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Formulation and development of spirulina enriched high protein bars for athletes

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Abstract
An athlete requires physical strength, agility, and stamina, and protein is a crucial factor for assessing sports performance. Protein helps in building and repairing muscles, which is necessary for recovery and enhancing performance. Studies suggest that 10g to 25g of high-quality protein is enough for maximum effect. A varied diet usually fulfills the protein requirement. The recommended dietary allowance of protein is 0.8g/kg/day for sedentary individuals. Athletes, marathon runners, and bodybuilders need a higher protein intake of up to 1.5 to 2 gm/kg/day, depending on age and activity level. 20 to 30% of calorie intake should be proteins.
Aim: The aim of the study is to formulate and develop protein bars infused with functional foods like spirulina, which will be high in protein, targeted at the sportsperson or athletes to meet their requirements.
Methodology: 5 spirulina-based protein bar combinations were created using high-protein and high-fibre ingredients such as whey protein isolate, inulin, and erythritol. Each serving of the protein bars ranged from 50-80g, providing 15%-30% of energy from protein, at least 3-5g of fibre, and 1-2g of spirulina. The recipes varied with sesame seeds, flax seeds, chia seeds, puffed rice, and almonds. The protein bars underwent standardisation in three phases. A semi-trained panel of nutrition experts evaluated the bars using a 9-point hedonic rating scale in each phase, and recipe modifications were made accordingly. In phase 3, the chocolate-coated almond protein bar was deemed the most acceptable and underwent shelf life evaluation, chemical, physical, and microbiological analysis, with data analysed using SPSS version 20.
Results: In Phase 1, five energy bars were evaluated for taste, texture, and color, with S-E receiving the highest mean taste rating, followed by S-A, S-D, S-B, and S-C. Overall, S-A, S-D, and S-E were found to be the most acceptable. In Phase 2, S-C (Spirulina + Almonds) was rated highest in taste and texture, making it the best option in terms of overall acceptability. However, S-A had the best color. From all three phases of sensory evaluation, S-A (Spirulina + Almonds - chocolate coated) had the highest mean score for all four sensory attributes, with a ranking scale score also the highest. The protein bar showed no major changes during the 30-day shelf-life analysis, and the taste was acceptable.
Conclusion: The protein bar S-A (Spirulina +Almonds- chocolate coated), which is high in protein and fibre is beneficial for the performance and to meet the protein requirements for athletes. The protein S-A (Spirulina + Almonds – chocolate coated) is not only a healthy on the go snack option meeting the high protein requirements for athletes, but it is also very filling and helps manage the hunger pangs, while keeping the person satiated for long period of time.

Keywords: athletes, spirulina, protein bars, high fibre, high protein

Introduction
Athletes, defined as individuals who participate in organized sports that require regular competition, intensive training, and value excellence, may require more protein intake than the recommended daily amount to promote muscle protein synthesis and support improved functioning and adaptation to exercise. Athletes are individuals who participate in organized sports that require regular competition, intensive training, and value excellence. The training and competition required for athletes put significant stress on their bodies, and the nutrition needs of athletes differ from those of sedentary individuals. Adequate nutrition is crucial for athletes to perform at their best, and protein is one of the essential nutrients that athletes require in larger amounts than the recommended daily intake to promote muscle protein synthesis and support improved functioning and adaptation to exercise (Phillips, 2015) [10].
Biochemical changes before, during, and after exercise are important for athletes' physiological control because blood responses in exercise physiology are permanent elements to bring exercise performance and competition to a high level. Athlete nutrition has been shown to have a beneficial impact on athletic performance and is the most important factor in achieving optimum-level response (Thomas *et al*., 2016) [16]. Moreover, a balanced diet, including the appropriate macronutrient distribution, is important in improving athletic performance (Stellingwerff & Cox, 2014) [15].
The goal of post-workout nutrition is to recover lost energy. Carbohydrates help maintain blood glucose levels during exercise as well as muscle glycogen replenishment and rapid recovery after exercise. Moreover, it is recommended to consume simple carbohydrates with high glycaemic indexes for glycogen synthesis, which plays an important role in athletic performance. (Elaine C. Lee *et al*., 2017) [6]
Protein is not the only essential nutrient for athletes, and carbohydrates also play a vital role in athletic performance. The goal of post-workout nutrition is to recover lost energy, and carbohydrates help maintain blood glucose levels during exercise as well as muscle glycogen replenishment and rapid recovery after exercise (Betts *et al*., 2015) [3].

Moreover, it is recommended to consume simple carbohydrates with high glycaemic indexes for glycogen synthesis, which plays an important role in athletic performance (Jeukendrup & McLaughlin, 2011) [7].

Apart from carbohydrates and protein, other nutrients such as vitamins and minerals also play a crucial role in athletic performance. Spirulina platensis is a nutrient-dense microalgae that contains a wide range of nutrients, including protein, carbohydrates, fats, vitamins, minerals, and phytochemicals. Spirulina is a popular nutritional supplement for humans and is accompanied by claims for antioxidant and performance-enhancing effects. These claims are extrapolated by the findings of in *vitro* and animal studies but have not been substantiated concerning humans (Sadeghi *et al*., 2022) [12].

