International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 17-06-2022, Accepted: 02-07-2022, Published: 20-07-2022
Volume 7, Issue 3, 2022, Page No. 52-61

Formulation & development of spirulina enriched, high protein, high fibre and low glycaemic index protein bars

Simran Seth[1], Rekha Battalwar[2], Madhuri Nigudkar[2]
[1] Department of Food Nutrition and Dietetics, Sir Vithaldas Thackersey College of Home Science, Shreemati Nathibai Damodar Thackersey Women's University, Mumbai, India
[2] Associate Professor, Department of Food Nutrition and Dietetics, Sir Vithaldas Thackersey College of Home Science, Shreemati Nathibai Damodar Thackersey Women's University, Mumbai, India

Abstract
Introduction: The Indian Council of Medical Research (ICMR) recommends a daily protein intake of 0.8 to 1 g/kg body weight and a dietary fibre intake of 40 g/day for a 2000 Kcal diet for a healthy Indian adult. Recommendations are difficult to meet, hence there arises a need for a supplementary protein and fibre source to complement the diet.
Aim: The aim of the study was to formulate and develop protein bars infused with the functional food spirulina and other ingredients which made the protein bar high in protein and fibre, and low in Glycaemic Index (GI), targeted at the general adult population.
Methodology: 10 different combinations of spirulina-based protein bars were formulated, using ingredients high in protein & fibre and low in GI, like whey protein isolate, inulin, erythritol. Depending on the recipe formulated, per serving of the protein bar weighed between 50- 80 g, which provided 15% - 30 % of energy from protein, at least 3-5 g of fibre along with 1-2 g spirulina. For the recipes, spirulina was constant and the variations included sesame seeds, pumpkins seeds, watermelon seeds, flax seeds, watermelon seeds ,chia seeds, amaranths, makhana, puffed rice, almonds, respectively Standardisation of the 10 protein bars was done in 3 phases. In each phase the developed protein bars were put in front of semi trained panel members, from the field of nutrition, who evaluated the protein bars using a 9- point hedonic rating scale. At the end of each phase the recipes were modified, as per the suggestions, given by the panel members. In phase 1, all the 10 protein bars were accepted and certain modifications were suggested in the recipes. In phase 2, based on the results of the hedonic rating scale, 4 out of 10 protein bars were selected for phase 3. At the end of phase 3, the protein bar which had sesame seeds in it, was considered the most acceptable by the panellists, and was subjected to shelf-life evaluation. The post development shelf-life changes were noted at day 0, day 10 and day 20. Data was analysed using the SPSS version 20.
Results: From the 3 phases of sensory evaluation, protein bar S-B, i.e., the protein bar with sesame seeds and spirulina had the highest mean score for all the 4 sensory attributes i.e., taste (7 + 1.054), texture (6.80 + 1.317), colour (6.40 + 1.265), overall acceptability (6.60 + 1.265). The ranking scale score was also the highest, for the Protein bar S-B (Spirulina + Sesame Seeds). No major changes in the protein bar were observed during the shelf-life analysis of 20 days, the taste was acceptable.
Conclusion: The protein bar S-B (Spirulina + Sesame Seeds), which is high in protein and fibre and low in GI is beneficial for the management of lifestyle diseases like metabolic syndrome, obesity, cardiovascular diseases, type 2 diabetes mellitus, which are becoming a burden on the society. The protein S-B (Spirulina + Sesame Seeds) is not only a healthy on the go snack option, but it is very filling and helps manage the evening hunger pangs, while keeping the person satiated for long period of time.

Keywords: spirulina, protein bars, low glycaemic index, high fibre, high protein

Introduction

The Indian Council of Medical Research (ICMR) recommends a daily protein intake of 0.8 to 1 g/kg body weight and a dietary fibre intake of 40 g/day for a 2000 Kcal diet for a healthy Indian adult. (ICMR, 2020). But it is generally seen that these recommendations are difficult to meet, hence there arises a need for a supplementary protein and fibre source to complement the diet (Sharma et al., 2020).
Protein bars are nutritious bars, which are a convenient high protein source. Due to their versatile nature, great nutritional profile, taste and texture, they are liked by all age groups. Unfortunately, looking at the current bars available in the Indian market, not many brands provide protein bars that have a good nutritional profile, and even if they do, not all of them have healthy ingredients and functional food in them. The bars are usually loaded with artificial preservatives and ingredients like high fructose corn syrup (HFCS) (Basciano et al., 2005), sugar,

flavourings, food colour, palm oil, etc. These ingredients generally pose a threat to the human body and end up causing more harm than good (Morenga et al., 2013) some of them being at an increased risk for developing insulin resistance, diabetes mellitus type 2, obesity, visceral adiposity, dyslipidemia, and fatty liver, cardiovascular diseases (CVD) to name a few (Stanhope et al., 2009)

