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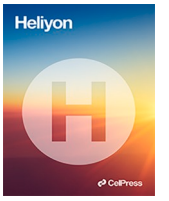

Research article

Physicochemical and sensory characterization of raw tuna muscle for plant-based fish analogs development purposes

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A B S T R A C T

Plant-based fish analogs are in trend for their potential to reduce overfishing and for providing enriched dietary options. Tuna is popular for raw food applications, especially the bluefin species group (*Thunnus thynnus, T. orientalis* and *T. maccoyii) and* yellowfin (*T. albacares*). The objective was to characterize the raw muscle of these species, identifying the most important traits and providing reference values for the development of plant-based analogs. Key factors to mimic in raw fish analogs include achieving a protein content of 25 %, emphasizing omega-3 DHA, and incorporating essential micronutrients. Visual attributes, such as color, translucency, and macrostructural aspects must be meticulously replicated. Additionally, capturing the subtle, delicate taste and aroma and replicating instrumental texture measurements are crucial yet challenging. This study highlights the importance of balancing nutrition, taste, texture, and visual appeal together to achieve consumer satisfaction, provides reference values, and establishes a pathway for developing plant-based raw tuna analogs.

1. Introduction

The consumption of fresh tuna has been traditionally popular in Japan and has been gaining popularity in other parts of the world, such as the US, Europe and Australia [1]. This global trend is a result of increasing consumer concern about healthy eating and the gain of popularity of international culinary experiences [2]. Global fish consumption has increased annually by 3.1 %, outpacing the average annual population growth rate of 1.6 % from 1961 to 2017 [3]. Specifically, tuna and tuna-like species are one of the most caught categories of fish, reaching 5 million tons in 2020 [4]. Because of the economic impact of overfishing, and the potential endangerment of tuna, it is important to provide other options to fish consumers that alleviates the strain on the tuna populations. For this reason, characterizing tuna muscle as a food product is the first step to develop desirable plant-based fish analogs.

There are seven major commercial tuna species: albacore (*Thunnus alalunga*), bigeye (*Thunnus obesus*), Atlantic bluefin (*Thunnus thynnus*), Pacific bluefin (*Thunnus orientalis*), southern bluefin (*Thunnus maccoyii*), yellowfin (*Thunnus albacares*), and skipjack (*Katsuwonus pelamis*). Skipjack (57 %) and yellowfin (30 %) are the most caught tuna species, followed by bigeye (8 %), albacore (4 %) and the bluefins (constituting the three bluefin species) (1 %) [4]. As shown in Table 1, the commercial uses of tuna depend on the fish size. Typically, the small species (skipjack and albacore) are used for canning purposes and the larger species are used for fresh or frozen applications. Bluefin species (Atlantic, Pacific, and southern) are most valuable due to their dark red muscle and fat content and are typically used for raw applications, such as sushi or sashimi. Bigeye and yellowfin tuna are less fatty but both are also widespread in the fresh fish industry due to their lower cost [5,6].

Bluefins (Atlantic, Pacific, and southern) and yellowfin tuna are the most vulnerable categories of tuna because they are larger, in

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Table 1
Major commercial tuna species and their characteristics.

Common name	Scientific name	Location	Common length (cm)	Common weight (kg)	Maturity age (years)	Common age (years)	Commercial use
Albacore	*Thunnus alalunga*	Temperate and tropical waters of all oceans	40-100 (130 max)	–	–	–	Canned, steaks
Bigeye	*Thunnus obesus*	Subtropical and tropical areas of the Atlantic (but not in the Mediterranean), Indian and Pacific Oceans	40-180 (230 max)	1.4–130 (210 max)	3–4	(17 max)	Steaks, sushi
Atlantic Bluefin	*Thunnus thynnus*	Subtropical and temperate waters of the Atlantic Ocean and the Mediterranean and Black seas	80-200 (458 max)	145-300 (679 max)	4–14	25+ (35 max)	Sushi
Pacific Bluefin	*Thunnus orientalis*	North Pacific Ocean — from East Asia to the North American West Coast — and with a more limited presence in the southern Hemisphere	120-210 (300 max)	100-260 (540 max)	3–5	15+ (20 max)	Sushi
Southern Bluefin	*Thunnus maccoyii*	Southern Hemisphere in the temperate and cold waters of the Atlantic, Indian and Pacific Oceans	160 (245 max)	180 (260 max)	8–12	14+ (40 max)	Sushi
Skipjack	*Katsuwonus pelamis*	Tropical areas of the Atlantic, Indian and Pacific Oceans	40-80 (108 max)	(33 max)	1–1.5	(6–10 max)	Canned
Yellowfin	*Thunnus albacares*	Subtropical and tropical areas of the Atlantic, Indian and Pacific Oceans	40-170 (205 max)	1.2–100 (194 max)	2–3	(18 max)	Canned, steaks, sushi

Source: ISSF (2022)

high demand, and are the species that take the longest to reach reproductive maturity (Table 1). One of the sustainable development goals (SDG) adopted by the United Nations in 2015 as part of the 2030 Agenda for Sustainable Development is to "Sustainably manage and protect marine and coastal ecosystems" [7]. This SDG addresses the protection of life undersea through enforcing effective management of fisheries and the monitoring of stocks.

The development of plant-based fish analogs would provide an ethical eating option for consumers who are concerned about the impacts of fishing and managing of farmed fish. Moreover, the widespread adoption of these analog product in the market could potentially reduce the pressure on the vulnerable tuna populations by offering an alternative solution.

In recent years, the plant-based food market has experienced substantial growth due to the increasing consumer inclination to reduce meat consumption, driven by environmental, ethical, and health concerns [8]. Specifically for fish, consumers' environmental concerns include overfishing, marine biodiversity reduction, and environmental destruction. The ethical concerns comprise animal rights protection and eating animals. From a health perspective, an increasing part of the population is giving up eating fish due to concerns related to fish diseases, the presence of microplastics, and elevated levels of heavy metals [9].

This trend has created significant business opportunities in the field [10]. Capitalizing on consumer familiarity, plant-based products that emulate their animal counterparts have gained popularity among consumers [11]. The size of the vegan food market was valued at USD 16.55 billion in 2022 and is forecasted to reach USD 37.45 billion by 2030, which will be an average annual growth rate (CAGR) of 10.6 % between 2022 and 2030 [12]. Another study from Allied Market Research shows that the global plant-based seafood market size was valued at $42.1 million in 2021, and is projected to reach $1.3 billion by 2031, growing at a CAGR of 42.3 % from 2022 to 2031 [13].

