



Nutritional, Biochemical and Sensory Properties of Snack Bars Enriched with Fish Protein Powder

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ABSTRACT

Ready-to-Eat (RTE) and Ready-to-Cook (RTC) are the new concepts in the food industry to define how quickly and conveniently food can be prepared and relished. Trials were conducted to develop a nutrition bar supplemented with fish protein powder extracted from carp (*Labeo rohita*) to improve protein in snack bar. The nutrition bar without Fish Protein Powder (FPP) was considered as control (C), whereas Fish Protein Powder was added @ 2.5% in T1, 5.0% in T2, 7.5% in T3, 10.0% in T4, and 12.5% in T5. Nutrition bars were analysed for proximate composition; the moisture content of the nutrition bars ranged between 6.22-6.25%, Protein 13.79% (in control) to 27.70% (T5). Fat 10.82% (T5) to 12.10% (control), Ash 2.51% (Control) to 2.87% (T5), and carbohydrate 52.36% (T5) to 65.33% (control). 'L*' & 'a*' values of colour analysis were increased, whereas 'b*' value decreased with the addition of FPP. Hardness of the nutrition bar was also increased due to the addition of Fish Protein Powder. Among all the treatments T3 (7.5% FPP) was highly accepted by the sensory evaluators. It can be concluded that FPP can be added to the nutrition bar up to 7.5% without significantly altering its sensory acceptability.

Key Words: Fish Protein Powder, Nutrition bar, Proximate Composition, Sensory properties Textural.

INTRODUCTION

A growing global population, combined with factors such as changing socio-demographics, is putting increased pressure on the world's resources to produce more production. Simultaneously, the supply of nutritious food is also a big challenge. Protein is such essential nutrient required for growth, body maintenance, enzyme formation, hormone synthesis, immunity, acid-base regulation, and fluid balance. Protein-energy malnutrition (PEM) remains one of the biggest nutritional challenges worldwide. Approximately 1 billion people worldwide do not get enough protein in their diets, leading to stunted growth and development (Wu *et al*, 2014). Malnutrition, mainly due to protein deficiency, is a problem worldwide. Proteins are essential biopolymers that can be obtained from both animal and plant sources. The outstanding bioactivities, as well as functionalities, have been studied in recent years, and these have gained more popularity by

contributing to health promotion and food processing industries (French *et al*, 2016 and Rehman *et al*, 2019).

Fish is considered an excellent source of protein due to the presence of essential amino acids and the demand for fish protein ingredients including dried fish protein is gradually increasing. Proteins from aquatic animals usually play important roles in improving cardiovascular and other associated health conditions (Torriss *et al*, 2018). Fish protein is known for containing essential amino acids (i.e., tryptophan, cystine, lysine, methionine, and threonine), as well as for improving digestibility. However, the presence of peptides and essential amino acids is much higher in proteins of aquatic animals than proteins in terrestrial animal meat such as lysine and methionine (Tacon and Metian, 2013). The composition and digestibility of protein ranging from 77.0 to 98.7% were determined by comparing it with the standard protein

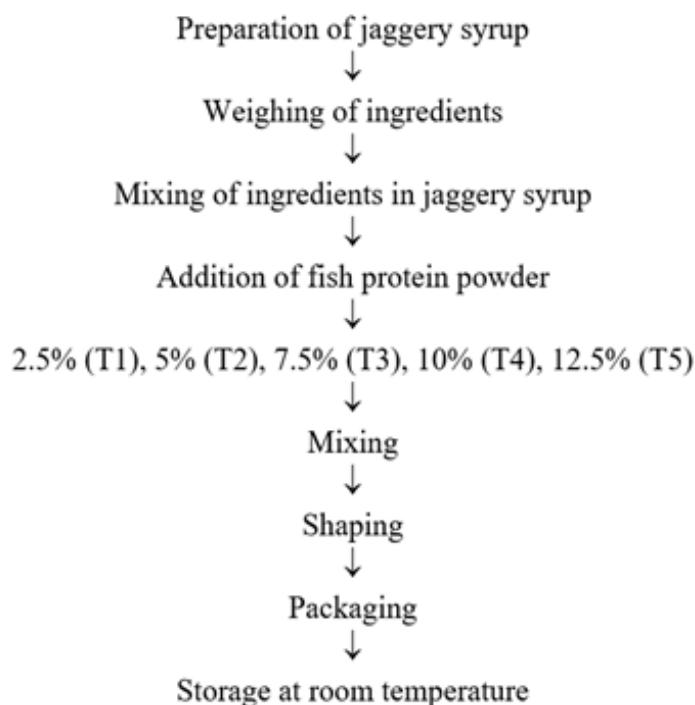
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Fig. 1 Flow diagram for preparation of carp (*Labeo rohita*) fish protein powder supplemented nutrition bar.



recommended by the World Health Organization (WHO).

