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Sustainable Methods and Nutritional Evaluation of High Protein Fish Meats via Kjeldahl and Sensory Analyses: A Case Study in Southwest El Salvador

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ABSTRACT

This study investigates the nutritional composition, chemical analytical methodologies, and functional potential of three underutilized aquatic species ― thornback ray (*Raja clavata* Linnaeus, 1758), eel (*Synbranchus marmoratus* Bloch, 1795), and tilapia (*Oreochromis mossambicus*). These species are explored as sustainable, high protein ingredients for the formulation of functional burger-type products designed to mitigate protein deficiency in vulnerable populations. All species are non-endangered and suitable for cultivation in controlled aquaculture systems, supporting environmentally responsible production. Three formulations were developed and subjected to proximate analysis, including moisture (oven drying), crude fat (Soxtec extraction), and total protein (Micro-Kjeldahl), alongside a sensory evaluation using a 9-point hedonic scale. Formulation A demonstrated the most favorable nutritional profile, exhibiting higher protein content (15.6 g/100 g) and substantially lower fat levels (18.4 g/100 g) compared to conventional beef patties (12.8 g/100 g protein; 29.1 g/100 g fat). Its elevated moisture content (60.5 g/100 g) contributed to improved juiciness, though potentially at the expense of shelf stability. Sensory results indicated strong consumer acceptance, with Formulation A achieving an average score of 8.06, closely approximating the beef control (8.37). Panelists expressed high preference for color and texture, while odor and flavor acceptance were moderate but positive, reflecting minimal fishy notes and a balanced flavor profile. These findings highlight the viability of seafood-based burgers derived from non-threatened species as nutritionally enhanced and sensorially acceptable alternatives to traditional meat products. Their adoption could strengthen nutritional security while promoting the valorization of underutilized hydro-biological resources. Future investigations should examine raw-material availability and develop robust preservation strategies to ensure feasibility and scalability for commercial production.

INTRODUCTION

The latest guidelines from the World Health Organization (WHO) emphasize the importance of reducing energy intake from fat and lowering saturated fat content in processed foods **(World Health Organization, 2014)**. Over the past decade, a persistent socio-gastronomic phenomenon has emerged within Salvadoran society, evolving into a generational legacy and contributing to notable changes in dietary patterns. Nevertheless, obesity remains a critical issue because it induces significant metabolic and endocrine alterations that may lead to severe health consequences **(Buttar *et al*., 2005; Dawes, 2014; Ruiz-Cano *et al*., 2015; Włodarczyk & Śliżewska, 2021)**. This raises the fundamental question of what truly underlies such unhealthy eating behaviors. This article addresses that concern by examining common practices and habits, including inadequate dietary behaviors and major nutritional challenges within the population **(Kraak *et al*., 2005; Riet *et al*., 2011; Roberto, 2020)**.

This phenomenon has had social, economic, and demographic impacts. One effective way to address this problem is to improve the quality of menus offered by so-called family restaurants and to enact stringent food regulations. Such regulations should include clear nutritional guidelines for the public and ensure that family eateries and fast-food establishments properly adhere to them. In addition, more than 30% of the global population, particularly in developing countries, consumes fish and other aquatic products, which provide at least 20% of protein intake **(Mohan Dey *et al*., 2005; Béné *et al*., 2015; Mohanty *et al*., 2019)**.

This research presents an innovative methodological framework characterized by four significant contributions to sustainable seafood product development. First, it proposes an unprecedented combination of three rarely utilized aquatic species — thornback ray (*Raja clavata*), eel (*Synbranchus marmoratus* B.), and tilapia (*Oreochromis mossambicus*), into a balanced seafood-based snack burger formulation. This multispecies approach contrasts with much of the existing literature, which has conventionally focused on single-species resources, isolated processing methods, or market-oriented analyses of consumption trends **(Eltholth *et al*., 2015)**. By integrating species with distinct biochemical and technological characteristics, the study establishes a comprehensive system for assessing synergistic nutritional improvements, functional properties, and processing efficacy. Thus, it expands the conceptual framework of multispecies valorization and provides empirical evidence supporting its viability as a novel approach for diversifying protein supplies in areas facing nutritional and resource limitations.

Second, the research employs advanced formulation optimization methods to determine the most effective ratios among the selected aquatic species, ensuring a scientifically balanced integration of protein yield, textural integrity, and sensory appeal. This optimization process is grounded in quantitative analysis and statistical modeling, allowing the identification of formulations that maximize nutritional value while preserving desirable organoleptic properties. By systematically adjusting component proportions, the study enhances the protein density and functional quality of the product while ensuring palatability and consumer acceptability. This methodological rigor provides a dual contribution: it validates the product as nutritionally superior to conventional alternatives and establishes a replicable framework for future food innovation aimed at addressing dietary deficiencies and promoting sustainable protein diversification.

Third, the uniqueness of this research is strengthened by a dual comparative framework that rigorously analyzes both proximate composition, including protein, fat, moisture, and ash content, and sensory or organoleptic qualities in direct comparison with traditional beef burgers. This scientific framework combines quantitative bromatological analyses with qualitative, consumer-focused assessments, generating a dataset that captures both nutritional value and sensory acceptance. The proximate analysis provides objective biochemical evidence of the nutritional superiority of Formulation A, supported by statistical assessment using ANOVA and non-parametric tests that corroborate consumer preferences. By integrating these two analytical dimensions, the study establishes a robust basis for evaluating seafood-based alternatives, demonstrating their capacity to diversify protein sources and their potential for broader consumer adoption. This integrated strategy strengthens the scientific discussion on functional food innovation by linking biochemical composition with sensory perception, thereby positioning mixed-species seafood burgers as a feasible and sustainable substitute for conventional beef products.

Finally, the study is situated within the context of El Salvador, where seafood consumption has declined significantly **(Guillén Henríquez *et al*., 1999; Ventura Santos *et al*., 2011)**. This trend highlights the potential to develop alternative production systems and sustainable agro-industrial initiatives that simultaneously promote conservation and the responsible use of marine resources. By contextualizing the research within a regional framework, this study emphasizes opportunities for diversifying protein sources, promoting sustainable aquaculture, and addressing nutritional challenges in a population vulnerable to diet-related health disorders.

The findings confirmed significant differences among the three formulations. Different proportions of ray, eel, and tilapia meat were processed into high-protein, hamburger-type seafood products and compared with traditional beef burgers. Sensory and organoleptic evaluations were subsequently conducted using untrained panelists. In addition, bromatological comparisons between the seafood snack burgers and traditional beef burgers were performed. The main objective of this research is to assess consumer preferences for hamburger-type seafood snacks and to propose a new by-product based on the combination of these seafood species as an innovative nutritional snack burger formulation.

Furthermore, according to Agreement No. 74 of the Law of Environment and Natural Resources of the Government of El Salvador, issued in 2009 and updated in 2015, none of the marine species used in this research are officially listed as threatened or endangered **(Naturales, 2009)**. However, to maintain environmental sustainability for these species, fishing overexploitation must be prevented **(Bizzarro *et al*., 2007; Cunningham *et al*., 2022)**. More importantly, production in controlled habitats or environments through sustainable aquaculture and marine production should be promoted to avoid habitat degradation.