While plant-based fish and seafood alternatives currently make up a small portion of the broader plant-based meat and seafood category — currently comprising less than 1 % — there is notable progress on this front. In 2021, sales in the US for these products increased to USD 13.9 million, or 42 % since 2019 [14]. These figures show a significant effort to bridge the market gap with plant-based alternatives for fish and seafood.

Current plant-based alternatives are formulated using ingredients such as proteins, carbohydrates and lipids coming from botanical sources. The molecular, chemical, and physical properties of plant-derived ingredients diverge substantially from those of animal-derived ones. It is key to understand the properties of plant-derived ingredients and how they can be used to design plant-based products [15].

As the industry is focusing on developing plant-based products that mimic their animal counterparts, the principal challenge lies in simulating the animal reference appearance, texture, flavor, and mouthfeel. Most plant-based fish products currently available in the market have focused on imitating the sensorial properties of processed fish products, such as breaded fillets, slices, sticks, or burgers using technologies such as extrusion, 3D and 4D printing, electrospinning, among others [9,16]. There are some alternative products that mimic raw whole-cut fish, but not to the level of similarity that consumers expect.

All these attributes are intertwined. The visual perception determines the expectation of a food's taste and flavor; therefore, it is imperative to create a product that has a good appearance to attract consumers [17]. In fact, based on the properties of current analogs, there will be a need for focus on the nutritional value and the physical and technological properties of fish analogs [9].

To successfully develop plant-based analogs, it is key to have a comprehensive understanding of the physicochemical and sensory attributes of the fish species prevalent in the market. Until the recent boom of the plant-based food market, the scientific community had not focused extensively on evaluating the multifaceted aspects of tuna. Previous tuna literature predominantly concentrated on quality, freshness, nutritional aspects, shelf-life extension, as well as fishing and farming patterns. Plant-based literature so far mainly focuses on the ingredients and technologies used for developing these alternative products.

Consequently, this study aims to characterize the four of the seven tuna species, which constitute the two primary commercial groups of tuna used for raw applications: bluefin (Atlantic, Pacific, and southern species) and yellowfin. The objective is to identify and quantify the essential traits that need to be considered for the successful development of a plant-based raw tuna analog. Ultimately, this study seeks to provide a comprehensive methodology and reference values to develop a plant-based product that closely resembles its animal model in terms of both nutritional value and sensory characteristics (appearance, flavor, aroma, structure, and texture).

2. Materials and methods

2.1. Sample collection

Samples of a total of 64 specimens were collected. Samples consisted of approx. 500 g of bluefin tuna (*Thunnus thynnus, T. orientalis* and *T. maccoyii, n = 31*) and yellowfin tuna (*Thunnus albacares, n = 33)*. The samples were from a variety of different geographical origins (Table 2) and were sourced from the local fish pier (3 fishmongers) and Japanese supermarkets (2 stores) in San Francisco,

Table 2
Geographical origins of tuna samples.

Species		n	Origins
Bluefin	Pacific bluefin	15	Mexico and USA
	Atlantic bluefin	5	Spain
	Southern bluefin	11	New Zealand, Philippines and Australia
Yellowfin		33	USA, Mexico, Philippines, Australia, Tahiti and Marshall Islands

California, United States in June and July 2022. According to the sellers, the fish were wild-caught, and the specimens had been kept on ice since capture. The samples came from the dorsal ordinary muscles of tuna, ranging from the head to the tail.

After purchase, the samples were transported under refrigeration to the laboratory located in San Francisco. At the laboratory, the subcutaneous fat was removed from all samples and they were immediately processed (besides sensory as described in the section below).

2.2. Composition, pH and water activity

The pH was measured with a PH60S pH meter (Apera Instruments, LLC, Columbus, Ohio, USA) and the water activity measurements were made with an Aqualab 4 TE water activity meter (Meter, Pullman, Washington, USA).

Proximate analyses were performed in an external lab using the standard methods described by the Association of Official Analytical Chemists [18]. Moisture and ash contents were determined gravimetrically, after being at 103 °C for 24 h (#950.46) and by igniting the samples in a muffle furnace at 550 °C for 4 h (#923.03), respectively. Total protein content was estimated by the Kjeldhal method (#992.15, #992.23) using 6.25 as the nitrogen-to-protein conversion factor. The acid hydrolysis method followed by Soxhlet extraction with ether was used for the analysis of total fat (#922.06, #948.15). Carbohydrates were calculated as: $\%\ carobohydrates = 100 - (\%\ moisture + \%\ fat + \%\ protein + \%\ ash)$.

The fatty acid profile, the concentration of mineral components and the B vitamin contents were analyzed on representative pool samples from each group, bluefin and yellowfin. The fatty acid profile was obtained by the Hydrolytic Extraction Gas Chromatographic method (#996.06) and expressed as a percentage of the total fat. Minerals and metals, Ca, K, Fe, P, Zn, and Mg were determined by the Inductively Coupled Plasma Emission Spectroscopic method (#984.27, #990.08) and Hg was determined by the Inductively Coupled Plasma-Mass Spectrometric Method (#993.14). Vitamin B12 content was determined by a microbiological assay (#952.20, #986.23) and vitamin B2 by a Fluorometric method (#981.15, #942.23).

All determinations were performed in triplicate.

2.3. Macro and microstructure

The muscular structure of tuna is composed of myocommata (layers of connective tissue) and myotomes (bundles of muscle fibers). Calipers (01407A, Neiko, China) were used to measure the perpendicular distance between myocommata. These measurements were taken from two different types of tuna cuts: the transverse cut of the loin, and the sushi cut as shown in Fig. 1. Slices of approximately 1 mm thickness were taken from the tuna samples and histological observations were made using a digital microscope (VHX970N, Keyence, USA).

2.4. Color and opacity analysis

The L*, a*, b* color coordinates were assessed on sample portions (30 mm × 30 mm x 15 mm) at room temperature with a ColorFlex EZ Spectrophotometer (Hunterlab, Reston, Virgina, USA) calibrated with standard black and white plates, with the following configuration: D65 illuminant, 10° standard observer angle and an aperture of 25.4 mm. The cylindrical coordinates, h° (hue angle in the CIE Lab color wheel), and C* (chroma, relative saturation) were calculated as follows: $h^\circ = \arctan\left(\frac{b^*}{a^*}\right)$; $C^* = \sqrt{a^{*2} + b^{*2}}$.

The opacity was measured on 30 mm × 30 mm samples, at two different thicknesses, 5 mm, and 15 mm, using the same equipment as for color measurement. The opacity was calculated from reflectance measurements of the sample with a black and a white backing, as $Opacity = \frac{Y_{\ black\ backing}}{Y_{\ white\ backing}}\ x\ 100$, where Y is the tristimulus value Y (luminance, or visually perceived brightness).

All measurements were made in triplicate.