Fortification of protein, to food products available most commonly in nearby areas can help to cater to such issues. Several new protein ingredients including fish protein powder (FPP) have been used as a source of protein energy to make diet nutritious. Several factors, including increased working hours, modern living, and rapid urbanisation, are influencing the way individuals consume food (Singh *et al*, 2023). Snack bars (SBs), also referred to as cereal bars or nutrition bars, are widely consumed because they give instant energy, can be a source of intake of beneficial nutrients, such as fiber, protein, minerals, and vitamins, and are easy to carry and transport (Zulaikha *et al*, 2021). The inclusion of fish protein can be a good alternative to improve the nutritional quality of available cereal bars. Further, in addition to improving their nutritional value, the inclusion of fish protein could promote fish consumption (Vitorino *et al*, 2020). The bars offer a readily available convenient food source

that requires no preparation, and a reasonably long shelf life at ambient temperature. Hence bars are popular as convenient food and can be consumed in between meals or along with lunch or dinner or even as meal substitutes (Constantin and Istrati, 2018) and can be a substitute to supply nutritious food to consumers.

In this regard, the trials were conducted to develop a nutrition bar supplemented with fish protein powder extracted from carp (*Labeo rohita*) which will help to serve as a healthy and nutritious alternative to the consumers.

MATERIALS AND METHODS

For the present study, fish protein powder was extracted from rohu (*Labeo rohita*) fish, which was procured from the Experimental cum Research Unit of the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Fishes were beheaded, eviscerated, de-scaled, de-skinned, and cut to obtain fillets. Further, the fillets were thoroughly washed and then it was passed through a locally

Table 1. Proximate composition (%) of fish protein powder extracted from rohu fish

Nutrient	Quantity (%)
Moisture	4.14
Protein	86.54
Fat	2.89
Ash	3.51
Carbohydrate	2.84

fabricated deboning machine to obtain bone-free mince. The mince was treated with 0.5% citric acid and washed twice with chilled water and then the mince was dried in a hot air oven at 55°C. After drying, meat was grounded mechanically in a grinder and sieved to obtain fish protein powder.

The bar was prepared by adding the ingredients jaggery @ 40.5% mixture of roasted peanut powder, oats flakes and corn flakes (1.5:1.4:1.0) @ 39%, roasted almond powder, cashew powder, sesame seed, dextrin @ 5% each and common salt 0.5% in control, whereas fish protein powder was added @ 2.5% in T1, 5.0% in T2, 7.5% in T3, 10.0% in T4, and 12.5% in T5 replacing mixture of roasted peanut powder, oats flakes and corn flakes (1.5:1.4:1.0). Shape of bar was maintained with the help of food-grade stainless steel bar frame. Method of carp (*Labeo rohita*) fish protein powder supplemented nutrition bar formation is given in Fig. 1.

The proximate composition of fish protein powder and bar prepared (Control and T1-T5) w.r.t. moisture, protein, fat, and ash were analysed as per AOAC, 2022 method, whereas carbohydrate content was calculated by numerical formula $[(\text{Carbohydrate } (\%) = 100 - (\text{Moisture } \% + \text{Protein } \% + \text{Fat } \% + \text{Ash } \%)]$. The biochemical parameters including pH, Free Fatty Acids (FFA), and Peroxide Value (PV) were analysed following the standard methodology described by Trout *et al* (1992) for pH and Koniecko (1979) for free fatty acid and peroxide value. Colour analysis of the nutrition bar was performed using Konica Minolta (Model: Chroma meter CR 400) to obtain colour values as L*, a* and b*. The Texture Profile Analysis of the bars was performed using TA-XT plus texture analyser (Stable Micro Systems, Surrey, UK). The sensorial attributes were evaluated by following the 9-point hedonic scale (Like extremely-9, Like Very Much-8, Like Moderately-7, Like Slightly-6, Neither Like nor

Dislike-5, Dislike Slightly-4, Dislike Moderately-3, Dislike Very Much-2, and Dislike Extremely-1) as per the methodology suggested by Popper *et al* (2004). The data were statistically analysed for Analysis of variance (ANOVA) using Duncan's Multiple Range Test (SPSS v. 20.0 for Windows, SPSS Inc., Illinois, USA) to determine the significant difference in treatments.

