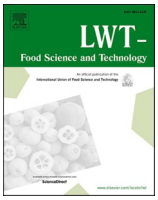
LWT - Food Science and Technology 215 (2025) 117247

Contents lists available at ScienceDirect

LWT

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



Development of nutritionally enhanced fish burgers: Integrating Atlantic bonito (*Sarda sarda*) with seaweed and hydrocolloids for sustainable food innovation

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ARTICLE INFO

Keywords:
Fish burger
Xanthan
Carrageenan
Spirulina
Fucus vesiculosus

ABSTRACT

This study developed an innovative fish burger using Atlantic bonito, an abundant but undervalued species rich in lipids and protein. The burger was enriched with chickpea, seaweed (Spirulina and *Fucus vesiculosus*), and hydrocolloids (xanthan and carrageenan). Using a Taguchi design, the effects of varying concentrations of these ingredients on the physicochemical properties, texture, phenolic content, and antioxidant activity (DPPH, ABTS) were evaluated. The optimal formulation (2 g/100 g Spirulina, 3 g/100 g *Fucus vesiculosus*, 0 g/100 g xanthan, 4 g/100 g carrageenan) increased fiber by 1.5 times, protein by 2.8 times, antioxidant activity (ABTS) by 1.2 times, and phenolic content by 3.4 times compared to the control. Seaweed concentration was linked to increased burger hardness, while cohesiveness, adhesiveness, and gumminess were unaffected by hydrocolloid concentration. No significant changes were observed in moisture content or DPPH antioxidant activity. The study demonstrates the potential of using undervalued fish species like Atlantic bonito to create nutritionally enhanced, sustainable food products.

1. Introduction

According to the Food and Agriculture Organisation (FAO) of the United Nations (UN), the presence of fish in the human diet has reached an average of around 17 kg per individual. This value shows that more than 3 million people in the world consume 15 % of fish proteins every day (FAO, 2016). The World Health Organisation (WHO) recommends eating fish three times a week, as it is a source of protein and rich in nutrients (fat-soluble vitamins (A, D and E) and omega-3 fatty acids) (WHO, 2024). Fish contains considerable levels of potassium, phosphorus, iodine and selenium, which are important for the healthy functioning of energy metabolism, as well as good thyroid function (IPMA, 2023). Consequently, consumers have tried to reduce their consumption of processed red meat products and have turned to fish-based products due to their lower cholesterol and saturated fat content (Tacon & Metian, 2013). The food industry is continuously flourishing and has faced soaring demand in the past decade. Meat and fish-based foods are of high nutritional value and important for human health, in line with Sustainable Development Goals (SDGs) to bring about nutrition and food security. Meat and fish-based food systems include zinc, heme iron, bioavailable B vitamins, and essential amino acids, and are often equated with different health promoting biological activities (Karwowska et al., 2021). According to the Fishing Statistics (Statistics Portugal, 2022), 165,801 tons of fish were caught by the Portuguese fisheries sector, which represents a reduction of 10.6% compared to 2021. This may be due to the lower volume of catches in national waters compared to catches in external fishing grounds. Of the relevant species with catch limitations in 2022, the most significant increases occurred in the quotas for horse mackerel on the mainland coast, megrim, anglerfish and cod in the traditional fishing grounds of the Northwest Atlantic Fisheries Organisation (NAFO). The Portuguese continue to be one of the biggest per capita consumers of fish in the

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https://doi.org/10.1016/j.lwt.2024.117247
Received 12 October 2024; Received in revised form 22 November 2024; Accepted 17 December 2024
Available online 19 December 2024

European Union (EU), according to a report by the European Market Observatory for Fisheries and Aquaculture Products (EUMOFA, 2021).

Atlantic bonito (*Sardasarda*) is a species of marine fish, characteristic of the Scombridae family, like tuna and mackerel. Despite its importance in sport fishing, it is not yet very commercialised, as it has not been studied and explored despite its rich composition in proteins, lipid nutrients, characteristic flavour and ease of use (Relini et al., 2005). The Atlantic bonito (*Sardasarda*) is an epipelagic, neritic, schooling scombrid of the Atlantic Ocean, the Mediterranean Sea and the Black Sea and is a commercially important species (Zaboukas et al., 2006). This fish stands out for its favourable aspects, such as its nutrient-rich lipid/protein composition, high meat yield and specific flavour (Altan et al., 2022).

Integrating seaweed into food products can serve as both a method of preservation and a means of nutritional enhancement. It has several beneficial constituents such as dietary fibre, amino acids, unsaturated fatty acids, vitamins and minerals, as well as bioactive compounds such as polyphenols, carotenoids and alkaloids demonstrating antioxidant, antibacterial and fungal activity (Salido, Soto & Seoane, 2024), According to European Council Regulation 258/97, seaweeds are food or food ingredients. Therefore, they can be used in the food industry without any health hazards. Seaweeds find suitability and application in manifold industries such as agriculture, biomedical, cosmetic and food industry. Seaweed-based biopolymers are an excellent option as polymer matrix to fabricate coatings and films, which can offer a great opportunity to decrease the devastating overuse of chemical alternatives and fruitfully preserve the quality and shelf-life of different food systems. Seaweed sourcing is sustainable and compared to terrestrial plants, seaweeds grow faster and do not require arable land, fresh water or contaminating fertilizers (Ebrahimzadeh et al., 2023).

Edible coatings can play a crucial role in enhancing food quality by delaying microbial growth, reducing lipid oxidation, and preventing quality loss because of their highly perishable nature (Dehghani, Hosseini & Regenstein, 2018). *Spirulina* is a micro seaweed that stands out due to its nutritional potential as protein source (55.8–77 g/100 g), emulsifying, foaming and gelling properties, among others (Menegotto et al., 2019). Spirulina has one of the highest protein contents found, as well as a high content of essential amino acids based on the FAO and World Health Organisation (WHO) composition. Bladderwrack (*Fucus vesiculosus*) is a macro seaweed that contains proteins, minerals, iodine, vitamins and monounsaturated and polyunsaturated fatty acids, but the main components that confer the reported health effects are non-digestible polysaccharides (dietary fibre) and polyphenols. The presence of functional components such as dietary fibre and antioxidants is higher than in other edible seaweed, which has led to the development of many functional ingredients and dietary supplements derived from this seaweed, such as fucoidan powder, capsules or antioxidant extracts. The use of the macro seaweed *Fucus vesiculosus* has attracted attention due to its nutritional content and bioactive compounds with antioxidant and antimicrobial activity (Kontominas et al., 2021). Freeze-dried powder of *Fucus vesiculosus* has a higher dietary fibre content and antioxidant capacity than commercial nutraceuticals from this seaweed and this suggests that drying processes at high temperatures or long periods of storage reduce the antioxidant properties of the original material.