2.5. Texture profile analysis

A mechanical texture analyzer (TA.XT plus, Texture Technologies, Hamilton, MA, United States) fitted with a 50 kg load cell was

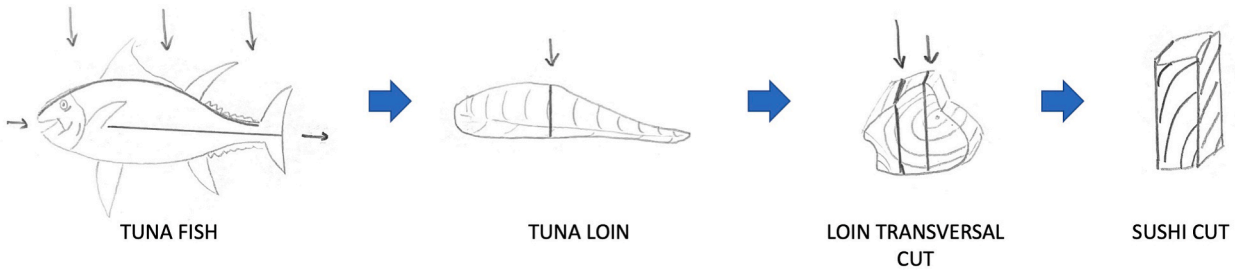

Fig. 1. Tuna cutting methodology.

used to perform a Texture Profile Analysis (TPA) test. Samples (15 mm cubes) were submitted to a two-cycle compression test to 75 % of their original height at room temperature (22–23 °C) using an aluminum cylindrical probe 76.2 mm diameter (TA-30). The parameter settings during the test were: trigger force 5 g, pre-test speed 1 mm/s, test and post-test speed 5 mm/s, time elapsed between cycles 5 s. The Texture Exponent Connect software (Stable Microsystems) was used to control the texture analyzer and to analyze the force-time deformation curves. The texture parameters considered were hardness (N), fracturability (N), adhesiveness (N·s), resilience (%), cohesiveness (%), springiness (%), and chewiness (N·s), as defined by Bourne [19]. TPA was performed with normal force perpendicular to the connective tissue layers.

All measurements were done in triplicate.

Table 3
Sensory attributes included in the descriptive profile of tuna meat with their description and evaluation procedure.

Attribute	Description
Appearance	
Color Intensity of Matrix	The intensity or strength of the color from light to dark
Shine	The degree to which light is reflected off the surface of the sample
Presence of Fibers	The presence of fibers in the sample (yes or no)
Aroma[a]	
Total Aroma Intensity	The total portion of aroma that is perceived by the sense of smell from a substance
Tuna Aroma	The slightly briny aromas associated with all species of tuna
Marine Aroma	The briny aroma associated with the ocean and ocean water
Protein Aroma	The aromas associated with whey, soy, pea, or other protein powders
Vegetative Aroma	The aromas associated with the components of green peas reminiscent of leaves, shoots, stalks, and other vegetative notes
Barnyard Aroma	The aromas associated with barn or barnyard: combination of manure, urine, moldy hay, feed, livestock odors.
Other Aromas	Other aromas that are not listed, to include description
Aromatics[a]	
Total Aromatics Intensity	The total portion of flavor that is perceived by the sense of smell from a substance in the mouth
Tuna	The slightly briny aromatics associated with all species of tuna
Fishy	The aromatics associated with fish and seafood not including tuna, may be reminiscent of oxidization
Vegetative	The aromas associated with the components of green peas reminiscent of leaves, shoots, stalks, and other vegetative notes
Metallic	(1) Aromatic associated with metals (tinny or iron), (2) a flat chemical factor
Other Aromas	Other aromatics that are not listed, to include description
Taste[a]	
Sweet	The taste on the tongue stimulated by sucrose, fructose, glucose, etc., and by other sweet substances, such as saccharin, aspartame and acesulfame-K
Sour	The taste on the tongue stimulated by acids, such as citric, malic, phosphoric acids, etc.
Salty	The taste on the tongue stimulated by sodium salts, such as sodium chloride and sodium glutamate, and in part by other salts, such as potassium chloride
Bitter	The taste on the tongue stimulated by substances such as quinine, caffeine, and hop bitters
Umami	Basic taste sensation stimulated by substances such as monosodium glutamate, and nucleotides
Aftertaste[b]	
Total Aftertaste Intensity	The total portion of aftertaste that is perceived by the sense of smell from a substance in the mouth
Tuna Aftertaste	The slightly briny aftertaste associated with all species of tuna
Fishy Aftertaste	The aftertaste associated with fish and seafood not including tuna, may be reminiscent of oxidization
Vegetative Aftertaste	The aftertaste associated with the components of green peas, such as pea protein and green pea aftertaste
Metallic Aftertaste	(1) Aftertaste associated with metals (tin or iron), (2) A flat chemical feeling factor
Other Aftertastes	Other aftertastes that are not listed, to include description
Texture – First chew[c]	
Hardness	The force required to bite completely through the sample (soft-hard)
Denseness	The compactness of the cross section of the sample when biting completely through with the molars (airy-dense)
Texture - Chew Down	
Number of Chews to Bolus[d]	The number of chews it takes for the mass to form a bolus
Fibrous Between Teeth[e]	The amount of grinding of fibers to get through the sample (no grinding-plenty of grinding)
Moisture Release/ Juiciness[f]	Amount of juice/moisture (oil, water) perceived in the mouth (none-very juicy)
Cohesiveness of Mass[g]	The degree to which chewed sample holds together in a mass (loose-tight)
Adhesiveness to Palate[g]	The force required to remove the product completely from the palate (no force-high force)
Residual[a]	
Oily Mouthcoating	The amount of oil coating the mouth surfaces

[a] **Evaluation methods:** Intensities are evaluated on a universal scale, in which panelists take into consideration all products and intensities they have experienced.
[b] Scores are determined 30 s after swallowing/expectorating.
[c] Place the sample between the molar teeth, and compress fully.
[d] Place the sample between the molars and chew into a bolus.
[e] Place sample between molars and chew to bolus. Press bolus between molars and compress until molars touch.
[f] Chew samples with the molar teeth for up to five chews.
[g] Chew sample with molars until phase change.

2.6. Sensory evaluation

Descriptive sensory analysis was conducted by the Addison Sensory Research Services of Mérieux Nutrisciences (Addison, IL, United States) using the Spectrum Methodology for appearance, aroma, flavor, texture, and aftertaste. The expert panel consisted of 8 highly trained assessors, who had previous experience in assessing fish products.

