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Development and Characterization of Tuna Peptide-Enriched Cookies

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Abstract

Fish is a vital component of the human diet, offering a rich array of essential nutrients. Among the various species consumed, tuna stands out due to its high protein content. These proteins, when hydrolyzed into peptides, find wide-ranging applications in both the food and pharmaceutical industries due to their enhanced bioavailability and functional properties. The present study investigated fortification of cookies with fish-derived peptides obtained from tuna meat (*Euthynnus affinis*), hydrolyzed using papain to a degree of hydrolysis of 24.07%. Cookies were prepared in five formulations: a control (C_0) and four peptide-enriched variants (C_1–C_4), containing 0.431 g to 1.7 g of peptide-derived protein per cookie. Compared to the control, fortified cookies showed significant changes. The diameter of fortified cookies increased from 4.1 to 5.3 cm, while the weight decreased from 20 g to 17.8 g. The spread ratio reduced from 4.0 to 3.65 with increasing peptide levels. Hardness increased from 17.46 to 25.77 N, while the breaking force rose from 3.3 to 4.01 N. Sensory evaluation confirmed that all peptide-enriched cookies were acceptable, with no bitterness detected even at the highest peptide concentration. These findings highlight the potential of tuna-derived peptides as a functional ingredient in bakery products, offering enhanced nutritional and textural properties without compromising sensory appeal.

Keywords: Fish peptide, tuna, *Euthynnus affinis*, cookies, fortification

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Introduction

The integration of protein content, nutritional richness, delicacy, versatility, and convenience makes tuna and its products highly valued and in demand among consumers seeking healthy and flavorful food options. Peptides are derived from the enzymatic or chemical breakdown of proteins into smaller peptides and amino acids. The production and utilization of peptides offer promising opportunities for innovation and product development in both the food and pharmaceutical industries, driven by their nutritional richness and bioactive properties (Parvathy et al., 2021). Enzymatic conversion allows for the extraction of valuable proteins from fish by-products and waste, which might otherwise be discarded. This process promotes sustainability in the seafood industry by reducing waste and maximizing the use of available resources. Further, the resulting bioactive peptides possess health-promoting properties (Peighambardoust, Karami, Pateiro, & Lorenzo, 2021).

Tuna peptides have broad potential for application in the food sector, where they can enhance nutritional value, biochemical properties, texture, and overall product stability (Parvathy, Binsi, Zynudheen, & Ninan, 2020a). The development of functional foods and nutraceutical products is another area where these peptides find valuable applications. Furthermore, they offer versatile and promising opportunities for pharmaceutical applications, ranging from the development of therapeutic peptides and drug delivery systems to tissue engineering and regenerative medicine (Karavasili & Fatouros, 2021). The shift in consumer preference towards nutritionally rich and healthier diets has led to increased demand for fortified food products. In response to health concerns associated with high-carbohydrate diets, the health food sector is focusing on developing protein-enriched alternatives. Cookies are popular for their delicious taste, variety of textures (from soft and chewy to crisp), and the convenience they offer as a ready-to-eat snack. Texture is the sensory and functional manifestation of a food's structural, mechanical, and surface properties (Szczesniak, 2002). It is one of the three main consumer acceptability factors to evaluate food, the other two being appearance and flavour. Their relatively long shelf life also makes them a convenient treat to have on hand. Cookies have traditionally been enjoyed as lenient treats, but concerns about their high carbohydrate content have driven interest in their fortification with healthy nutrients like protein, which can be popular choices for consumers seeking nutritious and satisfying treats (Agrahar-Murugkar, Dwivedi, Dixit-Bajpai, & Kumar, 2018).