Spirulina platensis is a type of blue-green algae that is widely used as a nutritional supplement due to its rich nutrient profile. It contains a high amount of protein (55-70%), carbohydrates (30%), and fats (8%) that include various essential fatty acids such as linoleic acid, oleic acid, gamma-linolenic acid, eicosapentaenoic acid, stearidonic acid, arachidonic acid, and docosahexaenoic acid. In addition, it also contains amino acids such as Lysine, methionine, and cysteine, vitamins like E, D, B, ascorbic acid (especially B12), minerals such as iron, potassium, zinc, calcium, selenium, magnesium, chromium, copper, phosphorus, manganese, and sodium, dietary fibers (3%), phytochemicals, and phycocyanin pigment. Spirulina is known for its antioxidant and performance-enhancing effects, which are supported by in *vitro* and animal studies. However, there is still a need for further research to substantiate these claims in humans. (Sadeghi *et al*., 2022) [12]

Nowadays, spirulina is a very popular nutritional supplement for humans and is accompanied by claims for antioxidant and performance-enhancing effects. These claims are extrapolated by the findings of in *vitro* and animal studies but have not been substantiated concerning humans. (Boukary *et al*., 2021) [4]

Recommended dietary allowance of protein is 0.8g/kg/day for sedentary individuals. 20 to 30% calories of your food intake should be proteins, athletes, marathon runners, body builders will require higher protein consumption up to 1.5 to 2 gm/kg/day depending on age and type of activity performed. The demands of physical exertion mean that protein requirements for athletes and active adults are now accepted as being greater than sedentary populations and those described by population reference intakes of ~0.8 g protein per kg of body mass per day (g/kg/day). Recent scholarly reviews and the latest guidelines for nutrition and athletic performance from the American College of Sports Medicine suggest intakes ranging from 1.2–2.0 g/kg/day whether performing aerobic or resistance exercise, with the higher range of intakes appropriate for the latter. Specific situations of hypo energetic diet or injury likely require greater protein intakes to preserve lean body mass (LBM), which is often paramount in athletes. The optimal dose of protein per meal is 0.25–0.40 g/kg when aiming to maximally stimulate muscle protein synthesis, a marker of repair, growth, and adaptation, but multifactorial interactions between protein source, meal timing and pattern of distribution, and macronutrient co-ingestion around exercise influence recommendations on a meal-by-meal basis. (Mariana Lares-Michel *et al*.,2022) [9].

According to a study conducted by (Gupta *et al*. in 2020), not many brands provide protein bars that have a good nutritional profile, and even if they do, not all bars are healthy. Most bars contain unhealthy ingredients such as artificial preservatives, high fructose corn syrup, sugar, flavourings, food colour, palm oil, etc., which pose a threat to the human body and can cause more harm than good. These ingredients increase the risk of developing insulin resistance, diabetes mellitus type 2, obesity, visceral adiposity, dyslipidaemia, fatty liver, and cardiovascular diseases. Therefore, it is essential to read the label and choose protein bars that contain healthy ingredients and functional food.

Hence, there is a need to develop bars using nutritious ingredients, which aim to make the bars high in protein, high in fibre and low in GI. The use of ingredients like Spirulina, Inulin, erythritol, whey protein isolate, peanut butter, nuts and oilseeds like flaxseeds, chia seeds, pumpkin seeds, sunflower seeds, will help formulae and develop, novel, unique and nutritious bars.

Aim

The aim of the study is to formulate and develop protein bars infused with functional foods like spirulina, which will be high in protein targeted to athletes for meeting their nutritional requirements for better performance.

Materials and methods

Study area

Food Product Development: Development of spirulina enriched protein bars.

Sample size

5 recipes for protein bars, high in protein and high in fibre were formulated and tested, out of which the best 3- recipes were taken for further evaluation for sensory attributes and finally one best protein bar was taken for shelf life based sensory evaluation and for physical, chemical and microbiological analysis.

For sensory evaluation sample size of maximum 10 semi trained panel (each phase had a same number of panel members) members from the field of nutrition, were recruited.

Inclusion criteria for panellists

The semi-trained panellists from the field of nutrition were recruited.

Study duration

The study was completed within 1 year from the date of receiving ethical approval.

Tools for data collection 9-point hedonic rating scale

A great tool for conducting a food product's sensory evaluation is the 9-point hedonic rating scale. The hedonic rating scale, which is used for phases 1, 2, and 3 as well as the shelf-life analysis. Hedonic scale used to rate how enjoyable or unpleasant an experience was. This test is used to gauge how well-liked food products are. The panel members are presented with the food products and asked to rate the product's acceptability on a scale of 1 to 9, with 1 denoting a strong dislike and 9 denoting a strong liking. The outcomes are then examined and results are then analysed.

Ranking scale

The ranking scale is a fantastic tool for determining which food product is the best. Appendix G mentions the ranking scale that was applied during standardisation phase three. When it's necessary to choose the best sample among those put in front of the panellists, this test is used. The panel members are presented with the food products and asked to rank them from best to worst, starting with first. The best product is then selected after careful analysis of the findings.

Study design ideation and prototype creation

To develop nutrient-dense protein bars infused with spirulina, the first step was to conduct a market survey to evaluate the nutrition labels of protein bars available in the market. The survey found that the average protein and fibre content of commercial protein bars is 11.6% and 1-2 grams, respectively, indicating a need for high-protein, high-fibre, and low-glycaemic index protein bars for the general adult population. Using Indian Food Composition Table (IFCT), the nutritional value of five different protein bar combinations was calculated, with functional foods and nutritional components such as spirulina, whey protein isolate, peanut butter, whole nuts and oilseeds like almonds, flaxseeds, inulin (for fibre), and a generally recognized as safe (GRAS) sweetener like erythritol being included. The developed recipes aimed to provide 15-30% of energy from protein and at least 3-5 g of fibre per serving. Standardized and modified recipes were created accordingly.