Xanthan and carrageenan, both widely used hydrocolloids, are crucial for food texture enhancement and stability. Xanthan gum, produced through bacterial fermentation, is known for its exceptional thickening, stabilizing, and emulsifying properties, making it a versatile ingredient in food formulations. Carrageenan, extracted from red seaweed, plays a vital role in forming gels and adding viscosity to foods, particularly in dairy and meat products. These hydrocolloids are commonly used in combination to improve the structure, texture, and water-holding capacity of food products, providing desirable qualities like smoothness and consistency, especially in processed foods. Additionally, their biocompatibility and safety make them suitable for use in innovative food systems focused on sustainability and health.

Previous studies have not yet explored the use of Atlantic bonito in combination with seaweed and plant-based ingredients such as chickpeas, highlighting the untapped potential for developing a sustainable food product like a fish burger that promotes both nutritional enhancement and environmental sustainability (Díaz-Rubio et al., 2009). Therefore, the aim of this study was to develop an innovative fish burger formulation using Atlantic bonito, enriched with plant-based ingredients such as chickpeas, seaweed (Spirulina and *Fucus vesiculosus*), and hydrocolloids, such as xanthan and carrageenan.

2. Materials and methods

2.1. Raw material

The fish Atlantic bonito (*Sardasarda)* was supplied by Docapesca (Viana do Castelo, Portugal) and captured on the North Atlantic Coast of Portugal, sent to the company Guimarpeixe, where the samples were subjected to ultra-freezing (−80 °C), transported to the laboratory under freezing temperatures and kept at −18 °C until processing. Eight fish samples were used in this study, with approximately 1–1.5 kg each. Prior to processing, the samples were thawed in a refrigerator for 14 h at 4 °C. Fish samples were washed, gutted and then filleted and stored at 4 °C until further processing. Garlic powder, pepper powder and chickpeas were purchased at a local supermarket in Viana do Castelo (Portugal). The hydrocolloids xanthan (E415) (Capri+, Portugal) and carrageenan (E407) (FormuLab, Portugal) were used. The seaweed Spirulina and *Fucus vesiculosus* were obtained from Supla (Portugal) and Celeiro (Spain).

2.2. Fish burger process production and experimental design

The Atlantic bonito (*Sardasarda*) burger was prepared as described in Fig. 1. Fish was decapitated, gutted, filleted manually, removing the skin layer, and immediately washed with tap water and then the fillets were grinded in an Ultra-Turrax (model T18D, IKA, Germany) to obtain a homogeneous minced meat. Chickpeas were cooked at 100 °C for 1 h and then grinded, weighed and added to minced fish, previously prepared, and then the garlic and pepper, already weighed, were added, according to Table 1. *Fucus vesiculosus*, previously grinded in a food processor (Termomix TM31, Vorwerk, Germany), Spirulina, carrageenan and xanthan were weighed into the appropriate quantities and added to each formulation according to Table 1 following a L9 orthogonal array of Taguchi design as describe below. The mixture was blended in a food processor (Termomix TM31, Vorwerk, Germany) for 1 min. Chickpeas were used to complete the final formulation. The mixture was placed into 6 cm diameter stainless steel mold. Each raw fish burger weighed approximately 80 g with a thickness of 6 mm. Preliminary experiments were conducted to identify the optimal temperature and cooking time for the fish burger, ensuring it achieved the desired organoleptic properties for a favourable sensory evaluation by the panellists. All samples were cooked in a convection oven (VPE-061, Fagor, Spain) at 180 °C for 15 min until the internal temperature reached 88.5 °C, packed in polyamide/polyethylene plastic bags (PA/PE - 20/70) (thickness 90 μm; permeability: O2 - 50 cm^3/m^2 dbar; CO2 - 150 cm^3/m^2 dbar; N2 - 10 cm^3/m^2 dbar; Water vapor transmission - 2.8 g/m^2 d) and refrigerated at 4 °C, for subsequent analysis.

The effect of seaweed (Spirulina, and *Fucus vesiculosus*) and hydrocolloids (xanthan gum, carrageenan) on Atlantic bonito (*Sardasarda*) burger was studied using the Taguchi method, a fractional factorial experimental design that uses orthogonal arrays for the optimization of different factors (Li et al., 2022). Orthogonal Array Testing Strategy can be used to reduce the number of combinations and provide maximum coverage with a minimum number of test cases. Each row represents a test case/combination, and the factors are combined pairwise rather than representing all possible combinations of factors and levels (Lazic, 2013).