MATERIALS AND METHODS

1.Study Area

A reconnaissance of the fishing sector in the region of El Salvador was carried out. It was taken into consideration the mapping carried out through the Fisheries and Aquaculture Development Center (CENDEPESCA), the Ministry of Agriculture El Salvador, and the Japan International Cooperation Agency (JICA), where they describe the most important zones and areas for the fishing sector in El Salvador, as well as other relevant information such as geography, limits, borders, and main aquifer and marine resources. Below, we present the fishing sector in the region of El Salvador (Fig. 1).

Fig. 1. The fishing industry in the El Salvadoran region. This map provides a comprehensive depiction of the key fishing sectors, geographical features, borders, principal aquifer resources, and limits in El Salvador.

There are 36 main zones (beach, coast, port, and island) where fishing activity, tourism, and recreation are carried out in El Salvador. The zones are listed below as follows:

1) Bola del Monte, 2) Garita Palmera, 3) Barra de Santiago, 4) Blue Coast, 5) Metalio, 6) Acajutla Port, 7) Los Cobanos, 8) Barra Salada, 9) Metayo, 10) El Sunzal, 11) El Majahual, 12) Los Filtros, 13) La Libertad Port, 14) Pimental, 15) San Marcelino, 16) Los Blancos, 17) San Luis La Herradura, 18) Tasajera Island, 19) Méndez Island, 20) El Triunfo Port, 21) Pirrayita Island, 22) Parada Port, 23) El Cuco, 24) Torola Beach, 25) Maculiz, 26) El Jaguey, 27) El Tamarindo, 28) Playitas, 29) Zacatillo Island, 30) La Union, 31) El Guisquil, 32) Chapernal, 33) Conchaguita Island, 34) Meanguera Island, 35) El Zope, 36) Avalos Port.

However, for this case study, the port of La Libertad was taken into consideration, where the samples of marine species were extracted, because it is a prominent hub for both tourism and industrial fishing. The purpose of this correlational study was to determine the relationship between the nutritional value, sensory and organoleptic evaluation of seafood snacks (hamburger type), the nutritional value as determined by analytical methods, and the acceptance of the snacks by untrained panelists.

La Libertad is a city and port located in the southwestern region of El Salvador. La Libertad's strategic position to the south of San Salvador, combined with its open roadstead port, facilitated its growth in the 19th century as a major shipping hub for "balsam" - a type of balsam extracted from El Salvador's coastal forests **(McKinley, 1947; Valadez González, 2007)** In the early 20th century, La Libertad served as one of the nation's largest ports. It continues to serve as a significant fishing port. Agriculture, encompassing livestock husbandry, sugarcane cultivation, and cotton production, along with the presence of beach resort amenities, serve to supplement fishing as a significant economic endeavor. The estimated population of the urban area in 2017 was 1.778 million.

Hence, the following species were utilized in the production of the seafood snacks (hamburger type): tilapia (*Oreochromis mossambicus*), eel (*Synbranchus marmoratus* Bloch, 1795) and thornback ray (*Raja clavata* Linnaeus 1758) (Fig. 2)

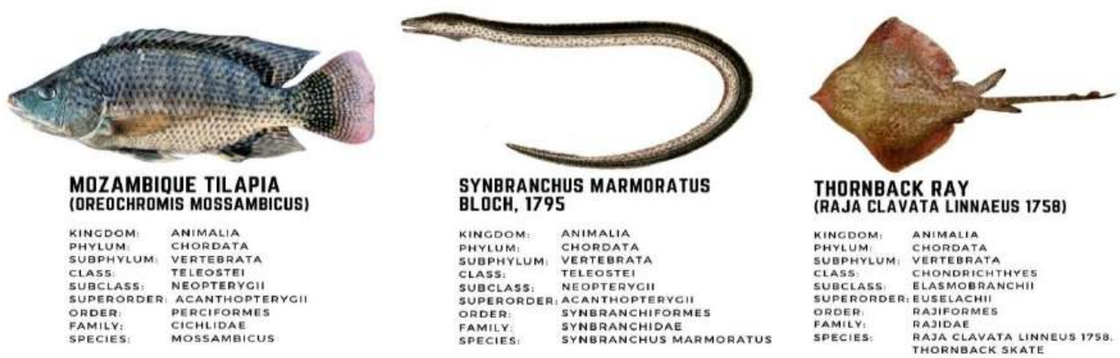

Fig. 2. Taxonomic classification of the species: tilapia (*Oreochromis mossambicus*), eel (*Synbranchus marmoratus* Bloch, 1795) and thornback ray (*Raja clavata* Linnaeus 1758) utilized in the production of seafood snacks (hamburger type).

According to a study conducted in 2020, the thornback ray, *Raja clavata*, is among the most abundant skate species in the Mediterranean **(Carbonara *et al*., 2020)**. The procedure began with the collection of bibliographic information related to the acquisition of the selected marine species. Furthermore, an analysis was conducted to evaluate the nutritional characteristics of these species. An assessment was also performed to determine whether the selected marine species are threatened with extinction, thereby supporting the objective of preserving marine biodiversity and its components.

Approximately 480 skate and ray species are categorized into 56 genera and 17 families. Nearly 230 of these species belong to the family Rajidae. The Central Eastern Pacific region contains 42 known species, organized into 11 families and 20 genera. Some of these species are frequently encountered as bycatch in other fisheries and are regarded as important components of associated fauna.

Furthermore, the marbled swamp eel, *Synbranchus marmoratus* Bloch, 1795, is a common and resilient freshwater fish species in tropical Latin America. It is often consumed by humans because of its high protein content and adaptability to various aquatic habitats. This species has a cylindrical and elongated body, rudimentary dorsal and anal fins, and lacks paired fins, which are characteristic features of the family Synbranchidae. Its ecological versatility allows it to inhabit both clear and muddy waters, as well as temporary wetlands, where it can endure seasonal desiccation by burrowing into the substrate. Classified as Least Concern by the IUCN, *S. marmoratus* is not considered endangered and maintains stable populations throughout Central America, including Panama and El Salvador. Its facultative air-breathing physiology and protogynous hermaphroditism provide adaptive advantages for colonization and reproduction under variable environmental conditions. Owing to its nutritional value and ecological prevalence, *S. marmoratus* represents a viable option for sustainable aquaculture and functional food innovation in tropical regions **(Kullander & America, 2003; Reis, 2003)**.

2. Kjeldahl Technique for Protein Chemical Characterization

This investigation involved bromatological analyses performed in triplicate, n = 3, representing independent analytical replicates derived from the same batch of the selected formulation. Each replicate was individually processed and analyzed to ensure methodological reliability and reproducibility of the chemical determinations. The sensory evaluation included 20 untrained panelists, n = 20, whose responses were treated as independent observations for statistical analysis. The replication values therefore refer to analytical replicates, not separate production batches, as the study aimed to evaluate the chemical and sensory performance of a single optimized formulation rather than manufacturing variability.

Only the selected formulation, Formulation A, underwent chemical characterization, as the study aimed to provide a viable prototype for the development of seafood-based snack burgers. This focus enabled resources to be allocated to the most promising formulation identified through sensory preference testing. The authors recognize this limitation and recommend that future studies include concurrent bromatological analyses of all formulations to improve comparative interpretation and strengthen the generalizability of the results.