Functional foods offer health benefits and have an extended nutritional profile. Spirulina is a functional food that is rich in high quality protein and lipids (Yifan et al., 2020) and has many functional benefits like having anti–obesity, anti-hypertensive, anti-diabetic and antioxidant properties(Machowiec et al., 2021) (Ogechi et al., 2021). Spirulina is a blue-green algae that is also termed a functional food (Liestianty et al., 2019) It is very potent, rich in high quality protein and lipids, has valuable bioactive compounds like polyunsaturated fatty acids (PUFA), carotenoids and vitamins, it is also rich in valuable pigments (Yifan et al., 2020) Spirulina has many functional benefits like, having anti – obesity, anti-hypertensive, anti-diabetic and anti-oxidant properties. Spirulina is also very useful for the prevention and treatment non communicable lifestyle disorders, such as metabolic syndrome, CVD, diabetes mellitus, obesity. Spirulina is also said to benefit in lowering the blood pressure, as seen in a studies by Machowiec et al., 2021 and Ogechi et al., 2021. The daily recommended safe to consume dosage of spirulina is 2-3g/day, and in order for spirulina to have any functional benefit in the body, an healthy adult must consume at least 1-2g of spirulina per day. Since spirulina has a fishy taste and odour, it is often difficult to consume it due to its decreased palatability. To overcome this, a variety of spirulina, called blue spirulina, also known as Phycocyanin extract spirulina, this is a neutral tasting, odourless version of spirulina, having the same functional benefits and has a good nutritional profile is used when it comes to develop food products infused with spirulina. The nutritional profile of the blue spirulina can be seen in table 1.1. Spirulina has gained popularity in the food development industry and many studies done by Lafarga et al., 2020,Kumar et al., 2018, Grahl et al., 2018, are present which show that it can be used and incorporated in food products.

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 was to formulate and develop spirulina enriched, high protein, high fibre and low glycaemic index protein bars.

Materials and Methods

Study Area: Food Product Development: Development of spirulina enriched protein bars.

Ethics Approval: Ethics approval was obtained.

Information Sheet and Consent Form: The participants were provided with an information sheet, and informed consent was obtained.

Sample Size: 10 recipes for protein bars, high in protein, high in fibre and low in GI were formulated and tested, out of which the best 4- recipes were taken for further evaluation for sensory attributes and finally the best protein bar was taken for shelf life based sensory evaluation.

For sensory evaluation A sample size of minimum 2 and maximum 10 semi trained panel (each phase had a different 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 4-6 months from the date of receiving ethical approval.

Tools for data collection

9-point Hedonic Rating Scale

The 9-point hedonic rating scale is a great tool for conducting sensory evaluation of a food product. The scale was used for phase 1, 2, 3 and for the shelf-life analysis. Hedonic rating scale related to a pleasurable or unpleasurable experience. This test is used to measure the acceptability of food products. The food products are placed in front of the panel members and they are asked weigh the acceptability of the product on the scale of 1 to 9 with 1 being dislike extremely and 9 being like extremely. The results are then analysed.

Ranking Scale

The ranking scale is also a great tool to understand the best product, out of all the food products. The ranking scale, which was used for phase 3 of standardization. This test is use when the best sample, out of all the samples placed in front of the panel members, needs to be determined. The food products are placed in front of the panel members and they are asked to rank the products as 1st (being the best), 2nd, and so forth. The results are then analysed and the best product is determined.

Study Design

Ideation and prototype creation

Initially a market survey was done, where the nutrition labels of the all the protein bars available in the market were assessed for the type of ingredients used, the amount of energy, protein and the GI (glycaemic index) per serving. As per the market survey and literature review, protein bars available in the market contain 11.6% protein and 1-to-2-gram fibre on average and thus, there was a need and scope of developing nutrient dense protein bars, infused with spirulina, protein bars which are high in protein and fibre, and low in GI, targeted for the general adult population. Ten different combinations of protein bars were formulated and the nutritive value was determined using Indian Food Composition Table (IFCT) The protein bars aimed to have functional foods and nutritional ingredients such as spirulina, whey protein isolate, nut butter like peanut butter, whole nuts and oilseeds like almonds, flaxseeds, inulin (for fibre), a Generally Recognized as Safe (GRAS) sweetener like erythritol, in various combinations. They aimed to be high in protein, high in fibre and low in GI with the targets set at 15 - 30 % of energy contributed by protein and each protein bar providing at least 3-5 g or more of fibre per serving. The formulated recipes were developed, standardized and modified, if required.

Development of Protein Bars and Standardization of The Recipes

All the ingredients for the protein bars, were procured online. Each protein bar had either a nut like almond, an oilseed like sesame seed or a cereal like puffed rice in it along with ingredients such as peanut butter, inulin, erythritol, whey protein isolate, spirulina etc. The nuts, oilseeds, cereal product was accurately weighed and dry roasted and if needed, were cut into smaller pieces. The other ingredients were accurately weighed and mixed together in a bowl; the nuts/oilseeds/ cereal were also mixed into the above mixture. For Phase 1 of standardization, the mixture was put in a bar shaped mould to get the desired shape, and then it was kept in the fridge to set for 10– 15 minutes. In phase 2 and 3, the steps mentioned during phase 1 were repeated and instead of putting the shaped protein bars in the refrigerator, the protein bars so obtained were baked for 8 minutes at 150 ^{0}C.