In this study, the experimental design was performed using a L9

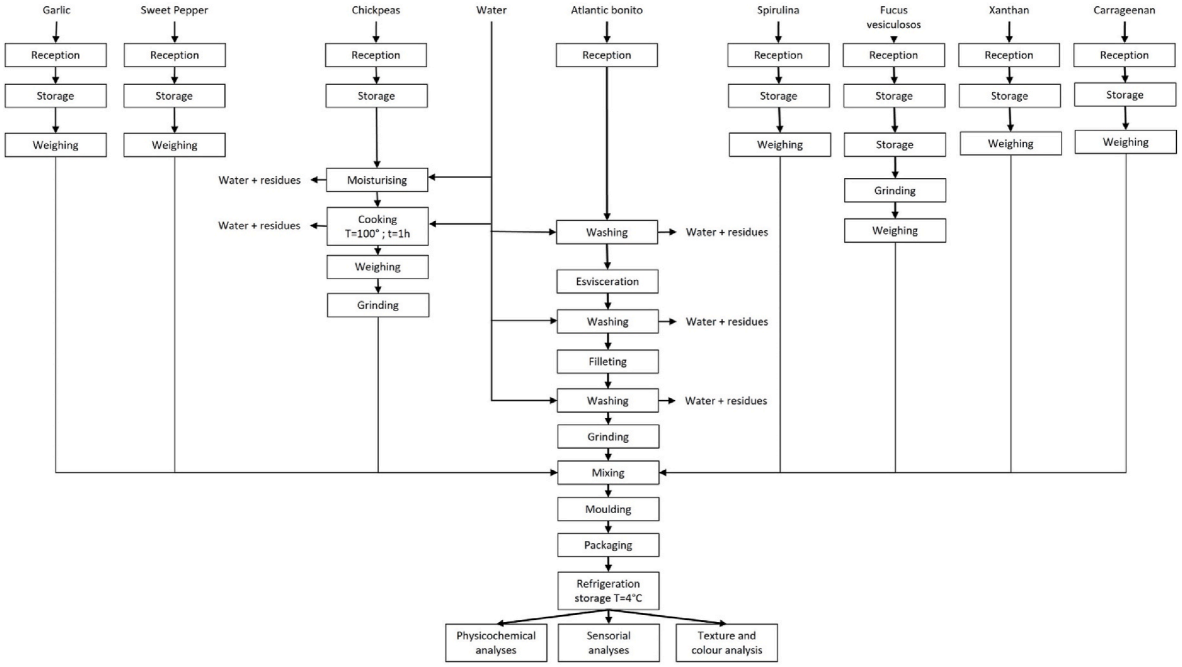

Fig. 1. Flowchart of the Atlantic Bonito (*Sardasarda*) burger production process.

Table 1
Raw material used in the experimental design for different Atlantic bonito burger following a L9 orthogonal array of Taguchi design. A control was also included.

Raw materials (g/100 g)	Runs									
	Control	1	2	3	4	5	6	7	8	9
Atlantic bonito	70	70	70	70	70	70	70	70	70	70
Chickpeas	28	26.5	23	19.5	23	19	21	22	19	20.5
Garlic	1	1	1	1	1	1	1	1	1	1
Pepper	1	1	1	1	1	1	1	1	1	1
Spirulina	0	0.5	1	0.5	1	1	2	2	2	0.5
F. vesiculosus	0	1	2	3	3	1	2	1	3	2
Xanthan	0	0	0	3	1	3	3	1	0	1
Carrageenan	0	0	2	2	0	4	0	2	4	4

orthogonal array (Qualitek-4 software, Nutek, Bloomfield Hills, USA). Four factors (xanthan gum, carrageenan, Spirulina, and *Fucus vesiculosus*) were combined and varied in three levels: xanthan (0, 1 and 3 g/100 g), carrageenan (0, 2 and 4 g/100 g), Spirulina (0.5, 1 and 2 g/100 g) and *Fucus vesiculosus* (1, 2 and 3 g/100 g). These factors and levels were selected considering a previously work. A control was also made with no addition of seaweed and hydrocolloids (0 g/100 g). Taguchi design was used to optimize the best formulation by the texture parameter (hardness) and antioxidant activity (ABTS and DPPH).

2.3. Chemical composition

The pH values were measured using a pH meter (pH 25+, Crison, Spain), previously calibrated with three standard solutions pH 4.00, pH 7.00 and pH 10.00.

The moisture content was determined according to the AOAC method 925.10:1995 (AOAC, 1995), three g of burger was weighed and dried in an oven (103 ± 2 °C) until a constant weight was reached (about 340 min).

Crude fibre was determined using AOAC Method 962.09:1995 (AOAC, 1995), A 2 g sample was accurately weighed into a reflux flask and dissolved using an acid and an alkali. After each boiling, the sample was filtered, and the filters were dried in an oven at 130 ± 2 °C for 2 h, weighed, and then transferred to a muffle furnace at 600 ± 15 °C for 30 min before being weighed again.

Sodium chlorides content was determined using AOAC Method 937.09:1995 (AOAC, 1995), approximately 1 g of the sample was weighed into an Erlenmeyer, then silver nitrate ($AgNO_3$ 0.1 N) and concentrated nitric acid (HNO_3) were added. After boiling for 15 min, it was allowed to cool to room temperature and water and iron (II) ammonium sulphate were added. Finally, the excess $AgNO_3$ was titrated until it reached a permanent light brown colour.

Ash content and AOAC method 938.08:1995 (AOAC, 1995), approximately 3 g of the sample was weighed and placed in the muffle furnace oven (Heraeus, M110, Hanau, Germany) at a temperature of ≤550 °C for 30 min, after they were weighed to determine the ash content.

Protein was determined using AOAC method 955:04:1995 (AOAC, 1995) by the Kjeldahl method, where the sample was digested to 420 °C for 90 min after adding 98 mL/100 mL H_2SO_4 and catalyst using DK6 Heating digester (Velp Scientifica, Usmate (MI), Italy). Then, the sample digested was distilled, after adding 40 g/100 mL NaOH, with the distillate being collected in a 4 g/100 mL boric acid solution in Velp UDK 140 distillation unit (Velp Scientifica, Usmate (MI), Italy). The nitrogen concentration was obtained by titration with 0.1 N HCl. The protein content was determined by multiplying the nitrogen content by the factor of 6.25.

Lipidic content was determined using AOAC Method 920.39:1995 (AOAC, 1995), 5 g of the fish sample was weighed, and hydrochloric acid (HCL 4 N) was added, then the sample was placed on a heating plate

and left to boil for 1 h. Once this stage was complete, the contents were filtered using a vacuum, then the filters were placed in an oven at 102 ± 2 °C for 1 h and once dry they were extracted for 4–6 h. After the extraction stage, the solvent was evaporated in a rotary evaporator and dried for 1 h in an oven at 102 ± 2 °C until constant weight, at the end of which the amount of fat retained was weighed.

The content of the polyphenolics in the Atlantic bonito burger was determined by Folin-Ciocalteu analysis according to the procedure described by Singleton & Rossi (1965) with adaptations from Ainsworth and Gillespie (2007).

2.4. Antioxidant activity determination

The antioxidant activity was evaluated by two different assays, namely DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method (Deng et al., 2011) based on Brand-Williams, Cuvelier & Berset et al. (1995) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging method.