Organic nitrogen analysis is one of the most widely used techniques for protein determination. In this method, proteins and other organic food components are digested using sulfuric acid and catalysts. Total organic nitrogen is converted into ammonium sulfate, $(NH_4)_2SO_4$. The digested mixture is then distilled after neutralization with a base, and the distillate is collected in boric acid. The nitrogen concentration of the sample is determined by titrating the borate anions with standardized HCl or H_2SO_4 **(International, 1995)**. This method provides a reliable estimate of crude protein content, although nitrogen may also originate from non-protein components. Currently, copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, is commonly used as the main catalyst.

Resources and Equipment

The equipment used included an analytical balance with a sensitivity of 0.1 mg, Kjeldahl equipment, a thermal mantle, a pH meter, and standard laboratory equipment.

Chemical Substances

The chemical substances used included concentrated sulfuric acid, H_2SO_4; potassium sulfate, K_2SO_4, or sodium sulfate, Na_2SO_4; 15% sodium hydroxide solution, NaOH; and 0.1 N sulfuric acid solution.

Kjeldahl Determination Protocol

The homogenized sample was first prepared for analysis. Approximately 1 g of the homogenized sample was weighed to the nearest 0.1 mg and transferred into a digestion flask.

Catalysts and acid were then added to the flask. Three glass beads, 10 g of potassium sulfate, K_2SO_4, or sodium sulfate, Na_2SO_4, 0.5 g of cupric sulfate, $CuSO_4$, and 20 mL of concentrated sulfuric acid, H_2SO_4, were added.

The digestion flask was connected to an absorption trap containing 250 mL of 15% sodium hydroxide, NaOH. The mixture was heated for 15–20 min until a clear solution was obtained. Stearic acid, $C_{18}H_{36}O_2$, or a few drops of silicone antifoam were added to reduce foaming, and the temperature was gradually increased.

After cooling, 200 mL of distilled water was added to the digested solution. The flask was then placed in the distillation apparatus, and 100 mL of 30% NaOH was slowly added through the funnel. The stopcock was secured before distillation.

At least 150 mL of distillate was collected with the condenser tube submerged. For Option A, 50 mL of 0.1 N H_2SO_4, 4–5 drops of methyl red, and 50 mL of distilled water were used, and the excess acid was back-titrated with 0.1 N NaOH to a yellow endpoint. For Option B, 50 mL of 3% boric acid, H_3BO_3, was used, and titration was performed with 0.1 N HCl to pH 4.6, verified using a calibrated pH meter or Tashiro's indicator (Fig. 3).

Fig. 3. Experimental setup for nitrogen quantification via the Kjeldahl Method.

Calculation:

$$\%\mathrm{N} = \frac{14 \text{ x N x V x } 100}{\text{m x } 100}$$

$$\%\text{ Proteins} = \frac{14 \text{ x N x V x } 100 \text{ x factor}}{\text{m x } 100}$$

Where:

V: represents the milliliters of H_2SO_4, NaOH, or HCl used;

M: indicates the mass of the material, measured in grams;

Factor: 6.25 for meat, fish, eggs, legumes, and proteins in general; 5.7 for cereals and soy derivatives; 6.38 for milk; 5.55 for gelatin; and 5.95 for rice.

The method's accuracy: The discrepancy between the outcomes of two consecutive measurements conducted by the same analyst must not surpass 0.06% for nitrogen or 0.38% for protein.

3. Soxhlet Technique for Lipid Content Determination

A previously homogenized, dried, and weighed portion of the food sample was subjected to extraction using petroleum ether, ethyl ether free from peroxides, or a mixture of both solvents. Complete extraction of free lipid matter was then performed using Soxhlet equipment **(Thompson & Merola, 1993)**.

Resources and Equipment

The equipment used included a Soxhlet Extraction apparatus, precision analytical balance, filter paper or cellulose thimble, thermoregulated bath, air oven set at 103 ± 2 °C, 1 mm mesh sieve, heating mantle or rotary evaporator, and standard laboratory equipment.

Chemical Substances

The chemical substances used included ethyl ether with a boiling point range of 40–60 °C and petroleum ether with a boiling range of 40–60 °C.

Soxhlet Determination Protocol

For samples with high moisture content, the sample was first homogenized and dried at 103 ± 2 °C in an air oven, with adjustments made according to sample type. Approximately 1 g of homogenized sample was dried under these conditions.

The dried material was then pulverized and passed through a 1 mm mesh sieve to obtain a uniform powder. Between 2 and 5 g of the prepared sample were accurately weighed in duplicate and transferred into the extraction thimble or onto pre-weighed filter paper coated with degreased cotton. The initial sample mass was recorded as **m**.

The extraction flask was dried for 30 min at 103 ± 2 °C, cooled, and weighed. The tare mass of the empty flask was recorded as $\mathbf{m_1}$. The extraction flask was then connected to the Soxhlet apparatus, the thimble was positioned inside the extraction tube, and the solvent was added to the flask.

The sample was extracted with solvent for 6–8 h, maintaining a condensation rate of 3–6 drops per second. After extraction, the solvent was removed by evaporation using a rotary evaporator or a water bath under a fume hood until the absence of ether odor was confirmed.

The flask containing the extracted fat was dried in an oven at 103 ± 2 °C for 10 min, cooled in a desiccator, and weighed. The final mass was recorded as $\mathbf{m_2}$.

4. Calculation and Expression of Results

Lipid content was calculated using the following equation:

$$\textbf{Lipid content (\%)} = [(m_2 - m_1) / m] \times 100$$

Where, m is the weight of the sample, m_1 is the tare weight of the empty flask, and m_2 is the weight of the flask containing the extracted fat.

The study emphasized the potential nutritional value of meat derived from selected marine species for hamburger production. Raw materials obtained from marine species were collected and systematically prepared for processing and production of the final agro-industrial product **(Lópeza, Sachdeva & Calderónb)**.

A sensory evaluation using a nine-point hedonic scale was conducted to obtain both qualitative and quantitative data at the Faculty of Agriculture and Agricultural Research, Dr. José Matías Delgado University. The evaluation included a panel of non-expert judges who independently assessed the tested formulations. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) within a randomized complete block design (RCBD), with formulations, samples A, B, and C, considered as treatments and panelists as blocks to account for inter-individual variability. This methodological approach enabled the identification of significant differences among formulations and determination of the most preferred sample based on sensory acceptance **(Montgomery, 2017)**.

By-product process

The bromatological analysis was conducted at the Quality Laboratory of the Faculty of Agronomy and Agricultural Research, Dr. José Matías Delgado University, using samples of a beef burger and a burger prepared from ray, eel, and tilapia meat, which received the highest approval from the panelists. Several studies have examined the protein quality of skate meat; however, specific practical data remain limited **(Li *et al*., 2017)**. This bromatological analysis therefore provides useful data for future researchers interested in reassessing the nutritional content of the final product.

The analysis involved three determinations: moisture percentage, determined by oven drying; crude fat percentage, determined using the Soxtec Method; and total protein percentage, determined using the Micro-Kjeldahl Method. These determinations formed part of a proximate analysis protocol.

The research was conducted using an experimental approach, with the aim of developing a functional food that could help address nutritional deficiencies. Functional foods are developed in response to the increase in certain diseases associated with modern lifestyles and are considered a valuable option for improving nutrition and public health **(Valenzuela & Valenzuela, 2015)**. Thus, three formulations were selected based on the results of sensory analysis, and the most preferred formulation was chosen for further bromatological investigation to assess whether it achieved the intended objectives.