Samples for sensory evaluation were stored frozen (−18 °C) and were moved to refrigeration (0–7 °C) 24 h before the evaluation. The samples were removed from the refrigerator and kept at room temperature for 2 h prior to the study to ensure that the cores of the tuna samples were completely thawed. The samples were cut into 12.7 mm cubes and 3–5 cubes were served to each panelist in Styrofoam cups labeled with 3-digit blinding codes. Samples were randomized across the panelists and presented one at a time. RedJade® software was used for data collection. Samples were analyzed in duplicate and rated from 0 to 15 using a 150 point scale. Some descriptors had alternative scales such as presence of fibers (1 = presence, 2 = absence) or number of chews to bolus. A descriptor list was developed based on the sensory panel's previous experience. The full list of the sensory terms and descriptions for evaluating the tuna samples is shown in Table 3.

2.7. Statistical analysis

The results were expressed as mean (standard deviation, SD). The SPSS software package version 25.0 for Mac (IBM SPSS Statistical software Inc., Chicago, IL, USA) was used for statistical analysis. Shapiro-Wilk and Levene's tests were used for testing normality and homogeneity of variances, respectively. Student-t tests or, alternatively, the non-parametric Mann-Whitney *U* test were performed to evaluate differences between bluefin and yellowfin groups. The significance level for all tests was established at $p < 0.05$.

3. Results and discussion

3.1. Composition and physicochemical characteristics

Physicochemical parameters and proximate composition of bluefin and yellowfin tuna are shown in Table 4.

The pH and water activity levels were comparable between bluefin and yellowfin tuna samples, and consistent with values reported in previous studies. The pH of both bluefin and yellowfin samples averaged around 5.9, aligning with the findings of Karamah et al. [20]. Tuna flesh typically exhibits a postmortem pH below 6.0, which is lower than that of most other fish species, attributed to the high initial concentrations of glycogen in the tuna muscle [21]. Regarding water activity, values above 0.95 were observed, falling within the normal range for fresh fish [22].

The proximal composition analysis revealed the following values for bluefin and yellowfin tuna: moisture (68.4 %, 70.7 %), protein (27.2 %, 26.3 %), fat (1.3 %, 1.0 %), ash (2.4 %, 2.0 %), and carbohydrates (0.7 %, 0.3 %). The protein content was notably high, averaging around 26 %, while the fat content was relatively low, approximately 1.1 %, in both bluefin and yellowfin tuna. These findings align with the traditional understanding of the dorsal muscles of these tuna species, which are recognized as lean fish meat with low-fat content and high-quality protein.

In the case of the bluefin tuna group, the moisture, protein, and fat values completely agree with those reported for bluefin tuna lean meat in the Standard Tables of Food Composition in Japan [23] with only the ash and carbohydrate contents being slightly higher. Giménez-Casalduero and Sánchez-Jerez [24] and Mišlov Jelavić et al. [25] also provided compositional values for wild-caught Atlantic bluefin tuna, and Nakamura et al. [26] or Pacific bluefin tuna, obtaining similar values to those of the present study. Atlantic bluefin tuna composition results reported in other studies [27,28] cannot be compared with those of this study, as they do not correspond to dorsal lean muscles, so-called *akami* meat, but to other parts of the fish, such as the tail and ventral muscles. For the yellowfin group, all the composition values were similar to those presented in the Standard Tables of Food Composition in Japan [23] except for moisture content, which was found to be slightly lower.

Statistics show no significant differences in any of the parameters between the two groups analyzed, which could be attributed to the fact that proximal composition values probably are mainly determined by factors other than the species, such as the raising pattern (wild caught vs. farm raised), the fish's diet, or the part of the body studied. In this sense, wild-caught tuna has been found to be lower in fat and protein and higher in moisture than farm-raised tuna [24–28], which can be attributed to the continuous high-energy diet and lower activity of aquaculture fish. It should also be noted that fat in tuna meat can exceed 25 % depending on the anatomical location of the sample analyzed. Thus, in the Japanese composition tables, values of 27–28 % are reported for the fatty meat of bluefin tuna, called *toro*, which is found in the belly portion of the fish.

It must be considered that fish is not only an important dietary source of some main macronutrients, like proteins and lipids, but also of micronutrients and polyunsaturated fatty acids (PUFAs). The concentration of some minerals and vitamins and the fatty acid

Table 4
pH, water activity (a_w) and proximal composition (%, wet basis) of the lean meat of two groups of tuna species (mean (SD)).

	pH	a_w	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
Bluefin	5.93 (0.31)	0.97 (0.02)	68.36 (2.15)	27.18 (1.32)	1.31 (0.74)	2.44 (0.48)	0.72 (0.49)
Yellowfin	5.90 (0.22)	0.98 (0.02)	70.67 (1.99)	26.33 (1.34)	1.01 (0.97)	1.99 (0.36)	0.26 (0.27)

* Statistics show no significant differences between the groups.

composition of the lipid fraction of tuna samples are shown in Tables 5 and 6, respectively.

The micronutrient values were comparable between both groups, and were consistent with the findings of other studies [23,29]. Tuna can be considered a "source of" omega-3 fatty acids and potassium, and "high in" phosphorus and vitamin B12 according to the European nutrition claims legislation [30]. This is because it provides 15 % or 30 % of the recommended daily allowance (RDA) of a vitamin or mineral per 100 g, respectively. It is to note that health claims are regulated differently in various regions and the RDA percentages might vary. Among many other benefits, vitamin B12 is believed to support red blood cell production and the development and normal function of the nervous system, while zinc and selenium play an important role in promoting a healthy immune system [29].

The mercury levels in the tuna samples were analyzed, with bluefin tuna showing 0.38 ppm and yellowfin tuna showing 0.35 ppm. These levels fall within the average range for commercial tuna according to the FDA [31]. Based on the US Environmental Protection Agency's maximum safe dose of mercury of 0.1 μg mercury/kg bodyweight/day [32] and a recommendation of one weekly fish serving, yellowfin tuna is considered an acceptable fish choice [33]. While specific data on bluefin tuna is not available, the values found in this study are similar to those of yellowfin.

Regarding the fatty acid profile, it is well known that oily fish (5–20 % of fat) is an important dietary source of omega-3 PUFAs, mainly eicosapentaenoic (EPA. 20:5 n-3) and docosahexaenoic (DHA. 22:6, n-3), which are essential nutrients needed for optimal growth and health. As can be seen in Table 6, short chain fatty acids were not detected and most of the long chain fatty acids were found in both groups of tuna: myristic (14:0), palmitic (16:0), palmitoleic (16:1, n-7), stearic (18:0), oleic (18:1, n-9), docosatetraenoic (22:4, n-6), EPA and DHA; whereas linoleic (18:2, n-6), a-linolenic (18:3, n-3) and docosapentaenoic (C22:5, n-6) were only detected in bluefin tuna, and gadoleic acid (C20:1, n-11) only in yellowfin tuna. The fatty acid composition depends on fish nutritional habits and varies not only among different species but also from specimen to specimen of the same species [34].