A total of 5 g of cooked burger was extracted with 15 mL of 80 mL/100 mL methanol and the mixture was filtered. Then, 15 mL of 80 mL/100 mL methanol were added to the residue and the mixture was filtered again. The last step was repeated one more time. The filtrate was centrifuged for 20 min, and the supernatant was completed to 50 mL with 80 mL/100 mL methanol (extract stock solution). Stock solution was diluted (1:2 and 1:4) for further analysis.

The antioxidant activity of each sample was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method based on Brand-Williams et al. (1995). A volume of 50 μL of diluted extract stock solution (in methanol) was placed in a clear 96-well microplate with 200 μL of DPPH in methanol and allowed to stand in the dark for 30 min before measuring the absorbance of the solution at 520 nm on the Varioskan LUX Multimode Microplate Reader (Thermo Scientific, Vantaa, Finland). Methanol was used as the blank to calibrate the spectrophotometer. The control was a DPPH solution containing absolute methanol instead of the sample.

The antioxidant activity of each sample was also determined by the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging method. A volume of 25 μL of diluted extract stock solution (in methanol) was placed in a clear 96-well microplate with 200 μL of ABTS in methanol and allowed to stand in the dark for 6 min before measuring the absorbance of the solution at 735 nm on the Varioskan LUX Multimode Microplate Reader (Thermo Scientific, Vantaa, Finland). Methanol was used as the blank to calibrate the spectrophotometer. The control was a ABTS solution containing absolute methanol instead of the sample.

The total phenolic content (TPC) was also measured due to its relationship with the antioxidant activity. The extract solution was added to 200 μL of the Folin-Ciocalteu phenol reagent followed by gentle shaking. Then, 800 μL of 7 g/100 mL sodium carbonate was added. After 2 h, the optical density at 765 nm of the resulting blue complex was measured using a Varioskan LUX Multimode Microplate Reader (Thermo Scientific, Vantaa, Finland) spectrophotometer. A mixture of water and reagents was used as a blank. The standard curve was prepared with gallic acid in water at 0, 5, 10, 20, 40, 60, 80, 100, 120 and 160 mg/L. The total phenolic contents were expressed as the g of gallic acid equivalents (GAE) per g of sample.

2.5. Texture determination

Texture properties (correlated with panellist's definitions) hardness, cohesiveness, adhesiveness and gumminess were determined 2 h after cooling at room temperature. Texture was measured using the TA-XT2i Texture Analyser (Stable Micro Systems Ltd, United Kingdom). Fish burgers were subjected to deformation with a single compression 150 cycle at room temperature (25 °C) using a stainless-steel cylindrical probe (10-P). The probe punched into the 151 bar with a constant crosshead test velocity of 0.5 mm/s and a test distance of 8 mm. Twelve replicates were performed for each run, using different areas of the sample.

2.6. Colour determination and sensory analysis

The colour of the samples was assessed using a Minolta CR300 (Konica Minolta, Japan) with the colour system CIE L*, a*, b*. Lightness (L *), redness to greenness (+a* to -a*) and yellowness to blueness (+b* to -b*). Fifteen replicates were performed, using different areas of the sample.

For sensory analysis, a quantitative descriptive analysis (QDA®) was carried out according to ISO 6658:2017, with seven semi-trained panellists. The panellists defined by consensus the meaning of each attribute related to the textural properties in order to correlate them with the results obtained with the instrumental texturometer. The attributes evaluated by the panellists were: colour, uniformity when cut, fish odour, seaweed odour, off-odour, hardness, adhesiveness, cohesiveness, fish flavour, garlic flavour, seaweed flavour and salty flavour. These attributes were evaluated using an intensity scale of 10 points (1 - lowest intensity, 10 - higher intensity). In addition, the samples were classified in terms of general taste (1–5 points: 1 - very bad, 5 - excellent). In another sensory analysis session, the panellists evaluated the attributes of each sample, which were served in individual plates and identified with 3-digit codes.

2.7. Statistical analysis

Statistical analysis was performed using the STATISTICA 7.0 for Windows (StatSoft, California, USA). Results are described as mean values of each determination ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA). The last significant difference (Tuckey HSD test) procedure was used to test for difference between means (significance was defined at $p < 0.05$).

3. Results and discussion

3.1. Physicochemical parameters

Table 2 shows the results of pH and the chemical parameters determined for the different formulations of Atlantic bonito burgers. A progressive decrease in pH values was observed as the proportion of seaweed in the formulation increased. The formulation of run 9 presented the lowest pH, 5.86, while the highest pH value was found in the control, 6.26. This trend aligns with findings reported by Hentati et al. (2019) where control burgers exhibited higher pH values compared to seaweed-supplemented burgers. The observed decrease in pH can be attributed to the acidic nature of the seaweed, which lowers the overall pH of the product when incorporated into the formulation. The obtained values are lower than the values reported by (Pachekrepapol, Thangrattana & Kitikangsadan, 2022) with fish burger prepared from salmon and striped catfish filleting by-product.

Table 2 shows that there were no significant differences in the moisture ($p > 0.005$), as in the study by Agregán et al. (2018). The fibre content increased in formulations with higher concentrations of Spirulina and *Fucus vesiculosus*, with the highest fibre levels observed in Run 8, 4.49 ± 0.45 g/100 g. Run 5 exhibited the lowest fibre content value at 1.10 ± 0.23 g/100 g, whereas the control formulation had 3.03 ± 0.71 g/100 g. This increase can be attributed to the inclusion of seaweed, which not only enhances fibre content but also promotes the formation of a more homogeneous structure. Similar findings were reported by Díaz-Rubio et al. (2009), who demonstrated that the incorporation of *Fucus vesiculosus* into food products significantly boosts fibre content.

The lipid and protein content remained consistent across formulations with the highest seaweed incorporation (Run 6, Run 7, Run 8, Run 9). The formulation that obtained the highest lipid content was Run 8

Table 2
Results of the pH and chemical composition (g/100 g) (Moisture, pH, Fibre, Lipids, Chlorides, Protein, Ash) of the Atlantic bonito burgers (average ± standard deviation). Means within the same column with different superscripts are significantly different at $p < 0.05$.