The study participants consisted of staff and students from Dr. José Matías Delgado University, specifically from the Faculty of Agricultural Research, "Julia Hill de O'Sullivan." The sensory evaluation panel consisted of 20 individuals of both genders, including 40% female participants, n = 8, and 60% male participants, n = 12. This balanced composition ensured gender representation, thereby enhancing the reliability of the sensory data and reducing potential bias in the evaluation process. The production process comprised approximately 15 distinct stages, beginning with raw material reception and ending with packaging. These stages are comprehensively described in the Results and Discussion section (Fig. 4).

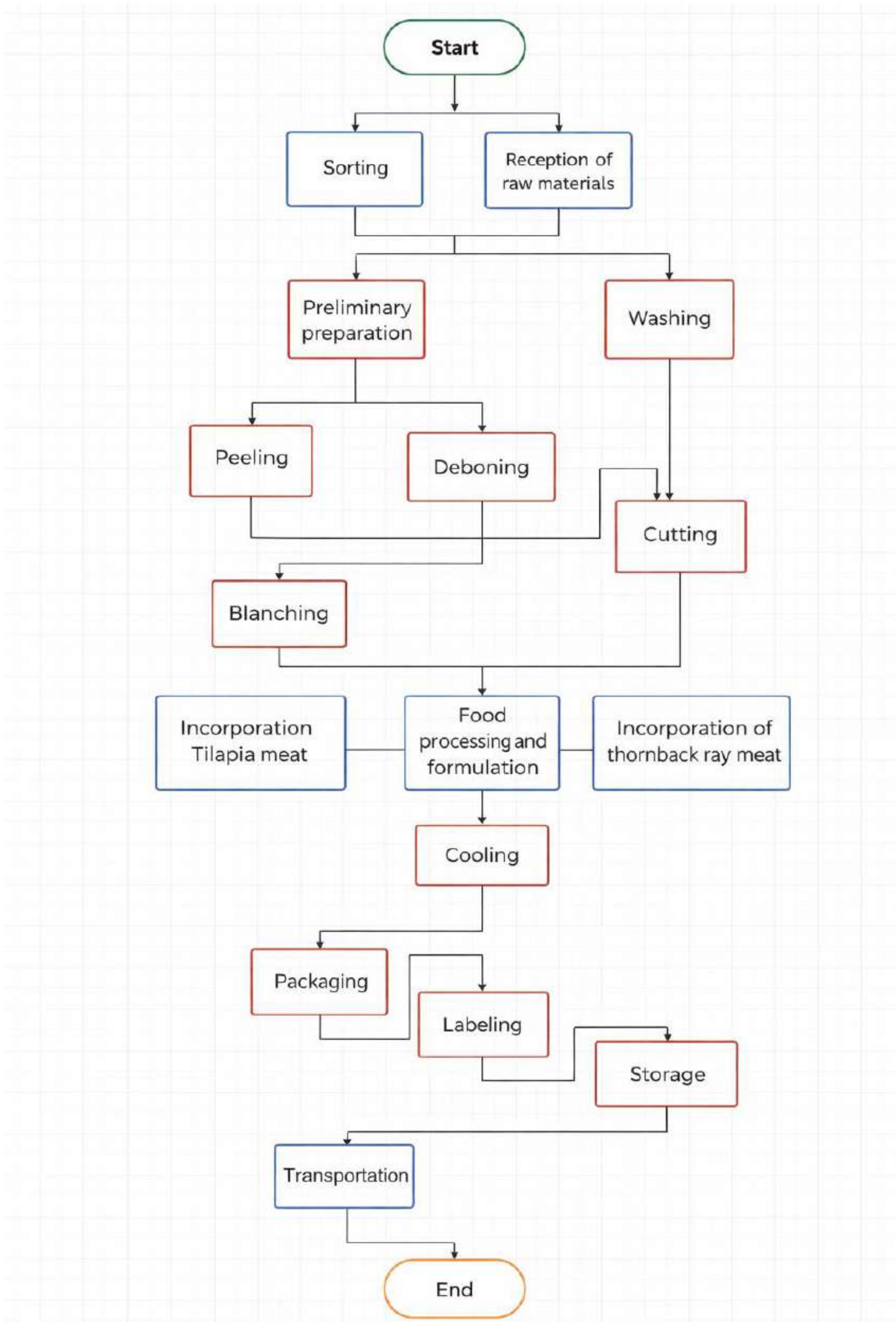

Fig. 4. Diagram illustrating the sequential steps involved in the manufacturing process of seafood snacks.

Moreover, a bromatological analysis was conducted on the winning sample to further ascertain its moisture content, ash content, and protein content. A sensory analysis was conducted at the mobile sensorial analysis laboratory, situated in the Faculty of Agriculture and Research Julia Hill of O'Sullivan. The analysis involved 20 judges, and their opinions were recorded and tabulated. Subsequently, the Analysis of Variance was performed to identify any significant differences between the two samples. Ultimately, the sample's product underwent bromatological and sensory analysis at the Industrial Quality Control Center (CCCI) in San Salvador. The investigation of the results involved the utilization of statistical techniques such as Analysis of Variance (ANOVA), Friedman Test, correction factor, and econometric functions. Below, a comprehensive description of these models/tests is illustrated.

5. Scaling Parameter (Multiplier)

The formula for calculating the coefficient of friction (CF) is CF = T2/N.

The sum of squares for a sample is calculated using the sum of squares formula, denoted as SSF.

The sum of the squares of the moments of forces (Σ MA) 2+ (Σ MB) 2+ (Σ MC) is equal to SSF. 2 - FCn

The root mean square of the sample values was calculated. Mean squares were obtained as follows: Mean squares = Sum of squares between groups / the corresponding degrees of freedom.

6. Sample Variability Ratio

The variability ratio, represented as $\mathbf{F_m}$, was calculated by dividing the mean square of the sample ($\mathbf{MS_{sample}}$) by the mean square error ($\mathbf{MS_{error}}$). This ratio served as the primary test statistic in the Analysis of Variance (ANOVA), enabling evaluation of whether the observed differences among sample means were statistically significant or due to random variation. The process provided the basis for hypothesis testing, in which the null and alternative hypotheses were defined as follows:

Assumptions

H_0 (null hypothesis): Sample A demonstrates superior performance compared with samples B and C across all assessed metrics.

H_1 (alternative hypothesis 1): Sample B demonstrates statistically significant improvement compared with Sample A across all assessed parameters.

H_2 (alternative hypothesis 2): Sample C exceeds samples A and B across all assessed parameters, demonstrating superior overall performance.

The hypotheses were evaluated using the computed $\mathbf{F_m}$ value, which was compared with the critical value from the F-distribution at a specified significance level, $\boldsymbol{\alpha}$. Rejection of $\mathbf{H_0}$ indicated that at least one sample mean differed significantly, confirming the presence of genuine variability among formulations.

Meat Preparation Process

The three types of meat were combined and finely minced. For ray, the fins constitute the edible portion, accounting for approximately 55% of the fish; in other words, about 55 g of every 100 g of fish are consumed. Ray is a white fish with few bones and low fat content; however, purchasing it already cleaned is the most efficient way to prepare it for consumption.