Proteins from both plant and animal sources have been extensively researched for their potential as fortifying agents in various food products (Parvathy et al., 2020a). Incorporating both plant and animal proteins into products like cookies allows manufacturers to cater to a diverse range of dietary preferences and nutritional needs. Additionally, enzymatic hydrolysis has been recognized as an effective method for improving the availability and digestibility of proteins, particularly those derived from fish sources (Parvathy, Zynudheen, Panda, Jeyakumari, & Anandan, 2016; Binsi et al., 2016). Even though many of these reports highlight the functional properties of peptides compared to their native proteins, limited data are available on their application as a fortifying agent in food products. Addressing the complexities, technological challenges, consumer acceptance, and regulatory considerations associated with peptides will be crucial for advancing their use in the food industry. The present work evaluates the effect of tuna peptide on the nutritional, physical, sensory, and textural properties of cookies prepared using standardized protocols. The study addresses key knowledge gaps and supports food scientists in developing innovative, nutritious cookie formulations that align with the changing preferences of health-conscious consumers.

Materials and Methods

Mackerel Tuna (*E. affinis*) was purchased in fresh form and brought in iced condition to the laboratory, wherein it was immediately processed by separating the meat from other waste and was used as initial raw material for peptide preparation. Hydrolysis was done enzymatically using papain derived from Papaya latex (Hi Media). Further, all quality analysis was carried out using analytical grade chemicals.

For deriving peptides from tuna meat, the following procedure was adopted with slight modifications (Parvathy et al., 2020b). Finely comminuted tuna meat was mixed with twice the quantity of water to get a slurry, which was further cooked (30 min at 80-90ºC) to completely inactivate the endogenous enzymes in the raw material. Enzymatic hydrolysis was initiated using the enzyme papain at physiological pH and a temperature of 60ºC with enzyme: substrate (E/S) concentration being 0.5% and hydrolysis time of 30 min. This optimum combination was selected based on previous literature (Gong, Aguirre, & Bassi, 2016; Nasri, 2017). Upon attaining the hydrolysis period, the sample was immediately heated to 80-90°C for 15-20 min to arrest the hydrolytic process. Further, the solution obtained was initially coarse filtered, followed by centrifugation (K-24A, Remi Instruments, Mumbai) (8000 g for 20 min at 10°C) to obtain a clear supernatant, viz., peptide solution.

Degree of hydrolysis (DH) of the derived tuna peptide was determined by evaluating the α -amino nitrogen in the protein peptide, determined by formol titration method (Taylor, 1957) and the total nitrogen content in the wet meat by Kjeldahl method (AOAC, 2023) and expressed as percentage of α - amino nitrogen to total nitrogen.

Cookies were prepared following the standard procedure described by Jeyakumari, Janarthanan, Chouksey, and Venkateshwarlu (2016) with slight modifications. Ingredients used for the cookie's preparation are indicated in Table 1. Refined wheat flour was mixed with hydrogenated vegetable fat, fine sugar, semolina, salt and baking powder to which fish peptide solution was added. Fish peptide solution was added at 0%, 25%, 50%, 75% and 100% replacement of water in five different formulations represented as C_0, C_1, C_2, C_3 and C_4, respectively. Additional water was added as required to make a dough. One lot was kept as control with the addition of water alone (C_0). All the ingredients were mixed using a food processor (Phillips intelligent food processor, HL 1659, India) till a soft dough was formed. The prepared dough was subjected to sheeting (6 mm thickness), which was further cut to 4.0 cm diameter by using a die with an average weight of 20 g and was subjected to baking at 180°C for 30-40 min, cooled and packed in low-density polyethylene pouches for further analysis.

Evaluation of the proximate composition of cookies was carried out as per AOAC (2023). The following methods were adopted for the analysis: Hot air oven method for moisture content, the micro-Kjeldahl method to analyse the crude protein content, the Soxhlet method for fat content and muffle furnace heating was adopted for ash determination. Total carbohydrate was evaluated from the weight difference of other constituents (protein, fat, water, ash) to the total sample weight (assumed as 100).

Caloric value of the sample was calculated as per Souci et al. (2000):

Total Calories (kCal) = (4 x Protein weight %) + (9 x Fat weight %) + 4 x Carbohydrate weight %)

The spread ratio was determined using the methodology described by Ayo, Ayo, Nkama, and Adewori (2007). The weight and the diameter of the cookies were measured. Three rows of the five well-formed cookies were made, and the height was measured. Spread ratio was derived using the formula:

Spread ratio = Diameter/Height

The colour of the cookies was measured using a Hunter colourimeter with a D-65 illuminant and a 10° standard observer. Results were recorded as L (lightness), a (redness), and b* (yellowness) values. The instrument was calibrated using standard white and black ceramic tiles, and readings were obtained through the built-in software.