Run	Moisture (g/100 g)	pH	Fibre (g/100 g)	Lipids (g/100 g)	Chlorides (g/100 g)	Protein (g/100 g)	Ash (g/100 g)
Control	58.96 ± 14.35	6.26 ± 0.01^{a}	3.03 ± 0.71^{bc}	5.93 ± 0.65^{cd}	0.33 ± 0.02^{g}	23.58 ± 0.40^{ac}	1.45 ± 0.01^{e}
1	58.43 ± 27.57	6.18 ± 0.004^{b}	4.57 ± 0.54^{a}	6.42 ± 0.02^{bc}	0.52 ± 0.03^{f}	25.38 ± 0.42^{ab}	1.63 ± 0.00^{e}
2	60.69 ± 0.87	6.07 ± 0.03^{cd}	1.74 ± 0.13^{df}	5.50 ± 0.02^{bce}	1.08 ± 0.03^{d}	22.04 ± 0.94^{cd}	2.71 ± 0.02^{cd}
3	56.74 ± 1.64	6.01 ± 0.06^{a}	1.34 ± 0.15^{ef}	4.94 ± 0.40^{d}	1.32 ± 0.02^{c}	20.72 ± 0.53^{d}	3.31 ± 0.05^{bc}
4	57.45 ± 0.53	6.09 ± 0.005^{c}	2.03 ± 0.61^{cde}	7.83 ± 0.04^{a}	0.83 ± 0.04^{e}	20.64 ± 1.6^{d}	2.59 ± 0.31^{cd}
5	56.62 ± 0.12	5.87 ± 0.03^{e}	1.10 ± 0.23^{ef}	5.77 ± 0.48^{ce}	1.61 ± 0.04^{b}	24.01 ± 0.43^{ac}	3.96 ± 0.08^{ab}
6	58.41 ± 0.10	6.11 ± 0.005^{c}	3.53 ± 0.20^{ab}	8.16 ± 0.17^{a}	0.51 ± 0.02^{f}	23.43 ± 0.24^{bc}	2.18 ± 0.00^{de}
7	59.60 ± 1.32	6.13 ± 0.005^{bc}	3.64 ± 0.46^{ab}	7.33 ± 0.47^{ab}	1.07 ± 0.01^{d}	25.09 ± 0.91^{ab}	2.69 ± 0.10^{cd}
8	57.17 ± 0.65	6.1 ± 0.01^{c}	4.49 ± 0.45^{a}	7.93 ± 0.05^{a}	1.62 ± 0.02^{b}	25.80 ± 1.13^{a}	4.51 ± 0.07^{a}
9	50.09 ± 4.49	5.86 ± 0.005^{e}	2.83 ± 0.18^{bd}	7.82 ± 0.02^{a}	2.06 ± 0.07^{a}	25.67 ± 0.20^{ab}	4.15 ± 0.95^{ab}

(7.93 ± 0.05 g/100 g) and the lowest was Run 3 (4.94 ± 0.40 g/100 g). Run 8 with the lower concentration of xanthan (0 g/100 g) and the highest of carrageenan (4 g/100 g), spirulina (2 g/100 g) and *Fucus vesiculosus* (3 g/100 g) was 1.33 times higher in lipid content than the control sample. Similar results were obtained by Smaldone et al. (2017) for horse mackerel (*Trachurus*) and rainbow trout (*Oncorhynchus* mykiss) burgers.

For the protein content, the formulation with the highest value was Run 8 (25.80 ± 1.13 g/100 g) and the lowest was Run 4 (20.64 ± 1.6 g/100 g), which means that formulation 8 the lower concentration of xanthan (0 g/100 g) and the highest of carrageenan (4 g/100 g), spirulina (2 g/100 g) and *Fucus vesiculosus* (3 g/100 g) was 1.09 times higher than the control sample.

These findings align with the results of fish burgers enriched with yerba mate extract (Tonet, Zara & Tiuman, 2019) and with fish burgers from common carp (*Cyprinus carpio*) and common barbel (*Barbus*) fortified with Spirulina platensis biomass (Barkallah et al., 2019), where reported that the inclusion of seaweed did not significantly affect fat and protein levels compared to the control.

Regarding ash content, the highest values were observed in Run 8 (4.51 ± 0.07 g/100 g). Run 8 with the lower concentration of xanthan (0 g/100 g) and the highest of carrageenan (4 g/100 g), spirulina (2 g/100 g) and *Fucus vesiculosus* (3 g/100 g) exhibited an ash content that was 3.11 times higher than that of the control. In contrast, the lowest ash content was found in Run 1 (1.63 ± 0.00 g/100 g), which also contained the smallest amount of seaweed, aside from the control. The results found in this work are consistent with the study carried out by Atitallah et al. (2019) which obtains 11.13 ± 0.11 g/100 g in control formulation and 11.29 ± 0.06 g/100 g, 11.33 ± 0.02 g/100 g, 11.29 ± 0.05 g/100 g for the formulations with the lowest percentage of seaweed and 11.77 ± 0.07 g/100 g, 11.9 ± 0.10 g/100 g, 11.49 ± 0.03 g/100 g for the formulations with the highest percentage. The same behaviour was observed for Barkallah et al. (2019), which obtained 11.12 ± 0.11 g/100 g for the carp control burger and 11.53 ± 0.03 g/100 g for the barbel control burger and the formulations with the lowest addition of Spirulina showed lower values (11.18 ± 0.01 g/100 g and 11.56 ± 0.32 g/100 g, respectively), while the formulations with a higher percentage of Spirulina showed higher values (1.69 ± 0.05 g/100 g and 11.84 ± 0.16 g/100 g, respectively).

The increase in the value of the chloride content was higher in the formulations with a higher seaweed concentration, due to the level of salt content.

3.2. Antioxidant activity

The antioxidant activity increased in formulations with higher concentrations of Spirulina and *Fucus vesiculosus* (Table 3) with the highest TPC levels observed in Run 8, 1.42 ± 0.02 mg GAE/g burger. Run 5 and 9 exhibited the lowest TPC content value at 0.35 ± 0.02 mg GAE/g burger, whereas the control formulation had 0.42 ± 0.95 mg GAE/g burger. Regarding the ABTS method, the run with the highest content was run 8, 0.71 ± 0.06 mg TE/g burger, and the run with the lowest ABTS content was run 2, 0.35 ± 0.02 mg TE/g burger, while the control formulation obtained 0.41 ± 0.14 mg TE/g burger.