During handling, strict care was taken to avoid bacterial contamination, particularly by *Escherichia coli* O157:H7, which can contaminate minced meat and cause foodborne illness. Spices, including oregano and cumin, were added to the meat mixture before final cooking at 90 °C.

The fish meat mixture contains certain B-complex vitamins, including vitamin B_1, thiamine; vitamin B_2, riboflavin; and vitamin B_3, niacin. These vitamins are essential for converting food into energy and supporting healthy skin, muscles, and nerves. Small amounts of vitamin A and retinol are also present.

RESULTS

This section describes and gives detailed explanation of the results obtained from the sustainable alternative, technical and food processing evaluation. Distinct processes were involved, such as those used for the production and characterization of raw materials, the equipment, and machinery used. It is very important to emphasize that the highest result obtained from the sensory analysis was the “Formulation A”.

1. The formulation

A total of three distinct formulations were developed, each of which featured a distinct variation in the proportion of eel, ray, and tilapia meat in that formulation. When compared to the other formulations, the formula "A" that is presented below is the one that demonstrates the greatest statistically significant difference; consequently, it is the formulation that is most widely accepted by the panel of judges. A sensory evaluation was performed on each formulation, and the formulation that was deemed to have the greatest significance was identified based on the results of the obtained test (Table 1).

Table 1. The development of an alternative meat made from ray, eel and tilapia.

Food Component	Percentage
Ray meat	14.4
Eel meat	7.2
Tilapia meat	14.4
Fat	24
Water	25.2

Protein	8.21
Salt	1
Egg	5
Oregano	0.05
Pepper	0.1
Cumin	0.03
Dehydrated garlic	0.3
Dehydrated onion	0.1
Liquid smoke	0.1
Total	100

A comparative analysis of the proximate composition of burger formulations was performed, revealing that the proximate composition of Formulation A (eel, tilapia, ray) exhibits a protein content of 15.6%. This is superior to certain beef-based formulations (10.16% as noted by **Ali and El-Anany** (**2025**) and comparable to the lower range of standardized beef patties (18–23% USDA), although it remains below the higher values reported for pangas fish burgers (20–22%). The lipid content (18.4%) is significantly lower than that of normal beef patties (8–22%), although it remains substantially higher than fish-based alternatives (4–8%), so categorizing it as an intermediate choice regarding fat reduction. The moisture level of 60.5% is comparable to beef patties (60–65%) but is lower than that of fish burgers (68–75%), potentially affecting juiciness and shelf-life stability. The prevalence of unsaturated fatty acids offers a distinct benefit for cardiovascular health; nevertheless, the lack of ash data limits the assessment of mineral benefits. In summary, Formulation A exhibits superior protein density and lipid quality compared to beef, although displays deficiencies in fat reduction and moisture retention when contrasted with fish-based formulations. Opportunities for nutritional enhancement encompass optimizing species ratios to increase protein content, integrating lean fish or plant fibers to decrease fat, augmenting moisture with natural humectants, and enriching with mineral dense ingredients to get a more balanced and competitive profile (Table 2).

Table 2. Comparative analysis of the proximate composition of burger formulations.

Study / Source	Product Type	Protein (%)	Fat/lipids (%)	Moisture (%)	Ash (%)	Notes
Formulation A (eel, tilapia, ray)	Seafood snack burger	**15.6**	**18.4**	**60.5**	n/a	Higher protein than beef; markedly lower fat; predominance of unsaturated fatty acids; juiciness enhanced by moisture.

Husein *et al.* (2019), Enhanced utilisation of nonmarketable fish: physical, nutritional and sensory properties of 'clean label'fish burgers. *Int. J. Food Sci. & Tech*) (Husein *et al.*, 2019)	Fish burgers (Sea bass, gilthead sea bream, and rainbow trout)	13.62–11.57 (Raw) 16.4–13.73 (Cooked)	4.58–4.07 (Raw) 4.68–4.02 (Cooked)	73.44–72.42 (Raw) 68.08–66.95 (Cooked)	1.93–1.79 (Raw) 2.52–2.29 (Cooked)	Clean label formulations; rich in essential fatty acids.
Ali, R. F., & El-Anany, A. M. (2025). Nutritional and qualitative characteristics of beef patties incorporated with hydrated coffee cherry pulp powder. npj *Science of Food, 9(1), 203*. (Ali & El-Anany, 2025)	Coffee derived by products / beef burger	10.16 ± 0.82	1.72 ± 0.13	8.33 ± 0.68	6.97 ± 0.40	Beef burger formulated with alternative coffee cherry by-products (pulp, mucilage, husk, silverskin), traditionally considered waste materials yet recognized for their nutritional and functional potential.
Mondol *et al.* (2025), A Comparative Assessment of Nutritional and Microbial Quality between Value Added Fish Burgers and Traditional Meat Based Fast Foods.*Microbiology Research Journal Int.*) (Mondol *et al.*, 2025)	Thai Pangas fish burgers vs beef & chicken	Fish: 20–22	4–5	72–75	1.5–2.0	Fish burgers significantly higher protein than beef/chicken.
USDA Food Data Central	Beef patties	18 g – 23 g	8 g –22 g	60–65	1.0–1.2	Standardized proximate values for beef burgers.

2. Results of the Bromatological Analysis

Formulation A, consisting of ray meat (*Raja clavata*), eel (*Synbranchus marmoratus* Bloch, 1795), and tilapia (*Oreochromis mossambicus*), was subjected to sensory comparison with a conventional beef burger. Both samples were evaluated through sensory analysis and bromatological analysis, and the results are presented in Table (3).

The seafood burger sample, prepared from fish, eel, and ray meat, contained **15.6 g protein/100 g**, **18.4 g fat/100 g**, and **60.5 g moisture/100 g**. In contrast, the conventional beef burger contained **12.8 g protein/100 g**, **29.1 g fat/100 g**, and **52.1 g moisture/100 g**. Therefore, the seafood burger contained **2.8 g/100 g more protein** and **10.7 g/100 g less fat** than the beef burger.

In theory, unsaturated fatty acids are more prevalent in fish-based burgers, providing greater nutritional value than saturated fatty acids, which are more commonly associated with beef products. The seafood burger also contained **8.4 g/100 g more moisture** than the beef burger.

The fish burger formulation represents an innovative contribution to meeting the nutritional needs of the population. Formulation A can be considered a food product with high nutritional value in terms of macronutrients, particularly protein and fat, and it may also provide a good supply of B-complex vitamins. These vitamins are important because they contribute to energy metabolism and help reduce fatigue. Therefore, this formulation offers a promising and innovative response to the population's health and nutritional needs.

Overall, the product represents a commercially valuable contribution by promoting the use of hydrobiological resources that are generally underutilized or less appreciated by consumers.

Table 3. Determination of nitrogen content in organic and inorganic compounds and bromatological analysis.

Analysis	Result	Equivalent copy	Technique/Method
Total fat	18.4g ± 0.5g / 100g	2	Soxhlet Extraction
Humidity	60.5 ± 0.1g / 100g	3	Gravimetric Method
Protein	15.6g ± 0.3g / 100g	2	Macro Kjeldahl

3. Findings from Sensory Analysis

In the sensory evaluation, 50% of the judges accepted the formula "A" based on the appearance of the meat, although only 10% indicated a moderate appreciation for it. Regarding color, the majority of judges, specifically 75%, expressed a strong preference for it. In the odor category, the sample received a 40% acceptance rate, considerably satisfying the judges. Regarding flavor, 30% of the judges expressed a strong preference for it, while only 10% had a slight dislike for it. Half of the evaluating judges expressed a strong preference for the sample texture, while the remaining half had a moderate liking for it.