The major fatty acids in this study were palmitic (16:0), which is reported to be a predominant source of potential metabolic energy in fish during growth [35], oleic (18:1), and DHA (22:6) acids. The latter being the most abundant in both groups, with 31.2 % and 33.9 % for bluefin and yellowfin tuna, respectively. EPA was present in lower amounts, always less than 5 %. Both tuna species showed to be rich in unsaturated fatty acids accounting for 70.9 % and 70 % for bluefin and yellowfin respectively.

The results for bluefin tuna show similar levels of saturated fatty acids to those found by Nakamura et al. [36], but the unsaturated fatty acids showed a trend towards a higher content of polyunsaturated versus monounsaturated compared to those obtained in the aforementioned work. The DHA content of our samples was much higher, practically double, than that of wild Atlantic bluefin tuna reported by Topic Popovic et al. [28]. However, the obtained profile would confirm that our samples come from wild tuna, considering the differences between them and farmed tuna highlighted in the same study, such as lower contents of myristic and palmitoleic acids, non-detection of arachidonic acid (20:4 n = 6) and high oleic acid content.

Based on these results, to develop an analog that is nutritionally comparable to fish, the analog should have a protein content of approximately 25 %. A combination of several complementary plant proteins may effectively address the deficiency of limiting amino acids in some plant protein sources to achieve a nutritional contribution close to that of animal protein. Despite the low-fat content (less than 2 %), the significant proportion of polyunsaturated fatty acids (45–46 % of total fat) especially omega-3 DHA (over 30 % of total fat), should be emphasized. Moreover, to preserve the nutritional claims established by the European legislation, the addition of some micronutrients, such as phosphorus and vitamin B12, should also be considered in the formulation of the analog.

3.2. Appearance

3.2.1. Macrostructure

There are significant differences between bluefin and yellowfin, due to the bluefin tuna being a bigger fish than yellowfin. The distances between myocommata in the two species are shown in Table 7. The variability observed could be due to variability in the location on the loin, whether that was closer to the head or the tail, however, these values provide a reference for the development of plant-based tuna analogs.

Histological observations of the samples are shown in Figs. 2 and 3, with bluefin and yellowfin images respectively. From a tissue perspective, both groups of species have a similar appearance with shiny surface, myocommata with white appearance, and a fibrous appearance given by the muscle fibers of the myotomes.

Table 5
Micronutrient concentration in bluefin and yellowfin pool samples.

	Bluefin	Yellowfin
VITAMIN B2 (μg/100 g)	80	100
VITAMIN B12 (μg/100 g)	1.11	1.25
POTASSIUM (mg/100 g)	466	426
CALCIUM (mg/100 g)	26.5	69.4
ZINC (μg/100 g)	424	422
IRON (mg/100 g)	0.66	0.51
MAGNESIUM (mg/100 g)	43.8	44.8
PHOSPHORUS (mg/100 g)	336.4	329.6

Table 6
Fatty acid composition in bluefin and yellowfin pool samples (% of total fat).

	Bluefin (% of total fat)	Yellowfin (% of total fat)
	Fatty acid profile	
C4:0 (BUTYRIC ACID)	NDLT 0.1	NDLT 0.1
C6:0 (CAPROIC ACID)		
C8:0 (CAPRYLIC ACID)		
C10:0 (CAPRIC ACID)		
C12:0 (LAURIC ACID)		
C14:0 (MYRISTIC ACID)	2.1	1.5
C16:0 (PALMITIC ACID)	21.1	18.9
C16:1 (PALMITOLEIC ACID)	3.5	2.5
C18:0 (STEARIC ACID)	6	9.5
C18:1 (OLEIC ACID)	21.8	18.1
C18:2 (LINOLEIC ACID)	1.6	NDLT 0.1
C18:3 (ALPHA LINOLENIC ACID)	1.6	NDLT 0.1
C20:1 (GADOLEIC ACID)	NDLT 0.1	3
C20:5 (EICOSAPENTAENOIC ACID)	4.8	4.9
C22:4 (DOCOSATETRAENOIC ACID)	2.1	3.1
C22:5 (DOCOSAPENTAENOIC)	0.9	NDLT 0.1
C22:6 (DOCOSAHEXAENOIC ACID)	31.2	33.9
	Totals	
SATURATED FAT	29.2	29.9
POLYUNSATURATED	45.6	46.4
MONOUNSATURATED	25.3	23.6

* NDLT=Not Detected Less Than.

Table 7
Distance between myocommata (connective tissue layers) in bluefin and yellowfin tuna lean meat (mean (SD)).

	Bluefin	Yellowfin
Distance (mm)	10.790 (2.236)[a]	7.026 (1.957)[b]

Different superscript letters in the same column indicate significant differences ($p < 0.05$) between samples.

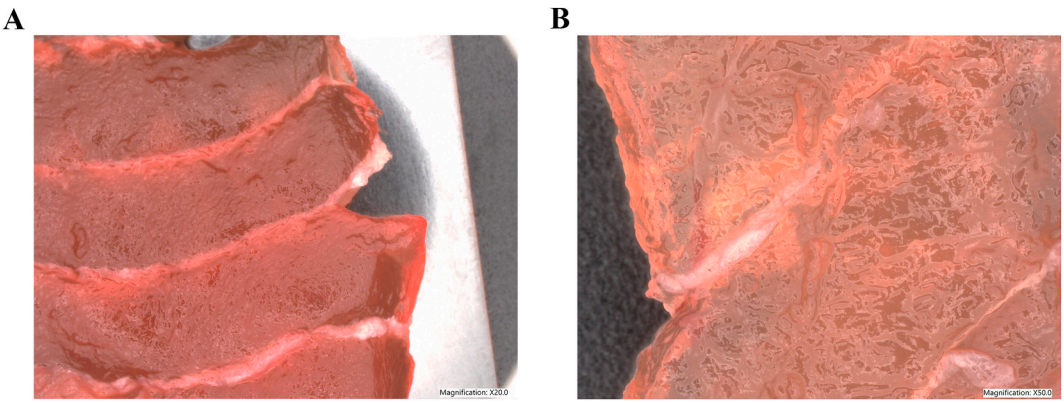

Fig. 2. Histological images of bluefin tuna, (A) magnification ×20, (B) magnification ×30.

3.2.2. Color, translucency and shine

The interaction of light with a product is one of the determining factors of its appearance. The color is related to the product's light absorption, reflection, emission spectra and interference. In addition to color, other features are also important, such as brightness and translucency, which are related to the product's light reflection or refraction properties.