Development of protein bars and standardization of the recipes

The protein bars' entire ingredient list was purchased online. Along with ingredients like peanut butter, inulin, erythritol, whey protein isolate, spirulina, etc., each protein bar contained either a nut, such as an almond, an oilseed, such as a sesame seed, or a cereal, such as puffed rice. The cereal, nuts, and oilseeds were precisely weighed, dry-roasted, and, if necessary, chopped into smaller pieces. The nuts, seeds, and cereal were added to the other ingredients, which had been carefully weighed and combined in a bowl. For Phase 1 of standardisation, the mixture was placed in an aluminium rectangular bar mould to achieve the desired shape, and it was then placed in the refrigerator to set for ten to fifteen minutes. In phase 2 and 3, the steps mentioned during phase 1 were repeated as with best of 3 bars and then best of 1 bar as done previously.

Post preparation in the respective phases, the protein bars were ready for the sensory evaluation by the panel members. a brief explanation of the 3 phases of standardization is given below standardization of recipes and phases of standardization

A standardised recipe is one that has been formulated, developed, adapted and retried several times and will produce the same good results and yield when produced using the same quality ingredients, quantity and method of preparation.

Phase 1

- Five Recipes were formulated, keeping certain ingredients like Spirulina, whey protein, inulin, peanut butter, erythritol constant.
- The formulated recipes were developed.
- The recipes were evaluated for the desired sensory attributes using a 9-point hedonic rating scale, by ten-semi-trained panel members and the bars were selected on the bases of all the sensory parameters including the overall acceptability score and considered for the next phase i.e. phase 2.

Phase 2

- Required modifications both in terms of proportion and method of preparation, i.e., changing the amount of Erythritol sweetener by reducing it to 5g from 10g, were done to the three recipes till the desired sensory attributes are obtained.
- The recipes were evaluated for desired sensory attributes using a 9-point hedonic rating scale, by ten trained same panel members and the bars were selected on the bases of all the sensory parameters including the overall acceptability score and considered for the next phase i.e. phase 3.
- The best one recipe, out of the prepared three, containing the desired sensory attributes, were selected for phase 3.

Phase 3

- According to the results of phase 2, one best recipe in two forms (chocolate coated & not chocolate coated) based on best sensory attributes were selected for sensory evaluation using a 9-Point hedonic rating scale, by 10 semi trained panel members.
- The panel members were also asked to rank the recipes using a ranking scale, based on the most acceptable to the least acceptable protein bar.
- Finally, one protein bar was chosen as the best protein bar, and was taken ahead for the shelf-life based day 0, 15 and day 30 sensory evaluation along with chemical, physical and microbiological analysis.

Sensory evaluation and product modification

10 nutrition experts conducted a sensory evaluation of the developed protein bars using a 9-point hedonic rating scale. The panellists were given instructions on how to conduct the sensory evaluation and were provided with protein bar samples, cutlery, tissues, and filtered water. They evaluated the protein bars' taste, texture, appearance, and general acceptability by taking a bite of each protein bar and filling out a sensory evaluation form. To avoid any flavour crossover, they were instructed to rinse their palates with water after trying each bar. The recipes were modified based on the feedback received, and the sensory evaluation was repeated two more times. The panel members were also asked to rank the protein bars from best to least favourite. The best protein bar, Spirulina + almonds with chocolate coat (S.A.), was subjected to chemical, nutrient, microbiological, and shelf-life analysis.

Shelf life

Upon evaluation and ranking of the best protein bar in phase 3 of standardization and sensory evaluation.by the panel member, protein bar S.A (table 3.4) was selected and the shelf-life analysis. The shelf life of that protein bar was conducted, i.e. the sensory attributed of the protein bar were checked on day 0, 15 and day 30 from the day of production of the protein bar. 2 sets of the selected protein bar were produced an on the day of production, the protein bars were

sealed in LDPE aluminium foil bags, and was delivered to the outsource for further evaluation for 15 and 30 days respectively.

Compositional analysis

Composition of PE-bars including moisture, protein and lipid contents were determined by following their prescribed methods of AOAC (Association of Analytical Chemist, 2012) and the results were expressed on a dry-weight basis.

Nutritional analysis

Nutrient analysis involves determining the nutrient content of food or beverages. This helps consumers and manufacturers make informed decisions about dietary goals and regulatory requirements. A protein bar's nutrient analysis determines the amount of protein, fat, carbohydrates, vitamins, and minerals. The analysis involves laboratory testing and helps calculate nutrient content per serving. A protein bar SA(Spirulina + almonds-chocolate coated) contained Calories (266 kcal), Total Fats (15.67g), Saturated Fat (2.7g), Unsaturated Fat (12.56g), Trans Fat (nd<0.5g), Total Carbohydrates (21.71g), Protein (10.3g), Cholesterol (0mg), Sodium (45.65mg), Dietary Fibre (4.4g), and Total Sugar (12.2g).

Chemical analysis

Chemical analysis is crucial for evaluating the quality and safety of protein bar. The protein bar involved testing various parameters, such as moisture content, acidity, and total sugar content, among others, to ensure regulatory compliance and desired quality specifications. Comprehensive chemical analysis helps to identify potential adulteration or contamination of the protein bar with harmful substances. Parameters considered for chemical analysis of the protein bar SA(Spirulina + almonds-chocolate coated) included moisture content, acidity, total sugar content, and other relevant factors. Manufacturers and consumers rely on this information to ensure thesafety and quality of the product. Thus, conducting comprehensive chemical analysis is an essential component of evaluating protein bars.