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

- Ten Recipes were formulated, keeping certain ingredients like Spirulina, whey protein, erythritol constant.
- The formulated recipes were developed.
- The recipes were evaluated for the desired sensory attributes using a 9-point hedonic rating scale, by two-semi-trained panel members.

Phase 2

- Required modifications both in terms of proportion and method of preparation, i.e., baking the protein bars instead of just setting them in the fridge, were done to the ten recipes till the desired sensory attributes are obtained.
- The recipes were evaluated for desired sensory attributes using a 9-point hedonic rating scale, by four- semi trained panel members.
- The best four recipes, out of the prepared ten, containing the desired sensory attributes, , were selected for phase 3.

Phase 3

- According to the results of phase 2, four recipes, 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 10 and day 20 sensory evaluation.

Sensory Evaluation and Product Modification

Sensory evaluation was conducted by a team of ten semi trained panel members, from the field of nutrition, using a 9-point hedonic rating scale. After the participant information sheet and consent forms were filled, the panel members on the day of the evaluation were briefed as to how the sensory evaluations should be conducted. Each member was given a pen, the sensory evaluation sheet, along with that bottle of filtered drinking water. The freshly prepared protein bar samples along with tissues and appropriate cutlery, were kept in front of the panel members. Each member had to take a bite of the protein bar and analyse it based on taste, texture, appearance and overall acceptability and fill the sensory evaluation form.

After tasting each protein bar, they were asked to cleanse their pallet with the water provided, this ensured that they get the true taste of the protein bar and that the taste of one protein bar, doesn't override the taste of the other. The recipe modifications, if any were made and a second and third round respectively of sensory evaluations were conducted for the modified recipes. In phase 3 of sensory evaluation, the panel members were also asked to rank the protein bars from the best protein bar to the least favourite protein bar, using a ranking scale sheet. Once the panel members decided upon the best protein bar, the shelf-life analysis of the best product i.e., of sample S.B was conducted.

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.B (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 10 and day 20 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 kept at in an air tight box, at ambient temperature for 10 and 20 days respectively. One protein bar was opened at day 10 and the other on day 20 and the shelf life based, sensory evaluation was conducted.

Shelf Life at day 10 and day 20 respectively

A team of 5 semi trained panel members conducted the shelf life based sensory evaluation of the protein bar. The panel members, used the evaluation, to evaluate the protein bar and determine, its shelf life. The panel members were given the same instructions as in phases 1-3, to conduct the sensory evaluation and additionally they were asked to see if they noticed any change in the mentioned sensory attributes, when tasking the protein bars on day 0, day 10 and day 20 respectively.

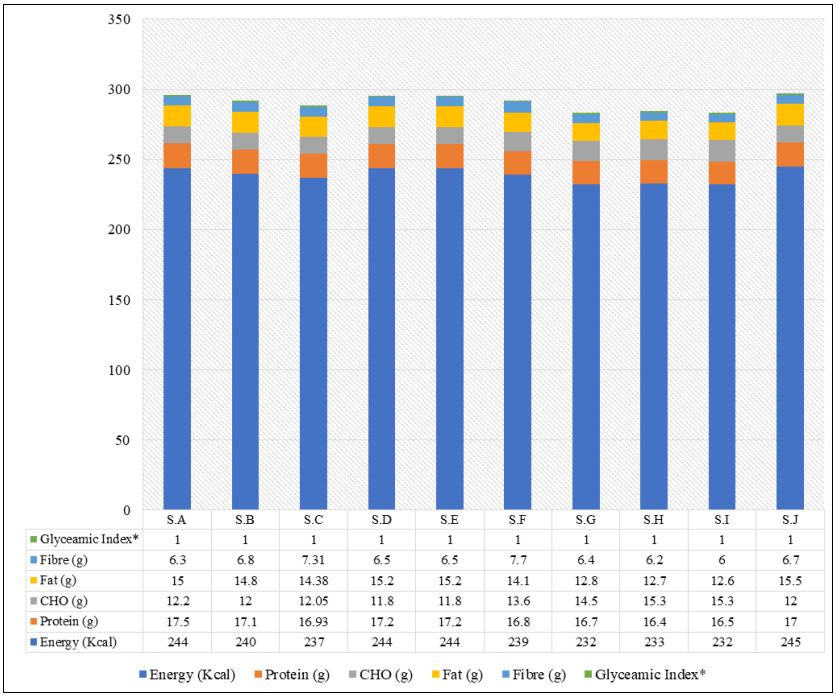
Statistical Analysis

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

Results and Discussion

Nutrient Composition of protein bars

Spirulina was the main functional ingredient used in the protein bars, along with whey protein as a source of protein, inulin as a source of fibre and erythritol as a low glycaemic index sweetener. The protein bars also contained nut butter, i.e., peanut butter as a source of healthy fat along with either cereals like puffed rice or nuts and oilseeds like almonds, chia seeds or sesame seeds, etc. The figure 1 represents the approximate nutritional composition of the protein bars, determined using IFCT. In case of products which were not mentioned in the IFCT, e.g., zero carbohydrate whey protein isolate, the nutritional labelling mentioned on the packaged food was referred to.