Table 3
Results of the chemical characterisation (Total phenolic compounds (TPC), ABTS and DPPH) of the Atlantic bonito burgers (average ± standard deviation). Means within the same column with different superscripts are significantly different at $p < 0.05$.

Run	TPC (mg GAE/g burger)	ABTS (mg TE/g burger)	DPPH (mg TE/g burger)
Control	0.42 ± 0.95^{cd}	0.41 ± 0.14^{c}	0.22 ± 0.02
1	0.36 ± 0.02^{d}	0.50 ± 0.01^{bc}	0.21 ± 0.00
2	0.82 ± 0.04^{b}	0.35 ± 0.03^{c}	0.20 ± 0.01
3	0.42 ± 0.03^{cd}	0.64 ± 0.06^{ab}	0.22 ± 0.05
4	0.63 ± 0.05^{bc}	0.53 ± 0.02^{abc}	0.26 ± 0.07
5	0.35 ± 0.02^{d}	0.40 ± 0.05^{c}	0.20 ± 0.07
6	0.38 ± 0.02^{d}	0.70 ± 0.07^{ab}	0.23 ± 0.04
7	0.44 ± 0.02^{cd}	0.54 ± 0.06^{abc}	0.19 ± 0.07
8	1.42 ± 0.02^{a}	0.71 ± 0.06^{a}	0.25 ± 0.05
9	0.35 ± 0.02^{cd}	0.60 ± 0.01^{abc}	0.17 ± 0.03

TPC - Total phenolic content; GAE – Gallic acid equivalent; DPPH - 2,2-diphenyl-1-picrylhydrazyl; ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ORAC - Oxygen radical absorbance capacity; TE – Trolox equivalent.

For the DPPH method, the highest levels were observed in run 4, 0.26 ± 0.07 mg TE/g burger. Run 9 shows a lower value, 0.17 ± 0.03 mg TE/g burger, and the control formulation presented 0.22 ± 0.02 mg TE/g burger. However, there were no statistically significant differences between samples.

Albertos, Martin-Diana, Burón & Rico et al., (2019) suggested that higher total phenolic content is likely linked to increased antioxidant activity, which explains why Run 8 exhibited the highest antioxidant activity among all formulations. In the ABTS assay, significant differences in antioxidant content were observed across formulations, with Run 8, containing the highest seaweed concentration, demonstrating the greatest antioxidant activity, consistent with findings from Díaz-Rubio et al. (2009). Table 3 shows that there were no significant differences in antioxidant parameters by the DPPH method ($p > 0.005$), as in the study by (Agregán et al., 2018)).

3.3. Colour parameters

Table 4 shows the values obtained from the colour analysis of the different Atlantic bonito burger formulations. Concerning the colour analysis, for the Luminosity (L) parameter, the run with the highest value was Run 1, 40.82 ± 2.86, and the run with the lowest value was Run 6, 28.19 ± 3.50, while the control formulation showed 53.21 ± 4.04.

For the a* parameter, which corresponds to the red/green coordinate, the formulation with the highest value, which means reddest, was Run 3, 1.29 ± 0.96, and the formulation with the lowest value, which means greenest, was Run 8, 4.22 ± 0.85, and the control formulation

Table 4
Results of the colour characterisation (L, a*, b*) of the Atlantic bonito burgers (average ± standard deviation). Means within the same column with different superscripts are significantly different at $p < 0.05$.

Run	L	a*	b*
Control	53.21 ± 4.04^{a}	10.67 ± 2.09^{a}	37.11 ± 1.63^{a}
1	40.82 ± 2.86^{b}	−1.57 ± 1.50^{ce}	24.24 ± 1.50^{b}
2	33.66 ± 2.48^{cde}	−2.49 ± 0.42^{ef}	17.85 ± 1.97^{e}
3	34.93 ± 2.37c^{d}	1.29 ± 0.96^{b}	21.67 ± 2.52^{cd}
4	30.60 ± 2.07ef	−2.14 ± 0.46^{def}	16.98 ± 2.06^{ef}
5	32.97 ± 2.41e	−0.540 ± 1.66^{c}	19.37 ± 3.09^{de}
6	28.19 ± 3.50^{f}	−2.60 ± 0.72^{ef}	14.09 ± 2.08 g
7	31.59 ± 3.50^{def}	−3.38 ± 0.68^{fg}	14.63 ± 2.19^{fg}
8	31.30 ± 3.20ef	−4.22 ± 0.85^{g}	13.73 ± 1.79^{g}
9	36.46 ± 2.90c	−0.97 ± 0.56^{cd}	21.87 ± 1.84^{bc}

obtained 10.67 ± 2.09, since the algae that promote the greenish colour were not added.

For parameter b*, which corresponds to the yellow/blue coordinate, the highest value, which means the most yellowish, is observed in Run 1, 24.24 ± 1.50. Run 8 shows a lower value, which means it is more bluish, 13.73 ± 1.79, for the control formulation the value obtained was 37.11 ± 1.63.

As shown in Table 4, the luminosity (L) parameter decreased with higher seaweed incorporation (R6, R7, R8), aligning with the findings of Barkallah et al. (2019), who reported similar results in Spirulina-fortified samples, where the darkest colours were associated with higher Spirulina content. Additionally, significantly lower a and b values were observed in the more enriched burgers, indicating a shift from a 'yellow' hue toward a greener colour. These results are consistent with (Senthil, Mamat ha & Mahadevaswamy, 2005), who also found that adding Spirulina reduced luminosity and resulted in a darker product.