Microbiological analysis

Microbiological testing is conducted to evaluate the safety and quality of the protein bar. Tests include total plate count, yeast and mold, E.coli, total coliform, Salmonella spp., and Staphylococcus aureus. The methods used for testing include IS 5402:2012; 2018, IS 5403:2012; 2018, IS 5887(I) 1976; 2018, IS 5887(III) 1999; 2018, and IS 5887(VIII-I) 2002; 2018. These tests are crucial for maintaining the quality and safety of protein bars and ensuring they meet regulatory standards. They provide information on microbial contamination levels that can affect shelf life, safety, and overall quality.

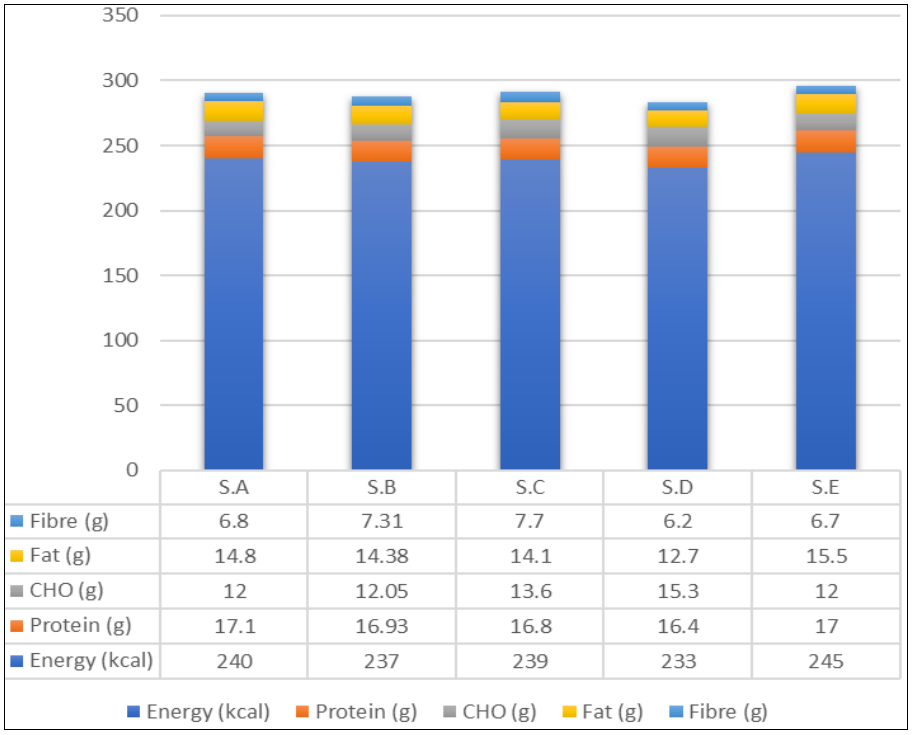
Statistical analysis

Data was analysed using SPSS version 20. Descriptive Analysis done to calculate Mean and Standard deviation of sensory characteristics of bars. Paired sample T test were used to compare between different bars. Significance for all the statistical measure was determined at 0.05 level.

Results and discussion

Nutrient composition of protein bars

Along with whey protein as a source of protein, inulin as a source of fibre, and erythritol as a sweetener, spirulina served as the protein bars' primary functional ingredient. Along with either cereals like puffed rice or nuts and oilseeds like almonds, chia seeds, or sesame seeds, among others, the protein bars also included nut butter, specifically peanut butter, as a source of healthy fat. A rough nutritional breakdown of the protein bars' ingredients, as determined by IFCT, is shown in figure 4.1. Products that weren't mentioned in the IFCT, like zero-carb whey protein isolate, were referenced using the nutritional information on the food's packaging.



	S.A	S.B	S.C	S.D	S.E
Fibre (g)	6.8	7.31	7.7	6.2	6.7
Fat (g)	14.8	14.38	14.1	12.7	15.5
CHO (g)	12	12.05	13.6	15.3	12
Protein (g)	17.1	16.93	16.8	16.4	17
Energy (kcal)	240	237	239	233	245

Fig 1: Proximate nutrient composition of the protein bars per serving

Figure 1 Graphical Representation of the Proximate Nutrient Composition of the Protein bars per Serving. Here the codes are as follows : - S-A (Spirulina + Sesame seeds), S-B (Spirulina + Flax Seeds), S-C (Spirulina + Chia Seeds), S-D(Spirulina + puffed rice), S -E(Spirulina + Almonds)

From figure 1 it was noted that The table displays the nutritional content of five different protein bars labeled as S.A through S.E. The columns include energy content in kcal, protein, carbohydrates (CHO), fat, and fiber content in grams (g). Energy content ranges from 233 kcal (S.D) to 245 kcal (S.E). Protein content ranges from 16.4 g (S.D) to 17.1 g (S.A). CHO content ranges from 12 g (S.A and S.E) to 15.3 g (S.D). Fat content ranges from 12.7 g (S.D) to 15.5 g (S.E). Fibre content ranges from 6.2 g (S.D) to 7.7 g (S.C). A total of 5 recipes were formulated, developed, and standardized in 3 phases. Sensory evaluation was carried out using a 9-Point hedonic rating scale for phases 1, 2, and 3. Additionally, a ranking scale was used for phase 3 to determine the best protein bar produced. Overall, the table provides a useful comparison of the nutritional content of different food products.

Sensory evaluation scores of all the developed protein bars

A group of 10 panel members from the field of nutrition who were semi-trained performed the sensory evaluation of the products. There were three standardisation phases. Each panellist tasted the various protein bars that were presented to them during each phase of standardisation to assess their sensory qualities and, if necessary, make suggestions for changes to the protein bars.

The protein bars were altered prior to each phase in accordance with any suggestions made by the panellists, and a new sensory evaluation was conducted. Three phases in total were carried out. To identify the best protein bar produced, the panel members were asked to rate the products using a 9-Point hedonic rating scale in each phase, and in phase 3, they were also asked to rate the products using a ranking scale. The protein bars were assessed for their taste, texture, overall acceptability, and appearance/colour.