RESULTS AND DISCUSSION

Protein content in fish protein powder was recorded 86.54%, fat 2.89%, Ash (as minerals) 3.51% and carbohydrate 2.84%. Details of the proximate composition of fish protein powder extracted from rohu fish are given in Table 1.

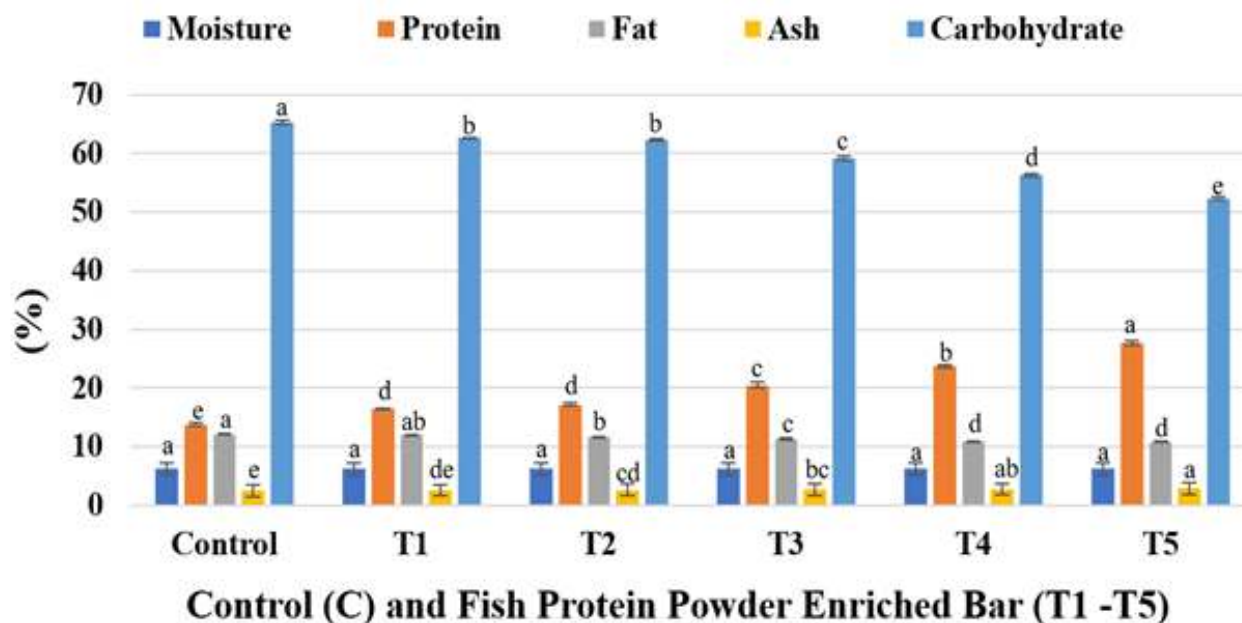
Jahan *et al* (2017) reported protein 66.90%, 68.46%, 62.18%, fat 19.33%, 15.71%, 18.65%, minerals 0.24%, 0.44%, 0.36%, and carbohydrate 1.75%, 4.85%, 1.98% in rohu, mrigal and silver carp respectively. Kabir (2022) quantified protein content in tilapia and silver carp fish powder as 63.6% and 73.5%, respectively. Kasozi *et al* (2018) analysed the nutrient composition of fish protein powder extracted from *Brycinus nurse* and reported 50.4% protein. Shaviklo *et al* (2012) reported 94.04% protein in freeze-dried saithe (*Pollachius virens*) protein isolate. According to Shaviklo (2015), the protein content in fish protein powder chiefly depends on the type of fish, moisture, and other additives, but it contains at least 65% protein. In the present study, the high protein content in fish protein powder may be due to the thorough washing and acid treatment which may help to improve the protein content in FPP, drying also plays a major role in the quantity of protein in the product.

Proximate Composition of Nutrition Bars

The proximate composition w.r.t moisture, protein, fat, ash, and carbohydrate content in the nutrition bar is given in Fig.2.

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Fig. 2 Proximate composition of bar control (C) and enriched with fish protein powder (T1-T5).