Formulation "A" was considered the most widely accepted by the judges due to its balanced mixture of meat, protein, and water proportions. This formulation demonstrated superior consistency, flavor, and appearance compared to formulation "B," which contained 10% more meat, and formulation "C," which contained 10% more protein. Further, both products received positive feedback from the judges during the evaluation. It was discovered that the distinct fishy smell in these hamburgers was either barely noticeable or completely absent (Fig. 5).

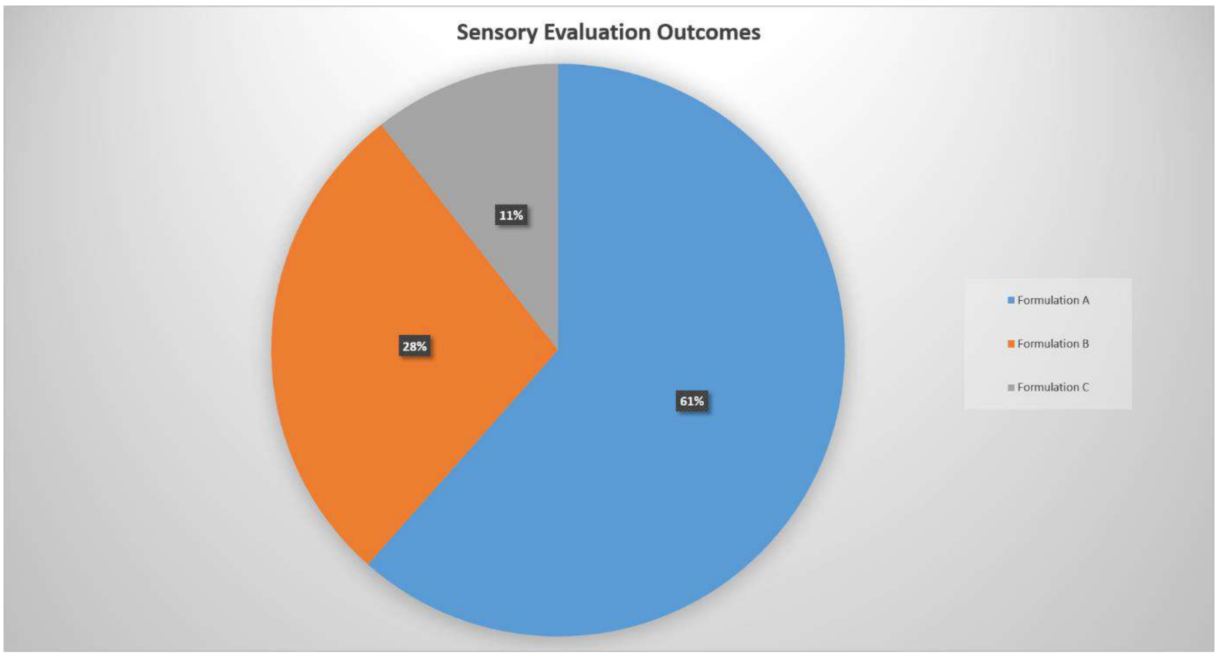

Fig. 5. Sensory evaluation outcomes.

The bromatological analysis discussion was conducted by comparing the beef burger with the fish, eel and ray meat hamburger, yielding the subsequent results:

a. The relative protein content of the fish, eel, and ray meat patty in comparison to the meat patty guarantees a greater protein content, thereby enhancing the nutritional value of the fish meat patty.

b. The fish meat patty is classified as a low-fat patty due to its comparatively lower total fat content in relation to the beef patty.

c. The meat patty contains a lower percentage of moisture than the fish, eel, and ray meat patty; this characteristic consequently prolongs the product's shelf life.

A limitation has been detected in this study. tThe raw material is difficult to obtain, and ray meat has a low level of commercialization in El Salvador. Despite the fact that fish, eel, and ray meat were more nutritious than beef, there is a limitation.

1. ANOVA Calculations Assessment

Table 4. ANOVA calculation assessment including judges' scores across samples.

Judge	Sample A	Sample B	Sample C	Total
1	7.8	8.4	7.6	23.8
2	8.6	8.6	8	25.2
3	8	8.4	8.8	25.2
4	9	7.8	7.4	24.2
5	8	8.6	7.4	24
6	8	7	7.6	22.6
7	8.4	8	8.4	24.8
8	8.8	8	8	24.8
9	8	7.8	6.8	22.6
10	7.8	8	7.6	23.4
11	8	8	7.6	23.6
12	8.6	8	8	24.6
13	8.4	9	8	25.4
14	8.8	7	7.6	23.4
15	8.8	7.4	7	23.2
16	9	8	7	24
17	8.4	7.6	7.4	23.4
18	8.4	7	6.2	21.6
19	8.6	7	6.8	22.4
20	8	7.6	7.4	23
Total	167.4	157.2	150.6	475.2
Mean	8.37	7.86	7.53	-

Calculation of the Correction Factor

$$Fc = \frac{(\Sigma G)^{2}}{k * n}$$

$$Fc = \frac{(475.2)^{2}}{3 * 20}$$

$$Fc = 3{,}763.584$$

Calculation of the sample-level sum of squares

$$SSs = \left[\frac{(\Sigma M1)^{2} + (\Sigma M2)^{2} + (\Sigma M3)^{2}}{n}\right] - Fc$$

$$SSs = \left[\frac{(167.4)^{2} + (157.2)^{2} + (150.6)^{2}}{20}\right] - 3{,}763.584$$

$$SSs = 7.164$$

Degrees of Freedom for Samples

$$df = number\ of\ samples - 1$$

$$df = 3 - 1 = 2$$

Sum of Squares for Judges

$$SS_{Judges} = \left[\frac{(0)^2 + (0_1)^2 + (0_2)^2 + \cdots + (0_n)^2}{3}\right] - Fc$$

$$SS_{Judges} = \left[\frac{(24)^2 + (22.6)^2 + (23.4)^2 + (25.2)^2 + \cdots +}{3}\right] - 3{,}763.584$$

$$SS_{Judges} = 6.8426$$

Degrees of Freedom for Judges

$$df = number\ of\ judges - 1$$

$$df = 20 - 1 = 2$$

$$df = 19$$

Quantification of the Total Sum of Squares

$$SSt = (O)2 + (O1)2 + \cdots + (On)2) - Fc$$

SSt = (7.42 + 7.62 + 5.42 + 7.02 + 6.62 + 8.62 + 9.02 + 7.82 + 7.22 + 8.22 + 7.62 + 8.02 + 7.42 + ⋯ +) – 3,763.584

