán, J.A. (2019). Eating fish in another way: development of functional pasta with added concentrates of farmed sea bass (*Dicentrarchus labrax*). *Cereal Chemistry*, **96**, 856–865.
Connell, J.J. (1995). *Control of Fish Quality*, 4th edn. London: Fishing News Books Limited.
Do, Q., Mishra, N., Colicchia, C., Creazza, A. & Ramudhin, A. (2022). An extended institutional theory perspective on the adoption of circular economy practices: insights from the seafood industry. *International Journal of Production Economics*, **247**, 108400.
EFSA (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, **8**, 1461.
Egerton, S., Culloty, S., Whooley, J., Stanton, C. & Ross, R.P. (2018). Characterization of protein hydrolysates from blue whiting (*Micromesistius poutassou*) and their application in beverage fortification. *Food Chemistry*, **245**, 698–706.
FAO (2022). *El Estado Mundial de la Pesca y la Acuicultura 2022*. Rome: FAO. https://doi.org/10.4060/cc0461es
Farzana, T., Hossain, F.B., Abedin, M.J., Afrin, S. & Rahman, S.S. (2022). Nutritional and sensory attributes of biscuits enriched with buckwheat. *Journal of Agriculture and Food Research*, **10**, 100394.
Field, C.J., Blewett, H.H., Proctor, S. & Vine, D. (2009). Human health benefits of vaccenic acid. *Applied Physiology, Nutrition, and Metabolism*, **34**, 979–991.
Fraterrigo Garofalo, S., Cavallini, N., Demichelis, F. *et al.* (2023). From tuna viscera to added-value products: a circular approach for fish-waste recovery by green enzymatic hydrolysis. *Food and Bioproducts Processing*, **137**, 155–167.
Fuentes, A., Fernández-Segovia, I., Serra, J.A. & Barat, J.M. (2010). Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. *Food Chemistry*, **119**, 1514–1518.
Gani, A., Broadway, A.A., Ahmad, M. *et al.* (2015). Effect of whey and casein protein hydrolysates on rheological, textural and sensory properties of cookies. *Journal of Food Science and Technology*, **52**, 5718–5726.
Gibson, S., Lambert, J. & Neate, D. (2004). Associations between weight status, physical activity, and consumption of biscuits, cakes and confectionery among young people in Britain. *Nutrition Bulletin*, **29**, 301–309.
Gómez, C., Bermejo, L.M. & Loria, V. (2011). Importancia del equilibrio del índice omega-6/omega-3 en el mantenimiento de un buen estado de salud: recomendaciones nutricionales. *Nutrición Hospitalaria*, **26**, 323–329.
Gómez-Guillén, M.C., Pérez-García, S., Alemán, A., Vázquez, J.A. & Montero, M.P. (2022). The role of the drying method on fish oil entrapment in a fish muscle protein-κ-carrageenan-fish protein hydrolysate wall matrix and the properties of colloidal dispersions. *Food Hydrocolloids*, **131**, 107799.
This research article evaluates precisely and concisely the reasons for lipid oxidation of fish protein hydrolysates and relates it to the main players in the technological process. It also studies techniques to minimise this process that could be useful in this research.
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**, 3656.
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*, **12**, 4437.
Iñarra, B., Bald, C., San Martín, D., Orive, M., Cebrián, M. & Zufía, J. (2018). *Guía de Valoración de Subproductos de la Acuicultura*. Derio: AZTI.
Instituto de la Galleta (2023). *Historia de la Galleta [WWW Document]*. Barcelona: Instituto de la Galleta. http://institutodelagalleta.com/historia.php?cl=2 (accessed 11.8.23).
ISO (2007). *ISO 8589:2007. Sensory Analysis – General Guidance for the Design of Test Rooms*. Geneva: ISO.
ISO (2016). *ISO 13299:2016. Sensory Analysis – Methodology – General Guidance for Establishing a Sensory Profile*. Geneva: ISO.
ISO (2020). *ISO 660:2020. Aceites y Grasas de Origen Animal y Vegetal. Determinación del Índice de Acidez y de la Acidez*. Geneva: ISO.
ISO (2023). *ISO 8586:2023. Sensory Analysis – Selection and Training of Sensory Assessors*. Geneva: ISO.
Javed, A., Imran, M., Ahmad, N. & Hussain, A.I. (2018). Fatty acids characterization and oxidative stability of spray dried designer egg powder. *Lipids in Health and Disease*, **17**, 282.
Kelley, D.F. (1988). The importance of estuaries for sea-bass, *Dicentrarchus labrax* (L.). *Journal of Fish Biology*, **33**, 25–33.
Kulp, K., Olewnik, M., Lorenz, K. & Collins, F. (1991). Starch functionality in cookie systems. *Starch-Stärke*, **43**, 53–57.
Larson-Powers, N. & Pangborn, R.M. (1978). Descriptive analysis of the sensory properties of beverages and gelatins containing sucrose or synthetic sweeteners. *Journal of Food Science*, **43**, 47–51.
Martins, S.I.F.S., Jongen, W.M.F. & van Boekel, M.A.J.S. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology*, **11**, 364–373.