Moisture content

Moisture content is an important parameter that plays a crucial role in shelf life and modifies the textural properties of the product. In the present study, moisture content was recorded 6.22% in control (C), 6.21% in T1, 6.23% in T2, 6.25% in T3, 6.22% in T4, and 6.24% in T5, and No-significant ($P < 0.05$) difference was recorded among the treatments. Moisture is a key influencing factor responsible for the shelf life of the product (Steele, 2004). Moisture content below 10 g/100 g is the normal requirement for dried fish powder products (Abbey *et al*, 2017; Kasozi *et al*, 2018 and Mahmud *et al*, 2019) and in the present study, moisture content was much lower than the suggested quantity by Abbey *et al* (2017), which indicates that the product was well dried.

Protein content

Significant ($P < 0.05$) variation in the protein content of the nutrition bar was recorded, it was highest in T5 (27.70%) and lowest in control (C) i.e., 13.79%. The quality and characteristics of fish protein ingredients are highly dependent on the source of the raw materials and the processing methods (Arason *et al*, 2010). The FPP is a dried and stable fish product, intended for human consumption, in which the protein is more concentrated than in the original fish flesh

(Shaviklo *et al*, 2015). It is well documented that the addition of FPP in conventional products improves the protein content in carbohydrate-rich processed products (Souza *et al*, 2017; Desai *et al*, 2019; Vitorino *et al*, 2020).

Fat content

The fat content showed a reducing trend from 12.10% in control (C) followed by T1, T2, T3, T4, and T5 (10.82%) in decreasing order. The reason behind the reduction in fat content in the bar w.r.t. increased quantity of addition of the FPP may be that the FPP contains very low levels of fat. Fat is an important nutrient of food, and products and affects the textural, sensorial, and other quality characteristics of the product. The amount of fat in the nutrition bar mainly comes from the ingredients added to it during formulation. Ash content was significantly high in T5 (2.87%) and low in control (C) (2.51%) and the trend shows that as the quantity of fish protein powder increases, the quantity of ash also increases.

Total Ash

Ash is an inorganic residue remaining after the water and organic matter have been removed through burning in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Elnovriza *et al* (2019) reported 4.44% ash content in the fish bar

Table 2. Biochemical parameters of nutrition bar control and enriched with fish protein powder

Parameter	Treatment					
	Control (C)	T1	T2	T3	T4	T5
pH	6.20±0.01 ^a	6.16±0.03 ^a	6.18±0.02 ^a	6.17±0.07 ^a	6.20±0.07 ^a	6.18±0.09 ^a
Free Fatty Acid (% Oleic acid)	0.098±0.002 ^a	0.088±0.002 ^b	0.083±0.002 ^{bc}	0.078±0.001 ^{cd}	0.071±0.002 ^{de}	0.066±0.002 ^c
Peroxide value (meq/kg)	0.198±0.00 ^a	0.198±0.00 ^a	0.197±0.00 ^a	0.198±0.00 ^a	0.197±0.00 ^a	0.195±0.01 ^a
Texture (Hardness as kg force)	3761.66±0.74 ^f	3861.20±0.50 ^e	4245.33±0.33 ^d	4918.06±0.43 ^c	5518.06±0.43 ^b	6918.73±0.41 ^a

when 50% bilih fish flour was added to them. The low ash content in the present study may be due to the addition of a high amount of jaggery which is high in carbohydrate and contains low levels of minerals.

Carbohydrate

Carbohydrate content was recorded 65.33% in control (C), 62.68, 62.26, 59.21, 56.33, and 52.36% in T1, T2 T3, T4, and T5, respectively. Jaggery and dextrin were the main sources of carbohydrates, which was reduced as the percent share of FPP increased in the bar. Fish is a very poor source of carbohydrates and that too stored mainly in the form of glycogen. Normally, a live fish contains glycogen at a level varying from 0.1 to 1%. However, carbohydrates can be said to be relatively stable in processing and storage compared to sensitive nutrients browning (Dandago, 2009). In the nutrition bar, high levels of carbohydrates were mainly because of the type and quantity of ingredients added, particularly jaggery and dextrin.