The following are the results of phase 3 of standardization.

Phase 3 Hedonic rating scale

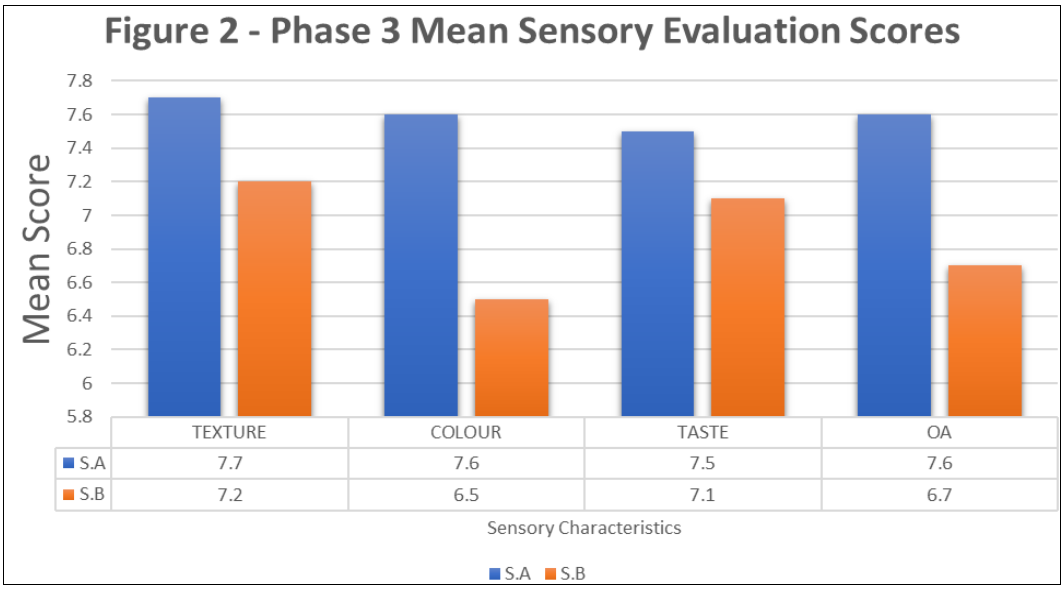


Fig 2: Graphical Representation of the phase 3 mean sensory evaluation scores

Here the codes are as follows: - SA (Spirulina + Almonds- chocolate coated), SB (Spirulina + Almonds- not chocolate coated)

Figure 2 shows mean scores on a 9-point hedonic rating scale for four sensory attributes (texture, colour, taste, and overall acceptability) of two protein bar samples (S.A and S.B). Sample S.A (Spirulina + almonds-chocolate coated) received higher ratings across all sensory attributes compared to sample S.B (Spirulina + almonds- non-chocolate coated). Sample S.A was rated as firm (7.7), attractive (7.6), palatable (7.5), and very acceptable (7.6), whereas sample S.B received lower ratings, indicating that it was moderately firm (7.2), less attractive (6.5), palatable (7.1), and acceptable (6.7).

Table 1: Paired Sample T-test for all the sensory characteristics evaluated for the protein bars in Phase 3 Here the codes are as follows : - SA (Spirulina + Almonds- chocolate coated), SB (Spirulina + Almonds- not chocolate coated)

		Mean	N	Std. Deviation	t	Sig. (2-tailed) (p value)
1	Taste SA	7.50	10	.850	1.809	.104
	Taste SB	7.10	10	.738		
2	Texture SA	7.70	10	.823	1.627	.138
	Texture SB	7.20	10	.789		
3	Colour SA	7.60	10	.966	3.161	.012
	Colour SB	6.50	10	.527		
4	Overall Acceptability SA	7.60	10	.699	5.014	.001
	Overall Acceptability SB	6.70	10	.422		
* p value is <0.05 i.e., statistically significant						

Tables 1 showed the paired sample T – Test scores for the sensory evaluation of protein bars in phase 3 of standardization, for taste, texture, appearance/ colour and overall acceptability of protein bars SA and SB. Although there was no significant difference in taste and texture between the two bars, bar SA was preferred for its color (mean rating of 7.6 compared to 6.5 for bar SB, p = .012) and overall acceptability (mean rating of 7.6 compared to 6.7 for bar SB, p = .001). As a result, bar SA (Spirulina + Almonds-chocolate coated) was selected as the best bar for shelf life, physical, and microbiological analysis.

Phase 3 Ranking scale

Table 2: Paired Sample T-test for the rank test for the protein bars in Phase 3

		Mean	N	Std. Deviation	t	Sig. (2-tailed) (P value)
1	S-A	1.20	10	.422	-2.250	.051
	S-B	1.80	10	.422		

* p value is <0.05 i.e., statistically significant

In the rank test, as seen in table 2, two samples were given to the panel members, and they had to rank the products on a scale of 1 and 2 with the best product being given the rank 1 and the least desirable product being given the rank of 2.
Table 2 shows the results of the paired sample t-test for the rank test conducted on the protein bars in Phase 3. The mean, standard deviation, t-value, and significance (p-value) are reported for the two bars (S-A and S-B) tested. The results indicate that there was a statistically significant difference between the mean rank scores of bars S-A and S-B (t = -2.250, p = .051). The mean rank score for bar S-A was 1.20, while the mean rank score for bar S-B was 1.80. This suggests that bar S-A was ranked higher compared to bar S-B.

Hence protein bar S- A (Spirulina + Almonds chocolate coated), was the best protein bar.