Mieszkowska, A. & Marzec, A. (2016). Effect of polydextrose and inulin on texture and consumer preference of short-dough biscuits with chickpea flour. *LWT*, **73**, 60–66.
Mohamed, G.F., Sulieman, A.M., Soliman, N.G. & Bassiuny, S.S. (2014). Fortification of biscuits with fish protein concentrate. *World Journal of Dairy & Food Sciences*, **9**, 242–249.
This research was focused on the biscuit making with added fish protein concentrate. This has made it possible to observe a certain similarity in the evolution of some physicochemical parameters depending on the level of supplementation, especially in colour (reddish-yellow tones) and sensory parameters.
Montero, D., Robaina, L., Caballero, M.J., Ginés, R. & Izquierdo, M.S. (2005). Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. *Aquaculture*, **248**, 121–134.
Moreno, J. (2001). The sweet transformation. The Spanish biscuit industry in the second half of the 20th century. *Revista de Historia Industrial*, **19**, 205–248.
Nirmal, N.P., Santivarangkna, C., Benjakul, S. & Maqsood, S. (2022). Fish protein hydrolysates as a health-promoting ingredient—recent update. *Nutrition Reviews*, **80**, 1013–1026.
Rainer, L. & Heiss, C.J. (2004). Conjugated linoleic acid: health implications and effects on body composition. *Journal of the American Dietetic Association*, **104**, 963–968.
Routray, W., Dave, D., Ramakrishnan, V.V. & Murphy, W. (2018). Production of high quality fish oil by enzymatic protein hydrolysis from cultured Atlantic salmon by-products: investigation on effect

of various extraction parameters using central composite rotatable design. *Waste and Biomass Valorization*, **9**, 2003–2014.
San José, F.J., Collado-Fernández, M. & López, R. (2018). Sensory evaluation of biscuits enriched with artichoke fiber-rich powders (*Cynara scolymus* L.). *Food Science & Nutrition*, **6**, 160–167.
Shaviklo, G.R., Thorkelsson, G., Sveinsdottir, K. & Rafipour, F. (2011). Chemical properties and sensory quality of ice cream fortified with fish protein. *Journal of the Science of Food and Agriculture*, **91**, 1199–1204.
Simopoulos, A.P. (2010). The omega-6/omega-3 fatty acid ratio: health implications. *Oléagineux, Corps gras, Lipides*, **17**, 267–275.
Singh, A. & Kumar, P. (2018). Gluten free approach in fat and sugar amended biscuits: a healthy concern for obese and diabetic individuals. *Journal of Food Processing & Preservation*, **42**, e13546.
Singh, A. & Kumar, P. (2019). Storage stability determination of calorie deficit gluten-free biscuit: Taguchi concern. *Journal of Food Processing & Preservation*, **43**, e13927.
Sinthusamran, S., Benjakul, S., Kijroongrojana, K. & Prodpran, T. (2019). Chemical, physical, rheological and sensory properties of biscuit fortified with protein hydrolysate from cephalothorax of Pacific white shrimp. *Journal of Food Science and Technology*, **56**, 1145–1154.
This innovative study has enabled a comparison between our enriched biscuits and those incorporating varying percentages of flour substituted with shrimp hydrolysate. It has revealed similar behaviours, particularly in terms of hardness and fracturability parameters.
Steinsholm, S. (2021). *Sensory and Physicochemical Properties of Enzymatic Protein Hydrolysates. Influence of Raw Material, Protease, and Downstream Processing*. Bergen: University of Bergen.
Sudha, M.L., Srivastava, A.K., Vetrimani, R. & Leelavathi, K. (2007). Fat replacement in soft dough biscuits: its implications on dough rheology and biscuit quality. *Journal of Food Engineering*, **80**, 922–930.
Szczesniak, A.S. (2002). Texture is a sensory property. *Food Quality and Preference*, **13**, 215–225.
Thiansilakul, Y., Benjakul, S. & Richards, M.P. (2011). Effect of myoglobin from eastern little tuna muscle on lipid oxidation of washed Asian seabass mince at different pH conditions. *Journal of Food Science*, **76**, C242–C249.
Valcarcel, J., Sanz, N. & Vázquez, J.A. (2020). Optimization of the enzymatic protein hydrolysis of by-products from seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*). *Chemical and Functional Characterization. Foods*, **9**, 1503.
Vijaykrishnaraj, M., Roopa, B.S. & Prabhasankar, P. (2016). Preparation of gluten free bread enriched with green mussel (*Perna canaliculus*) protein hydrolysates and characterization of peptides responsible for mussel flavour. *Food Chemistry*, **211**, 715–725.
Wang, H.-Y., Qian, H. & Yao, W.-R. (2011). Melanoidins produced by the Maillard reaction: structure and biological activity. *Food Chemistry*, **128**, 573–584.
XLSTAT (2016). *Addinsoft®*. Paris: XLSTAT.
Yuan, H., Luo, Z., Ban, Z. *et al*. (2022). Bioactive peptides of plant origin: distribution, functionality, and evidence of benefits in food and health. *Food & Function*, **13**, 3133–3158.