Shear strength or breaking force refers to the maximum force required to break a product in relation to its cross-sectional area. In this study, the breaking force of cookies was evaluated using a texture analyzer (Lloyd Instruments LRX Plus, UK) equipped with a crisp fracture blade. The test was performed with a force setting of 0.05 N and a test speed of 3 mm/s. Each cookie sample was placed on the platform, supported at two ends, while the blade, attached to the crosshead, applied pressure from above. This setup mimics how a consumer might assess cookie hardness by holding and bending the cookie until it breaks. The hardness of the cookies was measured using a texture analyzer (Lloyd Instruments LRX Plus, UK) fitted with a 50 mm diameter cylindrical probe and a 50 N load cell. Each cookie sample was placed on the platform, and the probe, attached to the crosshead, was lowered to apply force. The maximum peak force recorded during compression was taken as the measure of cookie hardness.

The sensory characteristics of the freshly prepared cookies were evaluated using 10 trained panellists. Evaluation of the sensory properties of cookies based on colour, aroma, taste, texture and overall acceptability was done using a 9-point hedonic scale (Meilgaard, Civille, & Carr, 2006).

The data generated was analyzed in triplicate and subjected to analysis of variance (ANOVA). The difference in means was evaluated by Duncan's multiple range test. SPSS Statistics programme (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL) was used for statistical interpretation.

Results and Discussion

The hydrolysis of food proteins represents a relatively recent innovation in the development of functional ingredients and nutritional supplements. This advancement began gaining commercial attention in the late 1940s, with the introduction of the first protein hydrolysate products. Among these, fish protein hydrolysates (FPH)/peptides have garnered significant attention due to their health-promoting bioactivities and desirable functional properties. These characteristics make them promising candidates for use in nutraceuticals and functional food formulations (Harnedy & FitzGerald, 2012). Optimization studies enable the generation of hydrolysates with targeted molecular structures and bioactive compounds that offer therapeutic and nutritional benefits (Chabeaud, Dutournié, Guérard, Vandanjon, & Bourseau, 2009). Further evaluation of protein availability is essential to assess the overall quality and functional efficacy of peptides across diverse applications. Enzymatic hydrolysis of parent protein facilitates their selective extraction by proper solubilisation, yielding higher protein content in the derived hydrolysate. In the present study, the protein content of TPH was 78.01 ± 1.09%. As the other unwanted components are removed during hydrolysis and the subsequent centrifugation process, generally protein hydrolysates will be a concentrated form of protein ranging between 60 – 90 % based on the process conditions adopted (Choi, Hur, Choi, Konno, & Park, 2009; Khantaphant, Benjakul, & Kishimura, 2011). Determination of the degree of hydrolysis (DH) is one of the most viable methods followed to evaluate the extent of hydrolysis undergone by a protein substrate. Recovery of protein from substrate to the final product is influenced by the degree of hydrolysis, with peptide bonds being cleaved, resulting in low molecular weight protein peptides, which are more water soluble, thereby increasing the protein recovery in peptide solution (He, Franco, & Zhang, 2013). This, in turn, is one of the most significant variables which influences the other attributes, viz., functional and bioactive ones exhibited by the peptides (Himonides, Taylor, & Morris, 2011). In the present study, the degree of hydrolysis (DH) was recorded at 24.15%, which corresponded with the protein recovery in the peptide solution.