	S.A	S.B	S.C	S.D	S.E	S.F	S.G	S.H	S.I	S.J
Glyceamic Index*	1	1	1	1	1	1	1	1	1	1
Fibre (g)	6.3	6.8	7.31	6.5	6.5	7.7	6.4	6.2	6	6.7
Fat (g)	15	14.8	14.38	15.2	15.2	14.1	12.8	12.7	12.6	15.5
CHO (g)	12.2	12	12.05	11.8	11.8	13.6	14.5	15.3	15.3	12
Protein (g)	17.5	17.1	16.93	17.2	17.2	16.8	16.7	16.4	16.5	17
Energy (Kcal)	244	240	237	244	244	239	232	233	232	245

*For Glycaemic index, the word Low GI is coded as number 1

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 + Pumpkin Seeds), S-B (Spirulina + Sesame Seeds), S-C (Spirulina + Flax Seeds), S-D(Spirulina + Sunflower Seeds), S -E(Spirulina + Sunflower Seeds), S-F(Spirulina + Chia Seeds), S-G(Spirulina + Amaranth),S-H(Spirulina + Puffed Rice),S-I(Spirulina + Makhana),S-J (Spirulina + Almonds)

From figure 1 it was noted that, per serving, the approximate energy of the protein bars, ranged from 232 kcal to 245 kcal, the protein in the bars was in the range of 16.4g to 17.5 g which is considered to be high in protein. Carbohydrates were low i.e., 11.8g to 15.3 g, making the protein bars low in glycaemic index. Fibre was high i.e., 6g - 7.7 g and the fat ranged between 12.6 g to 15.5g.

In total, 10 recipes were formulated, developed and standardized in 3 phases. Sensory evaluation was carried out using a 9-Point hedonic rating scale for phase 1, 2 and 3 and for phase 3 an addition tool, namely the ranking scale was used, to determine the best protein bar produced.

Sensory Evaluation Scores of all the Developed Protein bars

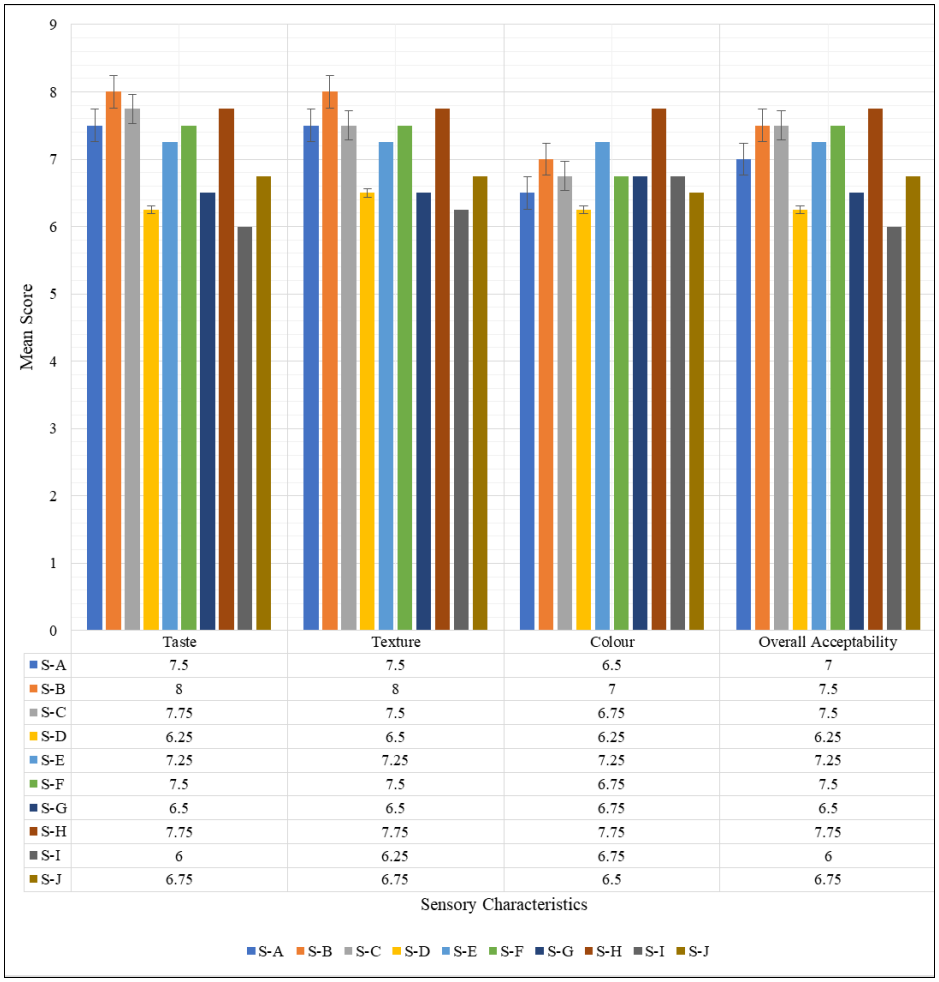
Sensory evaluation of the products was done by a team of semi trained panel members, from the field of nutrition. 3 phases of standardization were conducted. During each phase of standardization, each panel member, tasted the different protein bars presented to them for their sensory attributes and suggested modifications in the protein bars, if any.