3.4. Texture characterisation

Table 5 shows the results obtained in the texture profile analysis (TPA) of the Atlantic bonito burger. For the hardness parameter, the run with the highest value was Run 8, 32.04 ± 2.86 N, and the run with the lowest value was Run 2, 12.00 ± 1.01 N, while the control formulation showed 16.99 ± 1.65 N. For the cohesiveness parameter, the formulation with the highest value was Run 2, 0.50 ± 0.03, and the formulation with the lowest value was Run 4, 0.35 ± 0.05, and for the control formulation obtained was 0.50 ± 0.0, although there are no statistically significant differences between samples. Regarding the adhesiveness parameter, the highest value was observed in Run 9, -0.80 ± 0.78 N s, while the formulation with the lowest value was Run 6, -0.21 ± 0.18 N s, whereas the control formulation had −0.52 ± 0.31 N s, although there were no significant differences between the values obtained.

For the gumminess parameter, the formulation with the highest value was Run 8, 15.63 ± 2.43, while the formulation with the lowest value was Run 3, 4.43 ± 1.28, although the control formulation present 8.42 ± 0.90.

From Tables 5 and it is possible to observe that the increase in the seaweed incorporated increased the hardness of the burger by 1.9 times and the increase in hydrocolloids consequently increased the gumminess by 1.9 times. Barkallah et al. (2019) obtained the same result through the addition of Spirulina, where the 1 g/100 g concentration led to a 1.6-fold increase in hardness. This can be attributed to differences in the composition of Spirulina-fortified burgers, which result in protein/fat/water ratios that are determining elements in the consistency of the gel.

Table 5
Results of the texture characterisation (Hardness, Cohesiveness, Adhesiveness and Gumminess) of the Atlantic bonito burgers (average ± standard deviation). Means within the same column with different superscripts are significantly different at $p < 0.05$.

Run	Hardness (N)	Cohesiveness	Adhesiveness (N·s)	Gumminess
Control	16.99 ± 1.65^{de}	0.50 ± 0.02	−0.52 ± 0.31	8.42 ± 0.90^{bc}
1	19.29 ± 1.30^{c}	0.49 ± 0.04	−0.72 ± 0.49	9.51 ± 1.10^{b}
2	27.34 ± 2.44^{b}	0.50 ± 0.03	−0.72 ± 0.63	13.77 ± 1.66^{a}
3	12.00 ± 1.01^{f}	0.37 ± 0.13	−0.32 ± 0.31	4.43 ± 1.28^{de}
4	14.47 ± 1.41^{ef}	0.35 ± 0.05	−0.29 ± 0.23	5.16 ± 0.10^{de}
5	15.66 ± 1.67^{de}	0.39 ± 0.08	−0.42 ± 0.40	6.09 ± 0.10^{cde}
6	12.36 ± 2.31^{ef}	0.40 ± 0.06	−0.21 ± 0.18	5.07 ± 1.69^{d}
7	18.51 ± 1.54^{cd}	0.44 ± 0.05	−0.39 ± 0.37	8.10 ± 1.12^{bd}
8	32.04 ± 2.86^{a}	0.49 ± 0.04	−0.78 ± 0.63	15.63 ± 2.53^{a}
9	18.10 ± 1.41^{cd}	0.49 ± 0.03	−0.80 ± 0.78	8.82 ± 5.69^{bcd}

3.5. Sensory evaluation

A quantitative descriptive analysis (ADQ®) was carried out with a panellist of semi-trained tasters in order to classify several attributes namely colour, visual uniformity on cutting, fish odour, seaweed odour, off-odour, hardness, adhesiveness, succulence, cohesiveness, fish flavour, garlic flavour, seaweed flavour, salted, sweet. Fig. 2 shows the values attributed by the panellists through the sensory analysis of the burgers.

Run 7 presented higher adhesiveness and cohesiveness, while Run 8 presented higher hardness and seaweed flavour. At the level of the colour attribute, the Run 6, 7, 8 and 9 presented higher values which corroborates the results of the analysis to the colour of the parameters L, a*, b*. All formulations presented a low fish odour, absence of off-odour and low seaweed odour.

Thus, the Runs 1 and 3 were the most well scored, this because they have a more natural characteristic to what we are used to consume daily. According to Ribeiro et al. (2022), the control formulation obtained high values of acceptability, which was expected and agrees with several authors. However, the second-best evaluations were given to formulations with higher seaweed and hydrocolloids incorporation, Runs 7 and 8. The incorporation of seaweed, Spirulina, and *Fucus Vesiculosus* aimed to achieve a salty flavour, with Run 8 being the closest formulation to this goal.

3.6. Optimization of antioxidant content and texture parameters in fish burger

According to the experimental design, the results were studied by applying the Taguchi Method to optimize the formulation (soft texture and rich in antioxidant fish burger) according to improve the parameter antioxidants content (ABTS) and minimise the hardness parameter. Fig. 3 shows the optimal antioxidant content values of each factor

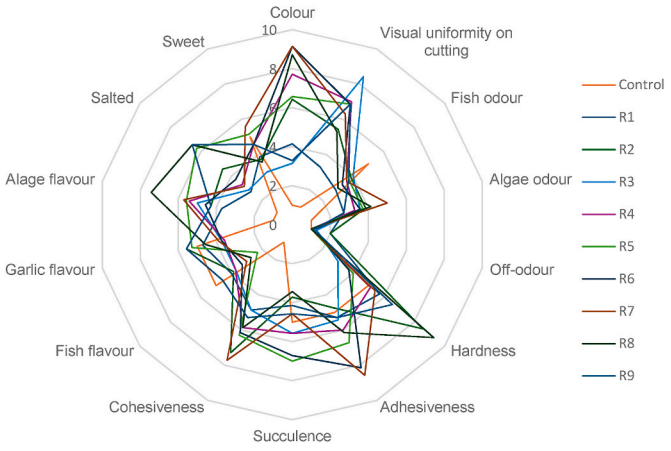

Fig. 2. Results of the sensory characterisation of the Atlantic bonito burgers. Composition of runs are shown in Table 1.