Proximate analysis provides essential information regarding the nutritional content of food products, which helps consumers, as well as producers and nutritionists, understand the dietary value of a particular product and make informed choices. In the present study, fish peptide solution was incorporated into five different formulations to enhance the nutritional profile of the cookies. A control batch was maintained without peptide addition (C_0). The amount of protein added to the cookies, based on the peptide solution's concentration, was approximately 0.431, 0.86, 1.3, and 1.7 g for samples C_1, C_2, C_3, and C_4, respectively. Moisture content ranged from 3.01% to 4.7% across samples and showed significant differences ($p < 0.05$). A significant increase in protein content was observed, from 6.42% in the control to 7.90% in the highest fortified sample, indicating a linear relationship with peptide concentration ($p < 0.05$). Fat content ranged from 18.48% to 19.60%, with the lowest value recorded in the most fortified sample, which was statistically significant ($p < 0.05$). Ash content varied between 1.38% and 1.65%, while carbohydrate levels ranged from 67.91% to 68.71% (Table 2). Ayo et al. (2010) reported 18.5% fat, 78.23% carbohydrates, 1.0% ash, and 7.1% protein in the soy-acha composite biscuit. In cookies incorporated with wheat, sweet detar and moringa leaf flour blends, Igbabul, Ogunrinde, and Amove (2018) reported a moisture content ranging from 10.89 to 13.10%, protein content from 6.21 to 8.43%, fat content from 19.50 to 23.42%, ash from 1.96 to 3.83% and carbohydrate from 57.94 to 47.59%. These variations in the proximate composition are on account of the different ingredients used for the formulation of the product. The calorific value of food is indeed a critical aspect of nutrition and health, as it directly relates to the energy content of the food. Understanding the calorific value allows individuals to manage their energy intake more effectively, which has several key implications. The present study indicated a calorific value in the range from 471.42 – 480.24 kcal/100g. Pasha, Butt, Anjum, and Shehzadi (2002) reported a calorific value ranging between 371.8 to 439.9 kcal/100g in cookies incorporated with different dietetic sweeteners. The calorific value ranged from 457–397 kcal/100 g in cookies prepared from sweet potato–maize flour blends (Adeyeye & Akingbala, 2016). As per USDA (2019), baked products present a calorific value of 514 kcal/100 g.

Spread ratio is a key quality parameter in cookies, as it affects their texture and visual appeal. Consumers often prefer cookies with higher spread ratios due to their crispiness and thinner profile (Suriya, Rajput, Reddy, Haripriya, & Bashir, 2017). In the present study, the spread ratio of cookies ranged from 3.65 ± 0.04 to 4.0 ± 0.2 (Fig. 1), indicating the influence of formulation on cookie spread during baking. Spread ratio and protein peptide concentration in cookies were found to be inversely related, and a linear decrease was observed from the control lot to the highest fortified sample. Ganorkar and Jain (2014), in their studies, reported a reduction in the spread ratio in flaxseed flour incorporated cookies, attributed to an increase in protein and dietary fibre content, which are known to have more water-binding capacity. Studies have also suggested that variations in the amount of water added during formulation, along with the moisture-binding capacity of different ingredients, can significantly influence cookie quality. These factors affect the dough's viscosity, which in turn impacts the cookie's diameter and spread ratio (HadiNezhad & Butler, 2009). Okpala, Okoli, and Udensi (2013) commented that higher spread ratios are desirable in cookies, and a reduction in spread

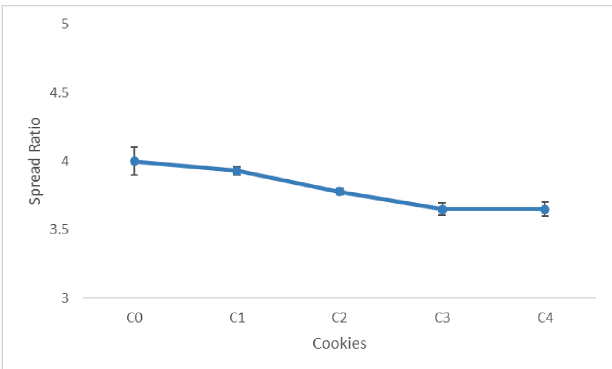

Fig. 1. Spread ratio of cookies

ratios is attributed to the hydrophilic nature of flours used in cookie production.