Before each phase, the protein bars were modified as per the suggestions of the panel members, if any, and the sensory evaluation was carried out again. In total 3 phases were conducted. In each phase the panel members were asked to rate the products based on a 9-Point hedonic rating scale and in phase 3 they were additionally asked to rate the products on the based on a ranking scale, in order to determine the best protein bar produced. The protein bars were evaluated for attributes based on, taste, texture, appearance/colour and overall acceptability.

Since there were only 2 panel members in phase 1 of standardization, the results for phase 1 were not represented statistically.

The following are the results of phase 2 and phase 3 of standardization.

Phase 2 Hedonic Rating Scale



	Taste	Texture	Colour	Overall Acceptability
S-A	7.5	7.5	6.5	7
S-B	8	8	7	7.5
S-C	7.75	7.5	6.75	7.5
S-D	6.25	6.5	6.25	6.25
S-E	7.25	7.25	7.25	7.25
S-F	7.5	7.5	6.75	7.5
S-G	6.5	6.5	6.75	6.5
S-H	7.75	7.75	7.75	7.75
S-I	6	6.25	6.75	6
S-J	6.75	6.75	6.5	6.75

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

Figure 2 represents the mean sensory evaluation scores of the 9 -Point hedonic rating scale, used in phase 2. From the figure 2, it was noted that, in terms of Taste, protein bar S-B (Spirulina + Sesame Seeds), had the highest mean score (8 ± 0.816), followed by S-C (Spirulina + Flax Seeds) which had a mean score of 7.75 ± 0.500. Next was S-H (Spirulina + Puffed Rice) with a mean score of 7.75 ± 1.258 and protein bar S-F (Spirulina + Chia Seeds) with a mean score of 7.5 ± 1.291.

When texture of the protein bars was compared, again, the four protein bars i.e., S-B (Spirulina + Sesame Seeds) mean score of 8 ± 0.816, S-C (Spirulina + Flax Seeds) mean score of 7.5 ± 0.577, S-F (Spirulina + Chia Seeds) mean score of 7.5 ±1.291 and S-H (Spirulina + Puffed Rice), mean score of 7.75 ±1.258 had the highest mean scores.

The same four protein bars dominated with the best mean scores for colour and overall acceptability, as well. Hence, the four protein bars S-B (Spirulina + Sesame Seeds), S-C (Spirulina + Flax Seeds), S-F (Spirulina + Chia Seeds) and S-H (Spirulina + Puffed Rice) were taken for the phase 3 of standardization and assessed for their sensory attributes, by the panel members in phase 3.

Phase 3 Hedonic Rating Scale

Table 1: Paired Sample T-test for all the sensory characteristics evaluated for the protein bars in Phase 3

		Mean	N	Std. Deviation	t	Sig. (2-tailed) (p value)
1	Taste SB	7.00	10	1.054	.557	.591
	Taste SC	6.80	10	1.135		
2	Taste SB	7.00	10	1.054	1.861	.096
	Taste SF	6.50	10	1.354		
3	Taste SB	7.00	10	1.054	1.964	.081
	Taste SH	6.40	10	1.350		
4	Taste SC	6.80	10	1.135	1.152	.279
	Taste SF	6.50	10	1.354		
5	Taste SC	6.80	10	1.135	1.078	.309
	Taste SH	6.40	10	1.350		
6	Taste SF	6.50	10	1.354	.361	.726
	Taste SH	6.40	10	1.350		
7	Texture SB	6.80	10	1.317	.480	.642
	Texture SC	6.60	10	.843		
8	Texture SB	6.80	10	1.317	.818	.434
	Texture SF	6.50	10	1.269		
9	Texture SB	6.80	10	1.317	2.236	.052
	Texture SH	5.80	10	1.135		
10	Texture SC	6.60	10	.843	.429	.678
	Texture SF	6.50	10	1.269		
11	Texture SC	6.60	10	.843	2.228	.053
	Texture SH	5.80	10	1.135		
12	Texture SF	6.50	10	1.269	1.909	.089
	Texture SH	5.80	10	1.135		
13	Colour SB	6.40	10	1.265	2.250	.051
	Colour SC	5.80	10	1.135		
14	Colour SB	6.40	10	1.265	2.333	.045*
	Colour SF	5.70	10	1.252		
15	Colour SB	6.40	10	1.265	.709	.496
	Colour SH	6.10	10	1.524		
16	Colour SC	5.80	10	1.135	.557	.591
	Colour SF	5.70	10	1.252		
17	Colour SC	5.80	10	1.135	-.758	.468
	Colour SH	6.10	10	1.524		
18	Colour SF	5.70	10	1.252	-.937	.373
	Colour SH	6.10	10	1.524		
19	Overall Acceptability SB	6.60	10	1.265	1.861	.096
	Overall Acceptability SC	6.10	10	.876		
20	Overall Acceptability SB	6.60	10	1.265	.557	.591
	Overall Acceptability SF	6.30	10	1.252		
21	Overall Acceptability SB	6.60	10	1.265	.709	.496
	Overall Acceptability SH	6.30	10	1.059		
22	Overall Acceptability SC	6.10	10	.876	-.557	.591