The instrumental color parameters of the tuna samples are shown in Table 8. Values of L and a* were significantly different ($p < 0.05$) between the two tuna groups, bluefin being redder (higher a* value) and yellowfin being lighter (lower L value), while both species showed the same levels of yellowness (b* values).

Lightness values for bluefin tuna agree with those reported by Nakamura et al. [26] for the dorsal ordinary muscles of cultured Pacific bluefin tuna; but differ greatly from the a* and b* coordinates, the redness being at least two times greater and the yellowness

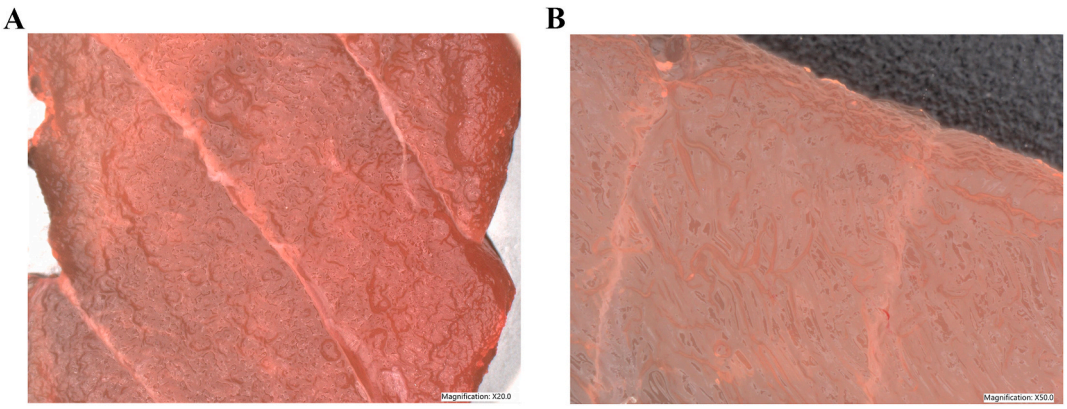

Fig. 3. Histological images of yellowfin tuna, (A) magnification ×20, (B) magnification ×50.

Table 8
Color parameters (CIE L*a*b*) and cylindrical coordinates: h° (hue angle), and C* (chroma, relative saturation) of raw tuna (mean (SD)).

	Lightness (L)	Redness (a*)	Yellowness (b*)	Hue (h°)	Chroma (C*)	n
Bluefin	25.65 (5.93)[a]	13.80 (3.59)[a]	11.08 (2.77)	39.03 (5.46)[a]	17.77 (4.24)[a]	26
Yellowfin	30.62 (3.90)[b]	9.76 (1.97)[b]	10.10 (1.20)	46.35 (5.65)[b]	14.10 (1.89)[b]	28

Different superscript letters in the same column indicate significant differences ($p < 0.05$) between samples.

much lower than our results. Nevertheless, some authors have pointed out that the ratio a*/b* is more appropriate than a* or b* values to indicate redness of bluefin and yellowfin tuna meat [37] and that it is better correlated with metmyoglobin formation than a* values in fresh and frozen yellowfin tuna steaks [38]. In this study, a*/b* was shown to be 1.24 and 0.96 for bluefin and yellowfin species, respectively; both values being higher than those reported by Bu et al. [37] for southern bluefin tuna. These high values may indicate a low metmyoglobin content, which increases during refrigeration storage and is responsible for meat browning.

Hue values were shown to be significantly different between bluefin and yellowfin samples ($p < 0.05$). Although the hue values indicated a red tone for both species, bluefin showed a tendency to pink while yellowfin tended to orange. The low color saturation, deduced from the Chroma values, indicated that none of the tuna species had a bright red color but rather a dull coloration, significantly more faded ($p < 0.05$) in yellowfin samples as compared to bluefin ones. The evaluation of the color intensity carried out by the experts of the sensory panel (Fig. 4) corroborated this result and proved that it is not a subtle difference only detectable by means of instrumental analysis, but that it can be also perceived visually.

It is to note that the color of fish is not stable, since it changes with decreasing freshness. Recently caught fish have more intense colors than those a few hours after death when they arrive at markets (considered fresh by the general consumer). After purchase, the color continues to become duller as the flesh deteriorates further (considered old by the general consumer). Our samples were analyzed fresh directly after purchase from the fishmongers, that is, in a state of freshness familiar and desirable to the general consumer [39].

The consumer often assesses the quality of a fish product by its color and appearance and forms assumptions about the sensory experience they will have when eating it. If a fish product fits within the consumer's impression of fresh, the consumer is biased

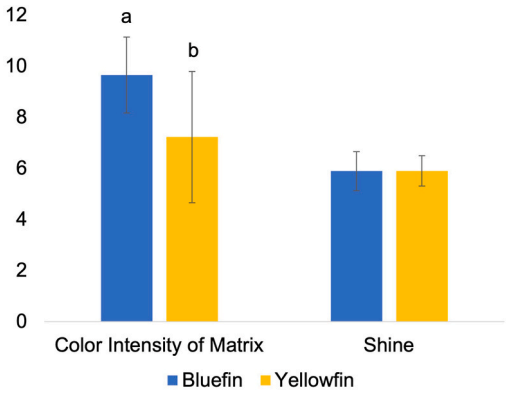

Fig. 4. Scores of the appearance attributes assessed in the sensorial analysis of bluefin and yellowfin tuna meat. Different superscript letters for the same attribute indicate significant differences ($p < 0.05$) between Bluefin and Yellowfin samples.

towards a positive sensory experience. On the contrary, if the fish does not fit within the fresh description, the consumer is more likely to perceive sensory defects [40].

Therefore, having a fresh-looking appearance is imperative for a positive sensory experience and will contribute to the acceptance of plant-based fish analogs by the consumer.

Opacity testing was conducted at two different sample thicknesses to simulate the visual appearance of raw fish products. Sashimi, typically presented as thin slices of fish flesh (approximately 5 mm), and poké or certain sushi preparations, which utilize thicker portions or cubes (around 15 mm), were considered for analysis. The opacity values for both presentations were evaluated for the two species under investigation, and no significant differences were observed (Table 9).

Thicker cuts of fish, resembling the poké or sushi-style portions, exhibited a near-complete opacity, reaching approximately 97 %. This indicates that they refract minimal light, appearing almost completely opaque. In contrast, thinner cuts resembling sashimi displayed a relatively lower opacity, measuring around 87 %. Consequently, these thinner cuts can be considered slightly translucent, allowing some light to pass through.