Shelf-life analysis at day 0, 15 and 30

The shelf life analysis was carried out at day 0, 15 and day 30, using a 9 – point hedonic rating scale. The bars were stored in LDPE (Low Density Polyethylene) aluminium foil bags. Overall, there was no change in taste, colour, and overall acceptability as noted by the outsource, and the protein bars had a safe for consumption shelf life of 30 days at ambient temperature, when kept sealed in LDPE (Low Density Polyethylene) aluminium foil bags.

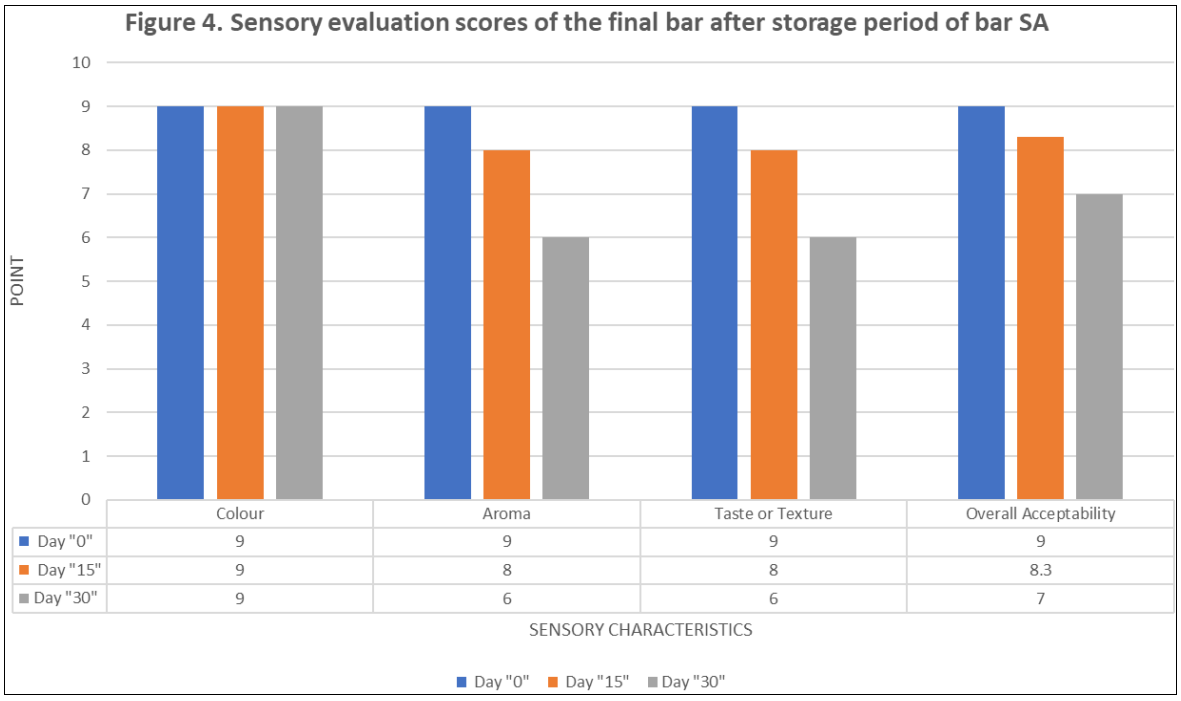


Fig 4: Graphical Representation of Hedonic rating scale values for Sensory characteristics for the final protein bar during shelf - life analysis at day 0, day 15 and day 30

Here the codes are as follows: - S-A (Spirulina + Almonds-chocolate coated)
In Fig 4 presents a comparison of specifications and results of organoleptic testing of food and agricultural products on day 0, day 15, and day 30. The parameters considered for testing are colour, aroma, taste or texture, and overall acceptability. The minimum specification for all parameters is 5.

On day 0, the results for all parameters meet the minimum specification of 5, with colour, aroma, taste or texture, and overall acceptability scoring 9.
However, on day 15 and day 30, the scores for aroma, taste or texture, and overall acceptability fall but are above the minimum specification of 5 on day 15 and 30, indicating that the quality of the food and agricultural products is good and fit for consumption.

Nutrient analysis of the final bar- sa (spirulina + almonds- chocolate coated)

Table 3: - Nutritional analysis of the protein bar –SA (Spirulina + Almonds- chocolate coated)

	Nutritional facts per 50gms	Bar weight - 50g	
Sr no	**Parameters**	**Result**	**Units**
1	Calories	266	kcal
2	Total Fats	15.67	Grams
3	Saturated Fat	2.7	Grams
4	Unsaturated Fat	12.56	Grams
5	Trans Fat	nd<0.5	Grams
6	Total Carbohydrates	21.71	Grams
7	Protein	10.3	Grams
8	Cholesterol	0	mg
9	Sodium	45.65	mg
10	Dietary fibre	4.4	Grams
11	Total Sugar	12.2	Grams

The table 3 displays the nutritional information of protein bar SA, with a weight of 50 grams. It has 11 parameters: Calories (266 kcal), Total Fats (15.67 grams), Saturated Fat (2.7 grams), Unsaturated Fat (12.56 grams), Trans Fat (nd<0.5 grams), Total Carbohydrates (21.71 grams), Protein (10.3 grams), Cholesterol (0 mg), Sodium (45.65 mg), Dietary fibre (4.4 grams), and Total Sugar (12.2 grams). All the results are per 50 grams of the food bar.