	Overall Acceptability SF	6.30	10	1.252		
23	Overall Acceptability SC	6.10	10	.876	-.612	.555
	Overall Acceptability SH	6.30	10	1.059		
24	Overall Acceptability SF	6.30	10	1.252	0.000	1.000
	Overall Acceptability SH	6.30	10	1.059		

*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. The p value of the pairs having <0.05 was taken to be statistically significant. From all the, protein bars were seen that, when, the mean scores of the pairs are compared, protein bar S-B (Spirulina + Sesame Seeds), was the best protein bar, with the most desirable and most appealing sensory characteristics, hence, it was selected for shelf-life 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-B	1.70	10	1.252	-2.121	.063
	S-C	2.70	10	.823		
2	S-B	1.70	10	1.252	-1.489	.171
	S-F	2.60	10	.966		
3	S-B	1.70	10	1.252	-1.857	.096
	S-H	3.00	10	1.155		
4	S-C	2.70	10	.823	.208	.840
	S-F	2.60	10	.966		
5	S-C	2.70	10	.823	-.580	.576
	S-H	3.00	10	1.155		
6	S-F	2.60	10	.966	-.840	.423
	S-H	3.00	10	1.155		

In the rank test, as seen in table 2, four samples were given to the panel members, and they had to rank the products on a scale of 1 - 4 with the best product being given the rank 1 and the least desirable product being given the rank of 4. From the above table, 2, as per the mean values, from the paired sample T test, protein bar S-B (Spirulina + Sesame Seeds), had the highest rank, mean value 1.70 $\pm$ 1.252, Hence protein bar S- B (Spirulina + Sesame Seeds), is the best protein bar. The p value of all the pairs is >0.05 so there was not much statistical difference between the rank of the protein bars.

Shelf-Life Analysis at day 0, 10 and 20

The shelf life analysis was carried out at day 0, 10 and day 20, 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 panel members, and the protein bars had a safe for consumption shelf life of 20 days at ambient temperature, when kept sealed in LDPE (Low Density Polyethylene) aluminium foil bags.

Table 3: Paired Sample T-test for all the sensory characteristics evaluated for the protein bars during shelf - life analysis at day 0, day 10 and day 20

Paired Samples Statistics						
For protein bar S-B		Mean	N	Std. Deviation	t	Sig. (2-tailed) (p value)
1	Day zero Taste	7.20[a]	5	1.095	NA	NA
	Day 10 Taste	7.20[a]	5	1.095		
2	Day zero Taste	7.20	5	1.095	0.000	1.000
	Day 20 Taste	7.20	5	.837		
3	Day 10 Taste	7.20	5	1.095	0.000	1.000
	Day 20 Taste	7.20	5	.837		
4	Day Zero Texture	7.40	5	.548	1.633	.178
	Day 10 Texture	7.00	5	1.000		
5	Day Zero Texture	7.40	5	.548	1.633	.178
	Day 20 Texture	6.60	5	1.140		
6	Day 10 Texture	7.00	5	1.000	.784	.477
	Day 20 Texture	6.60	5	1.140		
7	Day Zero Colour	7.20	5	.837	.535	.621

	Day 10 Colour	7.00	5	1.000		
8	Day Zero Colour	7.20	5	.837	2.138	.099
	Day20 Colour	6.40	5	1.517		
9	Day 10 Colour	7.00	5	1.000	1.500	.208
	Day 20 Colour	6.40	5	1.517		
10	Day Zero Overall Acceptability	7.20	5	.837	0.000	1.000
	Day10 Overall Acceptability	7.20	5	1.095		
11	Day Zero Overall Acceptability	7.20	5	.837	2.449	.070
	Day 20 Overall Acceptability	6.60	5	1.140		
12	Day10 Overall Acceptability	7.20	5	1.095	1.177	.305
	Day 20 Overall Acceptability	6.60	5	1.140		
a. The correlation and t cannot be computed because the standard error of the difference is 0.						

In table 3 the Paired Sample T-test for all the sensory characteristics evaluated for the protein bars in during shelf-life analysis at day 0, day 10 and day 20 is seen. From all the pairs it was observed that there wasn't a great change in the taste, texture, colour and overall acceptability of the protein bar from day 0 to day 20 and it was noted that the protein bar had a shelf life of 20 days at ambient temperature.