The experts from the sensory panel consider color intensity, shine, and the present of fibers as important descriptors of the appearance of raw tuna lean meat (Fig. 4). Shine was given values of 5 over 15 for both species. Presence of fibers was assessed on a different scale, 1 being presence and 2 being absence. The two types of tuna were both scored with values close to 1, indicating that fibrosity is also a visual character that has an impact on the appearance perceived by the consumer.

The key visual attributes to meticulously replicate in a plant-based analog are color and translucency, which are instrumental in mimicking the visual appeal of raw animal tuna. Bluefin tuna typically exhibits a deeper red color and less brightness compared to the lighter, yellow-hued yellowfin tuna. For development purposes, we provide a target average of both colors to utilize the general customer recognition of tuna color. Chroma, which measures the intensity of the color, is crucial for simulating the vividness associated with fresh tuna. Opacity varies with the thickness of the cut, with thicker cuts appearing more opaque and thinner ones more translucent. This variability in opacity must be carefully engineered in the analog to emulate the natural appearance of tuna slices. These parameters, along with the shine and the detailed macrostructural aspects like the presence of myocommata and the distance between them, are essential for creating a convincing plant-based alternative that visually resembles traditional raw tuna muscle.

3.3. Aroma and taste

The results of the aroma and taste evaluation of bluefin and yellowfin tuna are represented in Fig. 5. Aroma attributes refer to the perception by inhalation through the nose, and aromatic attributes to the retronasally perception, that is, into the mouth through the rhinopharynx (part of the pharynx located on the soft palate and behind the nostrils).

All aroma, taste, and aftertaste attributes evaluated in the sensory analysis received consistently low scores on a 150-point scale for both fish groups. The basic flavor attributes, except for umami, were rated below 1, indicating minimal perceived intensity. Similarly, other attributes related to taste and aftertaste were rated below 2.5, with the exception of total aromatics intensity, which averaged around 4. For clarity, Fig. 5 depicts the scale up to 5. These results provide valuable insights into the subtle sensory experience associated with the consumption of raw tuna. The low scores assigned to aroma, taste, and aftertaste attributes underscore the delicate and nuanced sensory perception associated with raw tuna. The marginal ratings for umami, as the most pronounced flavor attribute, further emphasize its pivotal role in shaping the overall flavor profile of raw tuna.

From our study results, a significant difference was observed between the tuna groups in terms of “total aroma intensity”, with bluefin tuna exhibiting higher scores compared to yellowfin tuna ($p < 0.05$). This finding aligns with the traditional knowledge that bluefin tuna is renowned for its stronger aroma and flavor profile. Previous research conducted by the US National Oceanic and Atmospheric Administration in 1992 assigned a score of 2 out of 5 for the aroma of raw bluefin tuna, while yellowfin tuna received a score of only 1 [41]. Early scientific literature, such as the work by Chartier [42], also supports this assertion. The difference in aroma intensity between the two tuna species may be attributed to synergistic effects resulting from subtle variations in other sensory attributes. Notably, applying a less stringent significance level ($p < 0.1$) reveals higher values for bluefin tuna in terms of marine aroma, total aromatics intensity, total aftertaste intensity, and fishy aftertaste.

The panelists in this study did not detect protein or vegetative aromas, flavors, or aftertastes (included in the descriptive profile in Table 3) and have not been included in Fig. 5 for the sake of clarity. Furthermore, no additional aromas or tastes beyond those specified in the provided list of descriptors were identified by the panelists.

Capturing the subtle aroma and taste profiles characteristic of raw tuna is essential for developing plant-based tuna analog. Sensory evaluations detailed above indicate that raw tuna generally exhibits a delicate flavor profile, with umami as the most pronounced taste. Reproducing the subtle characteristic flavors and aromas of raw fish presents significant challenges in the development of plant-based tuna analogs. One of the main difficulties lies in achieving a delicate, taste and aroma of low-intensity and without a strong aftertaste, which is often introduced by the base of plant ingredients [43].

Table 9
Opacity at different sample heights (%) (mean (SD)).

	Opacity at 5 mm (%)	Opacity at 15 mm (%)	n
Bluefin	87.36 (7.89)[a]	97.39 (1.99)[b]	26
Yellowfin	86.09 (5.83)[a]	96.86 (2.63)[b]	28

Different superscript letters in the same column indicate significant differences ($p < 0.05$) between samples.

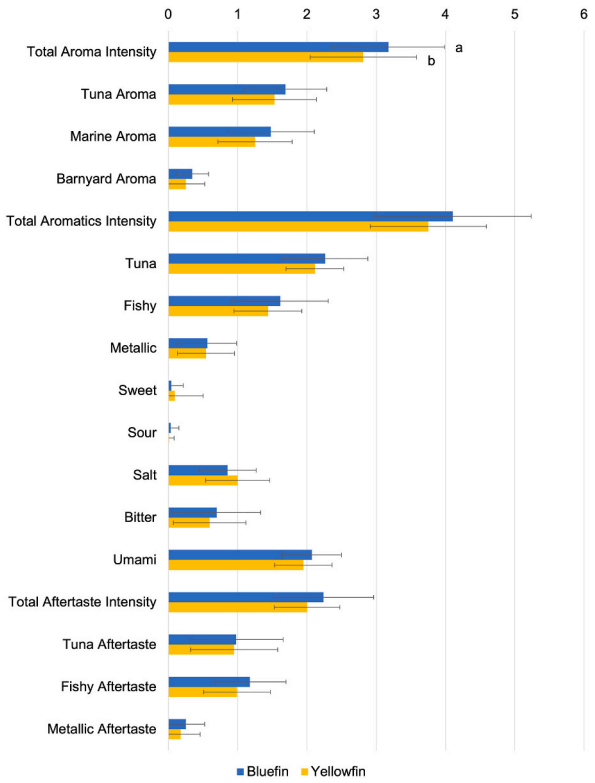

Fig. 5. Sensory analysis: scores of aroma, aromatics, taste and aftertaste attributes for bluefin and yellowfin tuna raw meat. Different superscript letters for the same attribute indicate significant differences ($p < 0.05$) between BF and YF samples.

3.4. Texture

The results obtained from the TPA test are presented in Table 10. Hardness values for both bluefin and yellowfin tuna were observed to be approximately 28N, indicating a similar level of firmness in the muscle tissue. Fracturability, which represents the ability of the samples to break under the applied force, was found to be around 20.5N in both sample groups. Despite claims by some authors suggesting that bluefin tuna muscle is firmer than yellowfin tuna [41], this study did not reveal any significant differences in traditional TPA parameters or fracturability between the two species. Additionally, the adhesiveness of the tuna samples was recorded to be approximately −0.3N, indicating a low level of stickiness or adhesion. The resilience of the samples was around 10.5 %, cohesiveness measured at 18N, springiness at 24 %, and chewiness at 1.3 N s. No comparable data has been found on the literature for these parameters for bluefin and yellowfin tuna. The variability in the data can be explained by the fact that the texture of raw fish is affected by many factors, such as the biological conditions of each individual, methods of catch or slaughter, storage, and processing conditions [44]. Additionally, obtaining homogenous samples of raw tuna muscle is difficult due to the inherent characteristics of fish flesh, the complexity and fragility of the overall structure, and the organization and orientation of the muscle fibers [44].