Colour is one of the first attributes noticed by consumers and plays a key role in determining the product's visual appeal, which can significantly influence their purchasing decisions (Ameur, Mathieu, Lalanne, Trystram, & Birlouez-Aragon, 2007). Composition and processing conditions like temperature, time, etc., determine and influence colour development and morphological changes in baked goods (Chen, Espinal Ruiz, Francavilla, Joye, & Corradini, 2024). Surface browning is a common phenomenon occurring during the baking process, and common colours in bakery applications include brown, yellow, and orange. The intensity of the pigments formed in the browning reactions can be measured by colour determination (Ramirez-Jimenez, Guerra-Hernández, & García-Villanova, 2000). The colour profile of the cookies was evaluated using L* (lightness), a* (redness), and b* (yellowness) values. In raw cookie dough, the values ranged from 61.82 ± 0.23 to 70.11 ± 0.12 (L*), 2.46 ± 0.01 to 3.37 ± 0.09 (a*), and 22.22 ± 0.02 to 23.67 ± 0.06 (b*). After baking, these values increased to 73.94 ± 0.03 to 76.99 ± 1.07 (L*), 4.17 ± 0.33 to 5.61 ± 0.45 (a*), and 28.37 ± 0.69 to 31.28 ± 0.01 (b*), indicating the development of a creamish-yellow hue in the final product. There was no noticeable increase or decrease in the colour values between the samples, which suggests that the added peptide did not have much effect on the cookie's colour. However, the baking process resulted in a significantly ($p < 0.05$) higher lightness, redness and yellowness for the product (Fig. 2). During the baking process, the initial temperature being low, colour change can be mainly attributed to a reduction in moisture content, which drove the transition of the colour from opaque to white. As baking progressed, reaching a maximum temperature, a significant increase in sample temperature allowed the Maillard reaction to proceed, with its concomitant generation of melanoidins contributing to a darker colour with increased redness. Further, reactions such as caramelization were reported with improved yellowness, particularly at the bottom region of the cookies where the temperature was higher (Chen et al., 2024). A similar trend was observed in thermally processed commodities also (Lukinac et al., 2017).

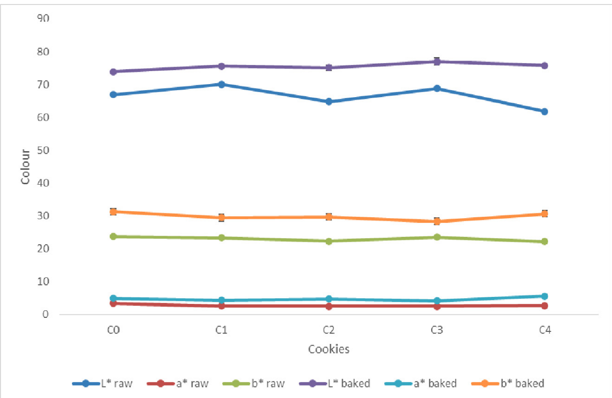

Fig. 2. Colour characteristics of cookies

The results with respect to textural characteristics of cookies, like hardness and breaking strength, are presented in Table 3. The average peak force, which reflects the hardness of the cookies, significantly increased ($p < 0.05$) from 17.46 to 25.77 N as the peptide concentration in the formulation increased (Table 3). Similar findings were reported by Kulthe, Thorat, and Lande (2017), who observed increased hardness in cookies incorporated with pearl millet. The hardness of cookies must be on account of the interaction of protein and starch in the flour by hydrogen bonding. Research has demonstrated that proteins, either alone or in combination with polysaccharides, can form gel matrices with varied microstructures, significantly enhancing their functional properties. This combination can lead to improvements in water retention, rheology (flow behaviour), and texture, which are important for food science and product development (Yu, Ren, Zhao, Cui, & Liu, 2020; Zhang, 2023).

Breaking strength, referred to as the force required to break a product, is a key indicator of texture, especially crispness or hardness and is widely measured in food science and quality control. It affects the structure and texture due to its role in gluten formation in wheat-based cookies or struc-

Table 1. Ingredients used for the preparation of cookies

Ingredients	Quantity
Refined wheat flour (g)	70
Semolina (g)	25
Powdered sugar (g)	25
Salt (g)	1.0
Baking powder (g)	1.0
Hydrogenated vegetable fat (g)	25
Water (mL)	20