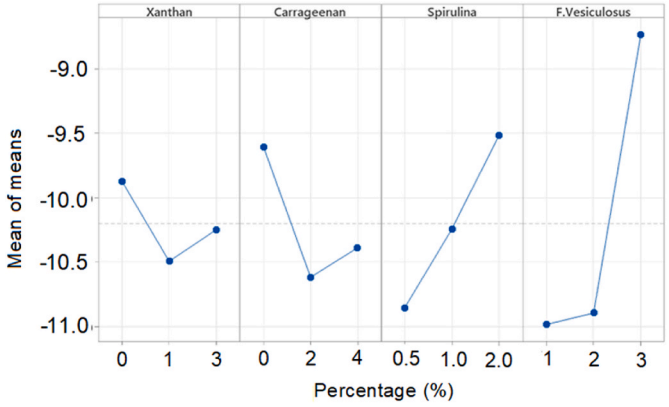

Fig. 3. Individual effects of concentration of xanthan, carrageenan, spirulina and *Fucus vesiculosus* on antioxidant (ABTS) influence at different levels.

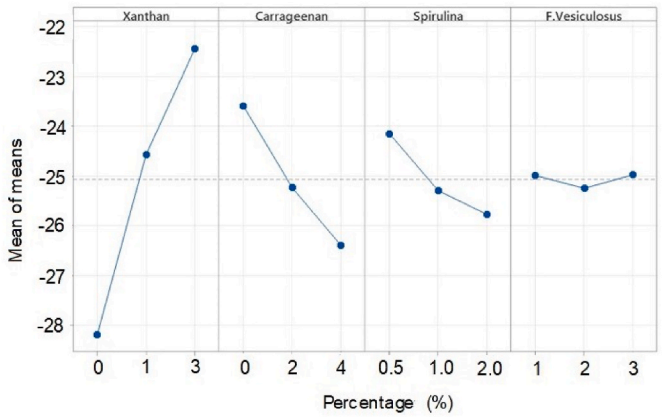

Fig. 4. Individual effects of concentration of Xanthan, Carrageenan, Spirulina and Fucus vesiculosus on hardness influence at different levels.

(Spirulina, *Fucus vesiculosus*, xanthan and carrageenan) at three different levels.

Supplemental Table S1 provides information on the relative importance of each factor, which means that the most significant values for the influence on the antioxidant capacity of the burgers can be identified. The individual contribution of each factor is represented by the %P, this value represents the relative influence that each factor has on the process, so the higher its percentage, the higher its influence on the product.

According to the results presented in Supplemental Table S1, the factors with the highest influence on the antioxidant capacity of the fish burgers were the seaweed *Fucus vesiculosos* and Spirulina. On the other hand, the hydrocolloids, xanthan and carrageenan, are the factors that have the lowest influence on the antioxidant capacity of the burgers.

Regarding the hardness parameter, Fig. 4 shows the hardness values for each factor (Spirulina, *Fucus vesiculosus*, xanthan and carrageenan) at three different levels. According to Supplemental Table S2, the factors that most influence the hardness of the burgers are the hydrocolloids carrageenan and xanthan. With the models obtained, it is possible to identify the optimal conditions for maximizing or minimizing a parameter that is important in the result of the characteristics of the product under study. The results showed that the optimal conditions for antioxidants are as following: xanthan at a level of 0 g/100 g with a contribution of 9.871%; carrageenan at a level of 0 g/100 g with a contribution of 9.601%; spirulina at a level of 2 g/100 g with a contribution of 9.515%; *Fucus vesiculosus* at a level of 3 g/100 g with a contribution of 8.735%.

The optimal conditions for hardness are as following: at a level of 3 g/100 g, *Fucus vesiculosus* with a contribution of the highest at 24.970%, followed by spirulina at 2 g/100 g, which contributes 24.144%. Carrageenan at 0 g/100 g provides a contribution of 23.586%, while xanthan at 3 g/100 g contributes 22.441%.

4. Conclusion

This study successfully developed an Atlantic bonito burger enriched with seaweed, such as Spirulina and *Fucus vesiculosus*, leading to increased antioxidant activity in the burger. According to the Taguchi experimental design it was concluded that the optimal burger formulation for maximizing antioxidant activity while minimizing hardness should contain 3 g/100 g xanthan, 0 g/100 g carrageenan, 2 g/100 g Spirulina and 3 g/100 g *Fucus vesiculosus, enhancing* several nutritional and functional properties of the Atlantic bonito burger. Specifically, compared to the control, the Run 8 formulation resulted in 1.5 times increase in fibre content, 2.8 times increase in protein content, 1.2 times increase in antioxidant activity (ABTS), and 3.4 times increase in total phenolic content (TPC). However, no statistically significant differences were observed in moisture levels or antioxidant activity measured by the DPPH assay.

Regarding the texture, the results revealed that higher seaweed concentrations led to increased burger hardness. Nevertheless, cohesiveness and adhesiveness and gumminess were not significantly affected by the type or concentration of hydrocolloids used. While formulations Run 1 and 3 received the highest sensory scores due to their more familiar and natural taste profiles, formulations with greater incorporation of seaweed and hydrocolloids, specifically Run 7 and 8, were also highly rated. This suggests that consumers are open to, and may prefer, burgers with enhanced nutritional profiles. Seaweed was the most influential factor in determining both the antioxidant content and the hardness of the burgers.

CRediT authorship contribution statement

Joana Solinho: Writing – original draft, Investigation. **Sofia Gonçalves:** Writing – review & editing, Methodology. **Sofia Machado:** Writing – review & editing, Formal analysis. **Ricardo Pereira-Pinto:** Writing – review & editing, Conceptualization. **Manuel Vázquez:** Writing – review & editing, Conceptualization. **Rita Pinheiro:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Ethical statement

During the execution of the research, appropriate protocols were used to protect the rights and privacy of all panellists, without coercion to participate, and including full disclosure of the requirements and absence of risk of the study, the written consent and non-disclosure of your data without your knowledge, and the ability to withdraw from the study at any time.

Funding

This research was funded by Iceland Liechtenstein Norway EEA grants. Blue Growth Programme. Call2 – Business, Development, Innovation and SMEs; to UIDB/05937/2020 and UIDP/05937/2020 – CISAS funded by national funds, through FCT – Fundacao para a Ciencia e a Tecnologia.

Declaration of competing interest

The authors declared that they had no conflict of interests with respect to their authorship or the publication of this article.

Acknowledgements

The authors thank: To Blue Project, Bioeconomy, People, Sustainability, Health (PT-INNOVATION-0105).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.117247.

Data availability

Data will be made available on request.

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