The TPA texture test, while providing valuable reference values for certain attributes such as hardness that often correlate well with sensory assessment, has limitations in accurately capturing other textural parameters. Some researchers argue that a single compression cycle may be sufficient to obtain measures of hardness and fracturability, questioning the necessity of a double compression step [44,45]. It is important to recognize that the complexity of raw fish texture cannot be fully captured by a single instrumental test [45]. While mechanical methods typically measure a single dimension of texture, sensory methods encompass multiple product attributes [37]. Consequently, there is a lack of consensus within the industry regarding the optimal methods for analyzing fish texture. Given the absence of a universally recommended method, it is advisable to use a combination of techniques to comprehensively assess fish texture [45,46]. By utilizing multiple approaches, the multidimensional nature of texture can be better captured and a more comprehensive understanding of the textural attributes of fish muscle can be obtained.

In the sensory analysis, no texture differences were detected between the two groups of tuna. The expert panel scored all texture-related parameters with virtually identical values for bluefin and yellowfin samples (Fig. 6). Some of the sensory attributes assessed go beyond those assessed with TPA, such as fibrosity, moisture release/juiciness or oily mouthcoating. Fibrosity was assessed directly through the attribute “fibrosity between teeth” and indirectly through “cohesiveness of the mass”. Respectively, these attributes were rated around 2.3 and 5.1 for both tuna species, indicating a slight fibrosity.

The methods used in this paper can provide a first approximation to animal tuna texture during the development of plant-based analogs. However, further mechanical texture characterization is required to obtain a deeper understanding of other aspects of raw

Table 10
Texture profile analysis parameters (mean (SD)).

	Bluefin	Yellowfin
Hardness (N)	28.49 (6.63)	29.68 (5.35)
Fracturability (N)	20.00 (7.42)	20.99 (5.68)
Adhesiveness (N·s)	−0.28 (0.13)	−0.28 (0.11)
Resilience (%)	10.01 (2.16)	11.99 (7.88)
Cohesiveness (%)	18.32 (2.97)	18.26 (2.63)
Springiness (%)	24.13 (3.24)	24.53 (3.17)
Chewiness (N·s)	1.30 (0.49)	1.34 (0.36)

* Statistics show no significant differences between the groups.

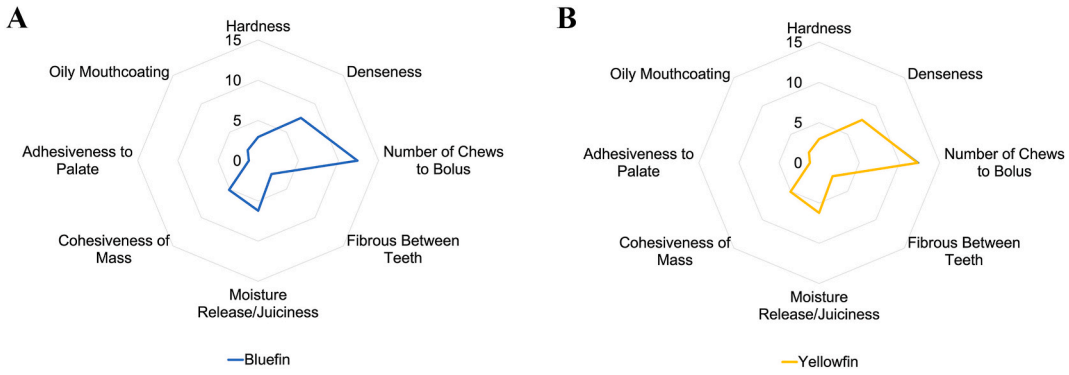

Fig. 6. Sensory analysis: texture attributes for bluefin (A) and yellowfin (B) tuna raw meat.

tuna muscle texture, such as how it cuts or breaks apart.

Replicating the instrumental TPA attributes in the plant-based analog is a good start towards achieving texture parity with animal fish.

4. Conclusions

This work presents a comprehensive characterization of tuna muscle, serving as a reference for developing plant-based raw tuna analogs. The study focused on bluefin and yellowfin species, which are the most common for raw applications, and established acceptable ranges for the composition, appearance, and texture of raw tuna alternatives. It is important to note that all attributes must be replicated holistically to meet consumer expectations, as research shows that the sensory expectations of consumers towards a plant protein substitute with visual patterns similar to that of fish can lead to disappointment after tasting them if the taste and texture are too far from those of animal meat [47].

Building on these findings, future research should prioritize refining the sensory, nutritional, and structural attributes of plant-based tuna analogs. The optimization of the nutritional profile, particularly in achieving a balanced amino acid composition, incorporating key macronutrients and replicating a similar fatty acid profile, is essential to ensure that the analogs meet established health claims. Additionally, capturing the subtle umami flavor and delicate aroma of raw tuna remains a significant challenge, necessitating innovative approaches such as the usage of natural or synthetic flavors or using advanced flavor encapsulation techniques. Textural parity, including complex attributes like fibrosity, moisture release, and cohesiveness, should be a priority, with a focus on refining mechanical texture characterization methods and exploring novel processing techniques. Moreover, visual realism in color, translucency, and macrostructural details must be advanced through food structuring technologies. Finally, addressing the scalability and sustainability of production processes will be crucial, focusing on sustainable plant protein sources and environmentally friendly production methods. By addressing these areas holistically, future research can significantly advance the development of plant-based tuna analogs, bringing them closer to achieving parity with their animal-based counterparts and enhancing their potential for consumer acceptance.

Data availability statement

Data will be made available on request.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because the sensory evaluation using human subjects

performed at the Addison Sensory Research Services of Mérieux Nutrisciences (Addison, IL, United States) was contracted.

CRediT authorship contribution statement

Daphne Jumilla-Lorenz: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tiffany Briones:** Writing – review & editing, Supervision. **Dolors Parés:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Daphne Jumilla-Lorenz reports financial support was provided by Current Foods, Inc. Daphne Jumilla-Lorenz, Tiffany Briones reports a relationship with Current Foods, Inc that includes: employment. We declare that all the sensory panel participants of the sensory evaluation perfomed at the Addison Sensory Research Services of Mérieux Nutrisciences (Addison, IL, United States) have expressed informed consent. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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