Discussion

The protein bar that was determined as the best protein bar was the protein bar S-B (spirulina + sesame seeds) i.e., the protein bar which had sesame seeds, spirulina, whey protein isolate, erythritol, peanut butter, dark chocolate, inulin in it, the main reason for this being , was the appealing contrasting look of the sesame seeds in the protein bar, the added crunchy texture of the sesame seeds along with the creamy aftertaste and mouthfeel of the sesame seeds upon biting and grinding them.

Studies by Normann et al., 2019 and, Capule et al, 2014, suggest that the first thing that attracts a person toward a food product is the appearance, and the is exactly the case in protein bar S- B (spirulina + sesame seeds). Compared to the other protein bars prepared, protein bar S-B (spirulina + sesame seeds), had the best physical looks.

Sesame seeds have a good fatty acid profile; hence they increased the taste, and overall acceptability of a food product Folasade & Oyenike, 2012.In the case of The, the Bar S-B (Spirulina + Sesame seeds), was the best ranked bar, because it had sesame seeds in it.

A study by Çakir et al., 2012, suggested that mouthfeel also determined the food choices and helps increase the likeability of the food product, in the case of the study, the crunchy texture added by the sesame seeds was the very fact behind the panel members choosing the sesame seeds protein bars over the other protein bars.

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, the taste of spirulina was also acceptable. Another Study by Batista de Oliveira et al., 2021, showed the incorporation of spirulina in chocolates milk, and was accepted by the panel members. The also successfully incorporated spirulina in protein bars, and the protein bars were considered to be acceptable by the panel members.

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, 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 can be one of the reasons why the protein bars had a good overall acceptability.

In the, it was seen that, the protein bars had a safe for consumption shelf life for 20 days at ambient room temperature. (24 – 35 degrees °C) , a study by S.N. Landge et al., 2009, also showed that when stored in LDPE lined aluminium foil bag, the food product compared to the other packaging techniques, stayed longer and fresher i.e., for a period of 20 – 28 days . Since The also used LDPE lined aluminium foil bags, similar results were obtained.

The, used LDPE (Low Density Polyethylene) aluminium foil bags, to seal and store the bars, for the shelf- life analysis. As per the results, there was no change in the mean score of taste from day 0 (7.20) to day 20 (7.20), however, the panel members did give a lower mean score for texture (from 7.40 to 6.60) and colour (from 7.20 to 6.40) at day 20, when compared to day zero. A study from S.N. Landge et al., 2009, has shown that when a food product is packed and stored in LDPE lined aluminium bags, over a period of time, i.e., 20 days, the colour and texture might get affected as certain gases from the environment might enter the packaging, but there will be no or negligible change in taste and overall acceptability by day 20. The same was observed in the current undertaken study.

Conclusion

In the, the protein bar which had the mean best score and overall best sensory evaluation and ranking scores, was the protein bar with the code S-B (Spirulina + Sesame Seeds) amongst the other protein bars which had, pumpkins seeds, watermelon seeds, flax seeds, watermelon seeds ,chia seeds, amaranths, makhana, puffed rice, almonds, respectively. The protein bar , S-B (Spirulina + Sesame Seeds) , weighed 57g, provided 240 kcal in

terms of energy along with 17.1 g (29% of energy) protein and 6.8g fibre, which made it high in protein and fibre. Compared to the other protein bars in the market survey, the best protein bar in the study provided 29% of protein, whereas the protein bars of the market survey provide 10% protein on an average. The protein bar was made using a low glycaemic index (GI) sweetener i.e., erythritol (10g was used). The use of the zero-carbohydrate sweetener, made the product low in glycaemic index as well.

The shelf life of the protein bar-coded S-B (Spirulina + Sesame Seeds) was carried out at day zero, day 10 and day 20, and it was observed that the protein bar had a safe for consumption shelf life at 20 days as well, when kept at an ambient temperature in (Low Density Polyethylene) aluminium foil bags. Hence, the study objective of developing a protein bar for the general adult population, low in glycaemic index and high in protein and fibre, enriched with spirulina was achieved successfully.

The protein bar S-B (Spirulina + Sesame Seeds), had 2 g of spirulina, which is the daily recommended intake in order to see any functional benefits of spirulina. Other Ingredients included, zero carbohydrate whey protein (protein source), inulin (fibre source), unsweetened peanut butter (fat source and binding agent), white sesame seeds (a balanced source of MUFA and PUFA and added the desired crunchy and palatable texture to the protein bar).

The protein bar S-B (Spirulina + Sesame Seeds), which is high in protein and fibre and low in GI is beneficial for the management of lifestyle diseases like metabolic syndrome, obesity, cardiovascular diseases, type 2 diabetes mellitus, which are becoming a burden on the society. The protein S-B (Spirulina + Sesame Seeds) is not only a healthy on the go snack option, but it is very filling and helps manage the evening hunger pangs, while keeping the person satiated for long period of time.