SSt = 3,786.88 − 3763.584

SSt = 23.296

Calculation of the Total Degrees of Freedom

$$df_{total} = number\ of\ observation - 1$$

$$df_{total} = 60 - 1$$

$$df_{total} = 59$$

Error Sum of Squares

$SSe = SSt - SSj - SSs$

SSe = 23.296 − 6.842 − 7.164

SSe = 9.289

Degrees of Freedom for Error

$$df_{error} = df_{total} - df_{Judges} - df_{samples}$$

$$df_{error} = 59 - 19 - 2$$

$$df_{error} = 38$$

Calculation of the Mean Square for $SS_{samples}$

$$MS_{samples} = \frac{SS_{samples}}{df_{samples}}$$

$$MS_{samples} = \frac{7.164}{2}$$

$$MS_{samples} = 3.5820$$

Calculation of the Mean Square for SS_{judges}

$$MS_{Judges} = \frac{SS_{Judges}}{df_{Judges}}$$

$$MS_{Judges} = \frac{6.842}{19}$$

$$MS_{Judges} = 0.36014$$

Calculation of the Mean Square for SS_{error}

$$MS_{error} = \frac{SS_{error}}{df_{error}}$$

$$MS_{error} = \frac{9.289}{38}$$

$$MS_{error} = 0.24446$$

Sample Variability Ratio

$$F = \frac{MS_{samples}}{MS_{error}}$$

$$F = \frac{3.5820}{0.24446}$$

$$F = 14.65$$

Judge Variability Ratio

$$F = \frac{MS_{Judges}}{MS_{error}}$$

$$F = \frac{0.36014}{0.24446}$$

$$F = 1.47$$

Table 5. Summarized tabulated data of the ANOVA results.

Results					
Source of Variation	df	SS	MS	F observed	F critical
Total (T)	59	23.30	-	-	-
Samples (S)	2	7.16	3.58	14.65	3.245
Judges (J)	19	6.84	0.36	1.47	1.867
Error (E)	38	9.29	0.24	-	-

2. Interpretation

The computed value of F is 14.65. The critical value of the F-distribution at the 0.05 significance level, using the appropriate degrees of freedom for the sample and error terms ($v_1 = 2$ and $v_2 = 38$), is derived from the F-table, resulting in $F_{(critical)} = 3.245$.

The criterion for decision making is outlined as follows:

If F observed exceeds F critical, the null hypothesis is rejected.

If F observed < F critical, the null hypothesis is accepted.

Considering the observed value $F = 14.65$ above the crucial threshold of 3.245, the null hypothesis is statistically rejected. This signifies that at least one group mean is significantly different from the others. Overall, the ANOVA confirms that Sample A was significantly preferred over samples B and C, validating the sensory superiority suggested by the descriptive statistics. These findings support the conclusion that Sample A exhibits a more favorable sensory profile and is the most promising formulation for further development.

To reinforce, validate, and further analyze the behavior of samples A, B, and C, the hedonic test results were subjected to a two factor ANOVA without replication. The outcomes of this analysis are presented in the following tables.

Table 6. Analysis of Variance (ANOVA two-factor, no replication) comparing samples A, B and C.

ANOVA (samples A & B).

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Rows	3.771	19	0.198474	0.671116	0.80374	2.168252
Columns	2.601	1	2.601	8.794981	0.007943	4.38075
Error	5.619	19	0.295737			
Total	11.991	39				

ANOVA (samples A & C).

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Rows	4.26	19	0.224211	0.779649	0.70358	2.168252
Columns	7.056	1	7.056	24.53587	8.82E-05	4.38075
Error	5.464	19	0.287579			
Total	16.78	39				

ANOVA (samples B & C).

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Rows	10.299	19	0.542053	3.612417	0.003717	2.168252
Columns	1.089	1	1.089	7.257454	0.014375	4.38075
Error	2.851	19	0.150053			
Total	14.239	39				

The hedonic evaluation revealed clear and statistically significant differences among the three formulations. Sample A obtained the highest mean acceptability score

(8.37), followed by Sample B (7.86) and Sample C (7.53). The two-factor ANOVA without replication confirmed that these differences were statistically meaningful. For the comparison between Sample A and Sample B, the sample effect was significant ($F = 8.79$, $p = 0.0079$), indicating higher preference for Sample A. Similarly, the comparison between Sample A and Sample C showed a highly significant sample effect ($F = 24.54$, $p < 0.001$), again demonstrating superior acceptability of Sample A. In the comparison between Sample B and Sample C, the sample effect remained significant ($F = 7.26$, $p = 0.0144$), confirming that Sample B was preferred over Sample C. Judge effects were non-significant in the A–B ($F = 0.67$, $p = 0.804$) and A–C ($F = 0.78$, $p = 0.704$) comparisons, indicating consistent scoring behavior across panelists, whereas a significant judge effect was observed in the B & C comparison ($F = 3.61$, $p = 0.0037$), reflecting greater inter-panelist variability for this pair. Overall, the results establish a clear preference hierarchy of A > B > C, with Sample A exhibiting the highest hedonic acceptance across the sensory panel.

3. SPSS Calculations Assessment

Further, the statistical analyses of samples A, B, and C were performed utilizing IBM SPSS Statistics, version 27.0.1.0 (64-bit edition). This software program was utilized in order to conduct descriptive statistics, inferential tests, and comparative analyses, assuring methodological consistency and repeatability of findings.

Table 7. Descriptive Statistics of Hedonic Evaluation for samples A, B, and C.

Within-Subjects Factors					
Measure: MEASURE_1		Descriptive Statistics			
Sample	Dependent Variable		Mean	Std. Deviation	N
1	Sample A	Sample A	8,370	,3962	20
2	Sample B	Sample B	7,860	,5807	20
3	Sample C	Sample C	7,540	,5986	20

Multivariate Tests

Table 8. Multivariate tests of sample effect.

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncentral. Parameter	Observed Power
Sample	Pillai's Trace	,562	11,571b	2,000	18,000	,001	,562	23,142	,983
	Wilks' Lambda	,438	11,571b	2,000	18,000	,001	,562	23,142	,983
	Hotelling's Trace	1,286	11,571b	2,000	18,000	,001	,562	23,142	,983
	Roy's Largest Root	1,286	11,571b	2,000	18,000	,001	,562	23,142	,983

a. Design: Intercept;

Within Subjects Design: Sample;

b. Exact statistics;

c. Computed using alpha = .05.

Table 9. Mauchly's Test of Sphericity for sample effect.

Mauchly's Test of Sphericity							
Measure: MEASURE_1							
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	Df	Sig.	Epsilonb		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Sample	,848	2,971	2	,226	,868	,948	,500

Mauchly's Test of Sphericity tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept

Within Subjects Design: Sample

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Table 10. Tests of within subject effects with sphericity corrections (sample factor).

Measure: MEASURE_1									
Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncentral Parameter	Observed Power
Sample	Sphericity Assumed	7,009	2	3,505	14,222	,000	,428	28,445	,998
	Greenhouse-Geisser	7,009	1,736	4,038	14,222	,000	,428	24,688	,994
	Huynh-Feldt	7,009	1,895	3,699	14,222	,000	,428	26,953	,997
	Lower-bound	7,009	1,000	7,009	14,222	,001	,428	14,222	,947
Error (Sample)	Sphericity Assumed	9,364	38	,246					
	Greenhouse-Geisser	9,364	32,982	,284					
	Huynh-Feldt	9,364	36,008	,260					
	Lower-bound	9,364	19,000	,493					

a. Computed using alpha = .05.

Table 11. Tests of within-subjects contrasts (linear and quadratic) for sample factor.