Table 2. Nutritional Composition of Cookies

Sample	Moisture%	Protein%	Fat%	Ash%	CHO	Calorific value (kcal/100g)
C_0	4.70^{a}±0.17	6.42^{e}±0.22	19.59^{a}±0.07	1.38^{c}±0.06	67.91^{b}±0.14	473.63
C_1	3.96^{b}±0.19	6.97^{d}±0.14	19.58^{a}±0.07	1.40^{c}±0.07	68.09^{b}±0.02	476.46
C_2	3.01^{e}±0.09	7.25^{c}±0.06	19.60^{a}±0.15	1.43^{c}±0.06	68.71^{a}±0.17	480.24
C_3	3.12^{d}±0.06	7.51^{b}±0.07	19.41^{a}±0.08	1.51^{b}±0.02	68.45^{a}±0.19	478.53
C_4	3.59^{c}±0.02	7.90^{a}±0.20	18.48^{b}±0.08	1.65^{a}±0.01	68.38^{ab}±0.18	471.42

Values are expressed as Mean ± SD, and values bearing different superscripts in the column are significantly different ($p < 0.05$)

Table 3. Textural Properties and Sensory Score of Cookies

Sample	Breaking Force	Hardness	Sensory Score
C_0	3.3^{c}±0.04	17.46^{d}±0.04	8.3^{a}±0.4
C_1	3.33^{c}±0.06	19.68^{c}±0.34	8.4^{a}±0.7
C_2	3.35^{c}±0.03	21.6^{b}±0.66	8.2^{a}±0.5
C_3	3.54^{b}±0.02	21.04^{b}±0.12	8.3^{a}±0.5
C_4	4.01^{a}±0.06	25.77^{a}±0.69	8.4^{a}±0.4

Values are expressed as Mean ± SD and values bearing different superscripts in column are significantly different ($p < 0.05$).

tural binding (in gluten-free/protein-fortified cookies). Higher protein in a product like cookies can lead to a tough texture and associated breaking strength. In the present study, along with increased hardness, the breaking force of the cookies also showed a rise with higher peptide levels, ranging from 3.3 ± 0.04 to 4.01 ± 0.06 N (Table 3). The breaking force of samples fortified with higher levels of peptide (C_3 and C_4) was significantly greater ($p < 0.05$) than other samples, viz., C_0, C_1 and C_2. Furthermore, the instrumental values for fracturability (breaking force) aligned with the sensory evaluation, where fracturability describes the perceived brittleness of the cookies during chewing. Soni, Kulkarni, and Patel (2018) in their studies commented that higher protein content in treated flours, viz., incorporating soybean, mothbean and chickpea rather than wheat flour, attributed to increased breaking strength of the cookies.

The taste and perceived texture of a food depend upon the flavour components contained in it, constituent materials used, and the pattern by which the structure behaves in the mouth and flavours are released (Lucas, Prinz, Agrawal, & Bruce, 2002). Sensory acceptance of a food product can be proven by consumer tests, and in the present study, a panel of ten were chosen, and a 9-point hedonic scale with appearance, texture (*viz*., bite hardness and fracturability), flavour, smell and overall acceptability of cookie samples was presented with number codes. The results of the sensory evaluation showed that all the cookies received overall acceptability scores between 8.2 ± 0.5 and 8.4 ± 0.7. There was no significant difference among the samples for the measured attributes, indicating a high acceptance for all the cookies irrespective of the peptide incorporation (Table 3). No traces of bitterness were detected even at the highest peptide concentration. Results from the study revealed the possibility of incorporating peptides in cookies without significant modification of their sensory characteristics and increasing their nutritional value.

Fish protein peptide, derived from the enzymatic hydrolysis of fish proteins, is rich in peptides and amino acids, making it a highly nutritious ingredient. They are increasingly being used in various food applications, including the fortification of snack products like cookies, due to their better digestibility, functional, bioactive and nutritional properties. However, a few challenges, including its flavour and odour, texture and appearance of the fortified product, allergenicity, as well as regulatory considerations, need to be sorted out. Measures like the use of masking agents or flavour enhancers for mitigating sensory issues, standardizing formulation and processing conditions to achieve the desired product quality, clear labelling regarding allergenic issues to sensitive populations etc., can be adopted as mitigation measures.

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