References

1. Basciano H, Federico L, Adeli K Fructose. insulin resistance, and metabolic dyslipidemia. Nutrition & Metabolism, 2005, 2(1). https://doi.org/10.1186/1743-7075-2-5
2. Batista de Oliveira, T T Miranda dos Reis, I Bastos de Souza, M da Silva Bispo, E Fonseca Maciel, L Druzian, et al.,2021. Microencapsulation of Spirulina sp. LEB-18 and its incorporation in chocolate milk: Properties and functional potential. LWT, 148, 111674. https://doi.org/10.1016/J.LWT.2021.111674
3. Çakir E Koç, H Vinyard, C J Essick, G Daubert, C R Drake M, et al. Evaluation of Texture Changes Due To Compositional Differences Using Oral Processing. Journal of Texture Studies,2012:43(4):257-267. https://doi.org/10.1111/J.1745-4603.2011.00335.X
4. Capule IB, Barcelon EG. Influence of Colour on The Sensory Perception of Ready-to-drink soy milk. Asian Journal of Science and Technology,2014:5(12):879-882. https://www.researchgate.net/publication/283645951_Influence_of_Colour_on_the_sensory_Perception_of_Ready-to-drink_soy_milk
5. Childs, J L, Yates M D, & Drake M A Sensory Properties of Meal Replacement Bars and Beverages Made from Whey and Soy Proteins. Journal of Food Science,2007:72(6):S425–S434. https://doi.org/10.1111/J.1750-3841.2007.00429.X
6. Folasade M, & Oyenike O. Effect of sesame seed addition on the chemical and sensory qualities of sorghum based kunun-zaki drink. In African Journal of Food Science and Technology, 2012, 3(9). http://www.interesjournals.org/AJFST
7. Grahl S, Strack M, Weinrich R, Mörlein D. Consumer-Oriented Product Development: The Conceptualization of Novel Food Products Based on Spirulina (Arthrospira platensis) and Resulting Consumer Expectations. Journal of Food Quality, 2018. https://doi.org/10.1155/2018/1919482
8. ICMR. A short note on nutrient requirements for Indians. National Institute of Nutrition, India., (2018) https://www.nin.res.in/RDA_short_Report_2020.html
9. Kumar A, Mohanty V, Yashaswini P. Development of high protein nutrition bar enriched with spirulina plantensis for undernourished children. Current Research in Nutrition and Food Science, 6(3), 835-844. https://doi.org/10.12944/CRNFSJ.6.3.26.
10. Lafarga T, Fernández-Sevilla, J M, González-López C, Acién-Fernández, F G. Spirulina for the food and functional food industries. Food Research International,2020:137:109356. https://doi.org/10.1016/j.foodres.2020.109356
11. Liestianty D, Rodianawati I, Arfah R A, Assa A, Patimah, Sundari, Muliadi. Nutritional analysis of spirulina sp to promote as superfood candidate. IOP Conference Series: Materials Science and Engineering, 2019, 509(1), 012031. https://doi.org/10.1088/1757-899X/509/1/012031
12. Machowiec P, Ręka G, Maksymowicz M, Piecewicz-Szczęsna H, Smoleń A. Effect of Spirulina Supplementation on Systolic and Diastolic Blood Pressure: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients,2021:13(9):3054. https://doi.org/10.3390/NU13093054
13. Morenga L te, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ (Online), 2013, 345(7891). https://doi.org/10.1136/bmj.e7492
14. Normann A, Röding M, Wendin K. Sustainable Fruit Consumption: The Influence of Color, Shape and Damage on Consumer Sensory Perception and Liking of Different Apples. Sustainability,2019:11(17):4626. https://doi.org/10.3390/SU11174626
15. Ogechi S, Soelaiman I-N, Chin K-Y. Health Beneficial Properties of Spirulina in Preventing Non-Communicable Diseases-The Green Metabolic Regulator from the Sea (Manfaat Kesihatan Spirulina dalam

Mencegah Penyakit Tidak Berjangkit-Pengatur Metabolik Hijau dari Laut). Sains Malaysiana,2021:50(3):803-819. https://doi.org/10.17576/jsm-2021-5003-21
16. Saharan V, Jood S. Effect of storage on Spirulina platensis powder supplemented breads. Journal of Food Science and Technology,2021:58(3):978. https://doi.org/10.1007/S13197-020-04612-1
17. Sharma M, Kishore A, Roy D, Joshi K. A comparison of the Indian diet with the EAT-Lancet reference diet., 2020. BMC Public Health, 20(1). https://doi.org/10.1186/S12889-020-08951-8
18. SN Landge, BR Chavan, DN Kulkarni, CD Khedkar. Effect of packaging materials, storage period and temperature on acceptability of milk cake. Asian Journal of Dairy and Food Research,2009:28(1):20-25. https://arccjournals.com/journal/asian-journal-of-dairy-and-food-research/ARCC2207
19. Stanhope K L, Schwarz J M, Keim N L, Griffen S C, Bremer, A A, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. The Journal of Clinical Investigation,2009:119(5):13220-1334. https://doi.org/10.1172/JCI37385
20. Yifan W, Maria Fides O, Bianca R, Jiwang C. Resveratrol and Spirulina: Nutraceuticals that Potentially Improving Cardiovascular Disease. Journal of Cardiovascular Medicine and Cardiology,2020:7(2):138-145. https://doi.org/10.17352/2455-2976.000129