Tests of Within-Subjects Contrasts									
Measure: MEASURE_1									
Source	Sample	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncentral Parameter	Observed Power
Sample	Linear	6,889	1	6,889	23,495	,000	,553	23,495	,996
	Quadratic	,120	1	,120	,603	,447	,031	,603	,114
Error (Sample)	Linear	5,571	19	,293					
	Quadratic	3,793	19	,200					

a. Computed using alpha = .05.

Tests of Between-Subjects Effects

Table 12. Tests of between-subjects effects (linear and quadratic) for sample factor.

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncentral Parameter	Observed Power
Intercept	3,766,753	1	3,766,753	10,472,388	,000	,998	10,472,388	1,000
Error	6,834	19	,360					

a. Computed using alpha = .05.

The hedonic assessment indicated statistically significant disparities among the three formulations, with Sample A exhibiting the highest acceptability (M = 8.37), succeeded by Sample B (M = 7.86) and Sample C (M = 7.54). Multivariate analyses validated a significant sample effect (Wilks' Λ = 0.438, F = 11.57, $p = 0.001$, $\eta^2 = 0.562$), corroborated by substantial observed power. Mauchly's test confirmed that the assumption of sphericity was satisfied ($p = 0.226$), hence verifying the ANOVA findings. The within subject's analysis indicated a significant effect of sample ($F = 14.22$, $p < 0.001$, $\eta^2 = 0.428$), while contrast tests showed a robust linear trend ($F = 23.50$, $p < 0.001$, $\eta^2 = 0.553$), confirming a gradual decrease in hedonic scores from A to B to C, with no notable quadratic component. The between subjects' effects further validated the model's robustness (F = 10,472.39, $p < 0.001$, $\eta^2 = 0.998$). These findings delineate a distinct preference hierarchy of Sample A > Sample B > Sample C, with robust statistical validation across multivariate, univariate, and comparison experiments.

DISCUSSION

The results obtained from the evaluation of the three formulations highlight the potential of fish-based alternatives as sustainable and nutritionally valuable products. Among the tested formulations, Formulation A demonstrated the most significant differences in both bromatological and sensory analyses, positioning it as the most promising candidate for further development.

From a nutritional perspective, Formulation A showed higher protein content, 15.6 g/100 g, than the conventional beef burger, 12.8 g/100 g, while simultaneously presenting markedly lower fat content, 18.4 g/100 g compared with 29.1 g/100 g. This reduction in fat, together with the predominance of unsaturated fatty acids in fish, eel, and ray meat, underscores the health advantages of the product. In addition, the higher moisture content, 60.5 g/100 g, contributed to juiciness and palatability, although it may reduce shelf life relative to beef patties.

Comparative assessment of proximate composition revealed that Formulation A, prepared from eel, tilapia, and ray, had a protein content of 15.6%, exceeding some beef-based formulations, 10.16%, as reported by **Ali and El-Anany (2025)**, and approaching the lower USDA threshold for beef patties, 18–23%. However, it remained lower than that reported for pangasius fish burgers, 20–22%. The lipid content, 18.4%, was lower than that of regular beef patties but substantially higher than that of standard fish-based formulations, 4–8%, categorizing it as an intermediate-fat product. The moisture content, 60.5%, was comparable to that of beef patties but lower than that of fish burgers, 68–75%, which may affect juiciness and shelf life. The predominance of unsaturated fatty acids provides a nutritional advantage, while the absence of ash data limits mineral assessment. Overall, Formulation A showed superior protein and lipid quality compared with beef but still requires improvement in fat reduction and moisture retention compared with fish-based alternatives.

The hedonic evaluation showed significant differences among the formulations, with a clear preference hierarchy of A > B > C. Sample A achieved the highest acceptability, and two-factor ANOVA without replication confirmed significant sample effects in all pairwise comparisons: A–B, $\boldsymbol{F = 8.79, p = 0.0079}$; A–C, $\boldsymbol{F = 24.54, p < 0.001}$; and B–C, $\boldsymbol{F = 7.26, p = 0.0144}$. Judge effects were non-significant for A–B and A–C but significant for B–C, indicating greater variability among panelists in that comparison.

The sensory evaluation reinforced these findings, with judges expressing strong preferences for the color, 75%, and texture, 50%, of Formulation A. Although odor acceptance was moderate, 40%, the absence or minimal presence of a distinct fishy smell was noted as a positive attribute. Flavor acceptance was also favorable, with 30% of judges strongly preferring the sample. Overall, the balanced composition of eel, ray, and tilapia meat contributed to superior consistency, flavor, and appearance compared with the other formulations.

Despite these promising outcomes, certain limitations must be acknowledged. The availability of raw materials, particularly ray meat, remains a challenge because of its low level of commercialization in El Salvador. This constraint may hinder large-scale production and market penetration. Furthermore, the higher moisture content, although beneficial for sensory attributes, could create challenges for storage and distribution, requiring further technological interventions to extend shelf life.

In summary, Formulation A represents a nutritionally superior and sensorially acceptable alternative to conventional beef burgers. Its development contributes to the valorization of underutilized hydrobiological resources, offering a sustainable and innovative solution to meet the nutritional needs of the population. However, future research should address raw material supply chains and preservation techniques to ensure the feasibility of commercial application.

CONCLUSION

This study assessed the nutritional and sensory characteristics of three alternative protein sources, thornback ray, *Raja clavata* Linnaeus, 1758, eel, *Synbranchus marmoratus* Bloch, 1795, and tilapia, *Oreochromis mossambicus*, integrated into a functional burger formulation designed to mitigate nutritional deficiencies. The comparative analysis revealed that fish-based formulations are sustainable and nutritionally superior alternatives to traditional beef burgers. Among the evaluated formulations, Formulation A proved to be the most promising, demonstrating exceptional bromatological and sensory attributes.

Formulation A exhibited higher protein content, 15.6 g/100 g, than beef, 12.8 g/100 g, and significantly lower fat content, 18.4 g/100 g compared with 29.1 g/100 g, complemented by a beneficial unsaturated fatty acid profile. The moisture content, 60.5 g/100 g, improved juiciness and palatability, although it may compromise shelf stability. Compared with similar fish-based products, Formulation A showed moderate fat content and slightly lower moisture levels, suggesting potential for further improvement.

The sensory evaluation established a distinct preference hierarchy, A > B > C. Formulation A had the greatest acceptability, with significant sample effects identified in all pairwise ANOVA comparisons: A–B, $\boldsymbol{F = 8.79, p = 0.0079}$; A–C, $\boldsymbol{F = 24.54, p < 0.001}$; and B–C, $\boldsymbol{F = 7.26, p = 0.0144}$. Panelists expressed a clear preference for its color, 75%, and texture, 50%, while moderate odor acceptance, 40%, and favorable flavor perception, 30% strong preference, further supported its sensory quality. These findings establish Formulation A as a nutritionally superior and sensorially accepted alternative that enhances the value of underutilized hydrobiological resources.

The study confirms the viability of cultivating these species through sustainable aquaculture systems, as none are classified as endangered and all remain ecologically available. Evidence suggests that *Raja clavata*, despite continued exploitation, sustains stable populations below the maximum sustainable yield, with broad distribution and relative abundance in the northeastern Atlantic supporting its classification as Near Threatened rather than Endangered **(Serra-Pereira *et al.*, 2011; Ferragut-Perelló *et al.*, 2023)**. These measures not only improve nutritional security in food-insecure areas but also support the conservation of wild marine populations and the protection of coastal ecosystems.

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