Biochemical Parameters and Hardness of Nutrition Bar

The biochemical analysis of the bar (Control and FPP enriched) was analysed for pH, free fatty acids, and peroxide value, and the details are given in Table 2. The pH value of bars ranged between 6.16 – 6.20 and no significant ($P<0.05$) differences were recorded. Free fatty acid (% oleic acid) was recorded significantly ($P<0.05$) low in T5 (0.066% oleic acid) and high in control (C) (0.098 % oleic acid). The free fatty acids formation itself does not lead to nutritional losses but such molecules are more susceptible to oxidative rancidity (Okaka and Okaka, 2001). Peroxide value which is an indicator of rancidity varied between 0.195 to 0.198 meq/kg, minimum and maximum. Peroxides are the precursors of breakdown products that cause rancid flavors in fat. Lipid oxidation produces off-flavor components called

hydroperoxides and peroxides which react chemically with fat-soluble vitamins rendering them nutritionally useless. Oils with peroxide values well below 10 meq/kg are considered fresh. When it is above 30 meq/kg, a rancid flavour becomes apparent (Gilbraith *et al*, 2021). Important factors influencing peroxide formation include oxygen, fatty acid composition, temperature, and the presence of metals.

Texture

The textural properties of bars were tested as Texture Profile Analysis (TPA) (*two-cycle compression test*) using a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), with a 50-kg load cell. A 75-mm-diameter compression platen was used with a speed of 1 mm/s throughout the process. A blade set (HDP/BSW) was used with a speed of 2 mm/s to measure the hardness of the bar. Texture value w.r.t. hardness was recorded maximum in T5 (6918.73 kg force) and minimum in Control (C) (3761.66 kg force) and the hardness of the bar increases with the increase in quantity of added FPP (Table 2). The texture profile method has been widely used as the standard method (ISO 11036) (Kohyama, 2020). The addition of protein affects the texture very much, a small quantity of protein may cause the formation of a liquid and ductile bar mass. On the other hand, overdosing the protein will result in a loose and crumbling structure (De la Fuente, 2002).

Colour Analysis of Nutrition Bar

Details of L^* , a^* & b^* values in the nutrition bar are given in Fig.3. In between the treatments, significantly ($P<0.05$) high L and a' value was recorded in T5 followed by T4, T3, T2, T1 and control (C) in reducing order, which indicates that when the amount of extracted fish protein powder increased in the nutrition bar, the product became lighter in colour and tint of the bar

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Fig. 3 Colour profile of bar control (C) and enriched with fish protein powder (T1-T5)

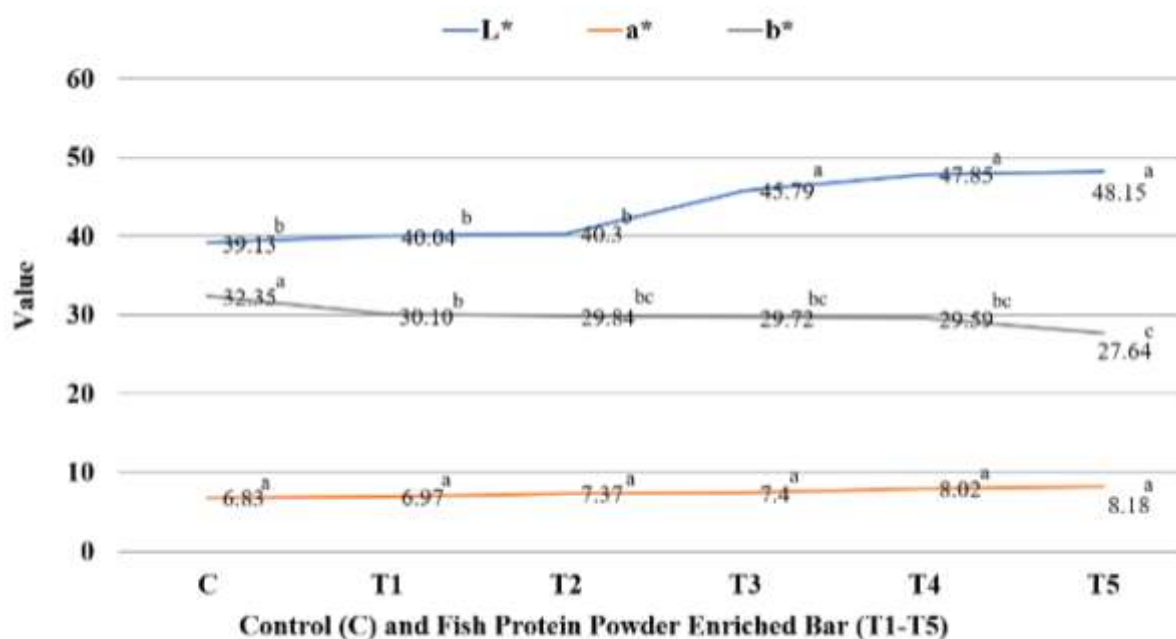
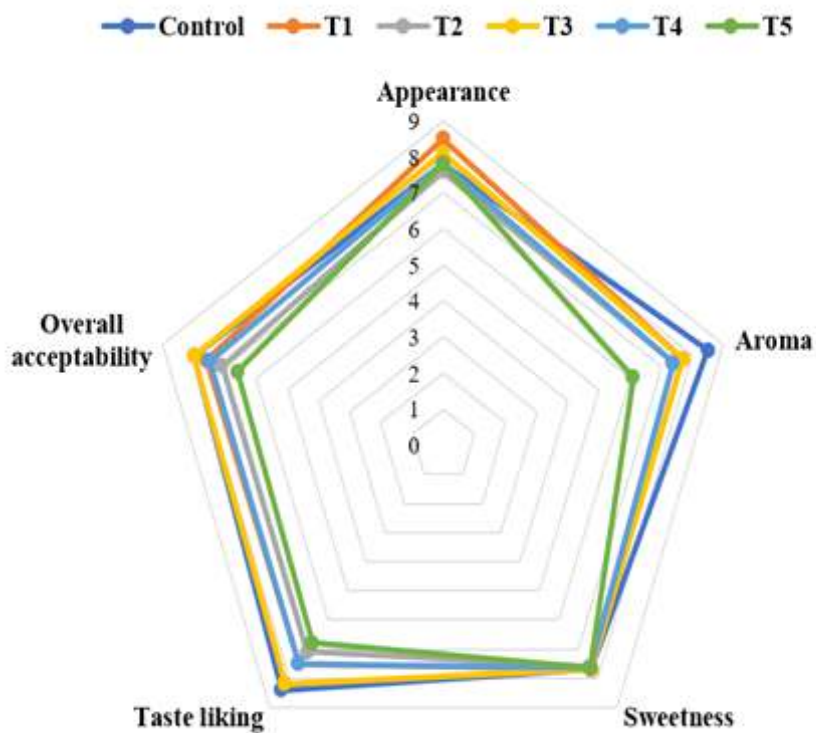