Chemical and Microbiological Analysis

Table 5: Shelf Life, chemical and microbiological analysis of the protein bar SA (Spirulina + Almonds- chocolate coated) for day "0", "15" and "30"

Sr. No	Parameters	Result on Day "0"	Result on Day "15"	Result on Day "30"	Unit	Specification
1. Discipline - Chemical Testing **1. Group** - Food & Agricultural products						
1	Fungus Spot	Absent	Absent	Absent	__	Absent
2	Acidity Of Extracted Fats	ND<0.01	0.42	0.47	%	NMT - 2.0
3	Moisture	3.15	3.27	4.02	__	__
2. Discipline - Biological Testing **1. Group** - Food & Agricultural products						
1	Total Plate Count	0.4×10^1	4.8×10^1	2.56×10^2	cgu/g	Max - 100000
2	Yeast n Mold	<10	<10	<10	cgu/g	Max - 100
3	E. Coli	Absent	Absent	Absent	cfu/g	Shall be Absent
4	Total Coliform	Absent	Absent	Absent	cfu/g	Shall be Absent
6	Salmonella spp.	Absent	Absent	Absent	cfu/g	Shall be Absent
7	Staphylococcus aureus	Absent	Absent	Absent	cfu/g	Shall be Absent

The table 5 shows the results of chemical and biological testing were conducted on the protein bar SA (Spirulina + Almonds-chocolate coated) on day 0, 15, and 30. The protein bar met all the specifications for chemical and microbiological analysis on day 0, and also on day 15, with a slight increase in acidity and moisture content and total plate count. On day 30, the protein bar sustained the shelf-life test for all parameters and met the specifications for fungus spots, total plate count, yeast and mold, and absence of harmful bacteria. The protein bar should have a "Best Before Date of 30 Days" from the date of manufacturing at room temperature condition.

Discussion

The protein bar industry has grown substantially in recent years, with consumers looking for convenient and healthy snack options This study aimed to develop protein bars using spirulina as the primary functional ingredient along with other ingredients such as whey, inulin, erythritol, nuts, and oilseeds which provides a unique and appealing contrast in appearance and taste.

Spirulina was one of the main functional ingredients used in bars and was accepted by the panel members. In a study conducted by Saharan & Jood, 2021 [13], the taste of spirulina was also acceptable. Another Study by Batista de Oliveira *et al*., 2021 [2], showed the incorporation of spirulina in chocolates milk, and was accepted by the panel members. The present study also successfully incorporated spirulina in protein bars, and the protein bars were considered to be acceptable by the panel members.

Moreover, a study by Sołowiej *et al*. (2021) [14] found that the addition of inulin to protein bars increased their dietary fiber content and improved their texture and mouthfeel. Similarly, the use of erythritol as a sugar substitute in food products has been found to be beneficial in managing blood sugar levels and reducing the risk of dental caries (Mamatha *et al*., 2021) [8].

Whey protein powder was used as protein source in the protein bars, and was seen to be acceptable in terms of the sensory characteristics. A study by Childs *et al*., 2007 [5], suggests that, whey protein enhances the taste and overall acceptability, when added to protein bars, as it contains vanillin flavour, which is one of the most liked flavours by the consumers. The whey protein powder, selected for the current study, was also vanilla flavoured protein, which was one of the reasons why the protein bars had a good overall acceptability.

In phase 3, the protein bar S-A(Spirulina + Almonds-chocolate coated) was the best protein bar in terms of color and overall acceptability, which is consistent with the findings of a study by Ramesh and Nagarajan (2018) [11], which reported that chocolate coating improved the sensory acceptability of spirulina-based snacks.

The protein bar S-A (Spirulina + Almonds – chocolate coated) contains a combination of almonds, spirulina, whey protein isolate, erythritol, peanut butter, dark chocolate, and inulin, which provides a unique and appealing contrast in appearance and taste. The combination of appearance, texture, and taste in this protein bar made it the best among the options tested. Moreover, the crunchy texture of almonds in the S-A (Spirulina + Almonds-chocolate coated) protein bar was found to be an important factor in its taste and appeal.

The study concluded that spirulina-based protein bars could be a healthy alternative to traditional protein bars, and further research could explore different flavour combinations and other functional ingredients. The protein bar S-A with spirulina and almonds as key ingredients has potential benefits in the field of

sports nutrition and sports performance. The combination of these two ingredients, along with other components such as whey protein isolate, erythritol, peanut butter, dark chocolate, and inulin, make the protein bar S-A a promising option for athletes and fitness enthusiasts. Inulin in the protein bar S-A could also be a contributing factor to its appeal, as research has shown that inulin can have prebiotic effects and improve gut health. Additionally, the use of erythritol as a sugar substitute in the protein bar S-A could be beneficial for those who want to reduce their sugar intake.

This study also evaluated the microbiological quality of the protein bar. The results showed that the protein bar was free of fungus spots and harmful bacteria throughout the 30-day shelf-life period. The study found that there was a slight increase in acidity and moisture content, and the total plate count had increased on day 15 and day 30. However, the protein bar still met the specifications for chemical and microbiological analysis on day 15 and day 30, indicating that it was fit for human consumption.

Several studies have also evaluated the microbiological quality of different food products. For instance, a study by Zhang *et al.* (2020) [17] evaluated the microbiological quality of different types of sushi sold in retail stores. The study found that some types of sushi had high levels of bacteria, including Salmonella and Listeria monocytogenes. Another study by Ahmed *et al.* (2021) [1] evaluated the microbiological quality of raw chicken meat sold in retail stores. The study found that the chicken meat had high levels of bacteria, including Campylobacter and Escherichia coli.