Fig. 4 Sensory attributes of bar control (C) and enriched with fish protein powder (T1-T5)



shifted from red to the greener side. The 'b' value in between the treatments reduced significantly ($P < 0.05$) and the value was maximum in control (C) followed by T1, T2, T3, T4, and T5 in reducing order, which indicated that the addition of extracted fish protein powder significantly decreased the 'b' value in the nutrition bar. The surface colour of a food product is the first quality parameter that is observed by the consumers and based on colour of the surface, consumers will accept or reject a food product (Leon *et al*, 2006).

Sensory Properties of Nutrition Bar

The sensory acceptability of bars was evaluated based on colour appearance, fishy aroma, sweetness, taste liking, and overall acceptability. Overall highest acceptability was recorded for control (C) and T3, followed by T4, T1, T2, and the least acceptability was recorded for T5. Details of the sensory properties of the nutrition bar are given in Fig 4. Akram *et al* (2020), studied the hedonic response of bars enriched with encapsulated fish and flaxseed oil and studied sensory attributes like colour, taste, flavour, crispiness, and overall acceptability based on a nine-point hedonic test scale. In the present study, the highest acceptability of the FPP incorporated bar was 7.5% (T3) and the protein content was also relatively good. Although protein content was maximum and carbohydrate was minimum in T5 and based on sensory parameters it was not much acceptable.

CONCLUSION

The nutrition bar enriched with FPP is acceptable, among nutrition bars, the highest acceptability was recorded for T3 (7.5% fish protein powder) as compared to control. In T3, protein content was recorded as 20.49%, fat 11.31%, ash 2.73%, and carbohydrate 59.21%. The protein and ash were 48.5% and 8.7% higher, respectively, whereas fat and carbohydrate were 7.6% and 9.6% lower, respectively as compared to control (C) Fish protein bars can play an important role in mitigating the issue of malnutrition among nutrition, particularly protein deficient population of the society.

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Abbreviations

FPP	-	Fish Protein Powder
PEM	-	Protein-energy malnutrition
WHO	-	World Health Organization
SBs	-	Snack bars
FFA	-	Free Fatty Acids
PV	-	Peroxide Value
ANOVA	-	Analysis of variance
TPA	-	Texture profile analysis
kg	-	Kilogram
ISO	-	International Organization for Standardization
%	-	Percentage

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