This study evaluated the shelf-life and microbiological quality of a protein bar containing spirulina and almonds, coated with chocolate. The results showed that the protein bar was safe for consumption up to 30 days at ambient temperature. The study also evaluated the sensory and microbiological characteristics of the protein bar and found that it met the specifications for chemical and microbiological analysis

Conclusion

This study successfully developed a spirulina-infused protein bar targeted at athletes. The study involved formulating and standardizing five combinations of spirulina-based protein bars through three phases of hedonic rating evaluation by a panel of semi-trained nutritionists. The protein bar-coded S-A, which contained spirulina, almonds, and was chocolate coated, was considered the most acceptable by the panellists and was subjected to shelf-life, chemical and microbiological evaluation. The protein bar S-A had 2 g of spirulina, which is the daily recommended intake to see any functional benefits of spirulina, along with zero carbohydrate whey protein (protein source), inulin (fibre source), unsweetened peanut butter (fat source and binding agent), and almonds (a balanced source of MUFA and PUFA). The results of the chemical and biological testing analyses conducted on the protein bar-SA indicate that the product is fit for human consumption within the recommended limits of each parameter.

The protein bar S-A is not only high in protein and fiber but also beneficial for meeting the nutritional goals of athletes. It is a healthy on-the-go snack option that helps manage evening hunger pangs and keeps the person satiated for a long period of time. The study provides valuable information for ensuring the quality and safety of food and agricultural products, particularly protein bars. The chemical and biological testing is essential for ensuring the safety and quality of the product, and the results showed that the protein bar-S-A is safe for consumption for up to 30 days of manufacturing, when kept at an ambient temperature in (Low Density Polyethylene) aluminum foil bags.

References

1. Ahmed A, Rahman A, Rahman SMA, Farzana T. Microbiological evaluation of raw chicken meat sold in retail stores of Dhaka city, Bangladesh. Scientific reports,2021:11(1):1-9. https://doi.org/10.1038/s41598-021-82333-2
2. Batista de Oliveira L, Rodrigues Faria F, Lannes SCS. Milk chocolates with Spirulina platensis biomass: Physicochemical properties and consumer acceptance. Food Research International,2021:141:110-118. https://doi.org/10.1016/j.foodres.2020.110118
3. Betts JA, Williams C, Duffy K, Gunner F, Theodorescu D. Energy expenditure and intake during high-intensity intermittent exercise: the role of carbohydrate availability. International Journal of Sport Nutrition and Exercise Metabolism,2015:25(5):426-434. https://doi.org/10.1123/ijsnem.2014-0203
4. Boukary A, Ibrahim O, Issoufou M, Malam YA. The effectiveness of spirulina on athletes' performance and its side effects: A systematic review. Journal of Nutrition and Metabolism, 2021, 9872403. https://doi.org/10.1155/2021/9872403
5. Childs J, Yates LA, Drake MA. Modification of sensory properties of a whey protein fortified nutrition bar. Journal of Sensory Studies,2007:22(1):81-98. https://doi.org/10.1111/j.1745-459X.2006.00075.x
6. Elaine C, Lee K, Nieman DC, Manore MM, Henson DA. Post-exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glucose uptake and glycogen synthesis. Amino Acids,2017:49(4):609-618. https://doi.org/10.1007/s00726-017-2379-6
7. Jeukendrup AE, McLaughlin J. Carbohydrate ingestion during exercise: effects on performance, training adaptations and trainability of the gut. Nestlé Nutrition Institute Workshop Series,2011:69:1-12. https://doi.org/10.1159/000329276
8. Mamatha BS, Nivedhitha MS, Shravya K. Sugar substitutes–A review. Journal of food science and technology,2021:58(1):1-18. https://doi.org/10.1007/s13197-020-04506-3
9. Mariana Lares-Michel J, Vega-Vega O, González-Vargas LD, Medina-Inojosa JR. Protein intake in athletes and active adults: From requirements to optimal intake. Journal of Clinical Medicine,2022:11(3):455. https://doi.org/10.3390/jcm11030455
10. Phillips SM. Nutritional supplements in support of resistance exercise to counter age-related sarcopenia. Advances in Nutrition,2015:6(4):452S-460S. https://doi.org/10.3945/an.115.008367
11. Ramesh P, Nagarajan S. Microencapsulated spirulina incorporated snacks: An approach towards value addition. Journal of Food Science and Technology,2018:55(4):1264-1274. https://doi.org/10.1007/s13197-017-3011-x
12. Sadeghi A, Parsaeimehr M, Ebrahimi S, Ghalavand M. Spirulina platensis: A nutrient-rich microalgae with antioxidant and performance-enhancing effects. Journal of Dietary Supplements,2022:19(1):92-107. https://doi.org/10.1080/19390211.2021.1989218
13. Saharan BS, Jood S. Nutraceutical potential of Spirulina: A review. Journal of Food Science and Technology,2021:58(6):1847-1859. https://doi.org/10.1007/s13197-020-04566-5
14. Sołowiej BG, Kowalska H, Krystyjan M, Ziobro R. Effect of Inulin Addition on Nutritional and Sensory Properties of Whey Protein Bars. Foods,2021:10(1):109. https://doi.org/10.3390/foods10010109
15. Stellingwerff T, Cox GR. Systematic review: carbohydrate supplementation on exercise performance or capacity of varying durations. Applied Physiology, Nutrition, and Metabolism,2014:39(9):998-1011. https://doi.org/10.1139/apnm-2014-0027
16. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. Medicine and Science in Sports and Exercise,2016:48(3):543-568. https://doi.org/10.1249/MSS.0000000000000852
17. Zhang J, Li S, Liu T, Li J, He X, Li F, *et al*. Microbiological quality evaluation of retail sushi in Hangzhou, China. Food Control, 2020, 112, 107129. https://doi.org/10.1016/j.foodcont.2020.107129