In this study, chia seeds were incorporated for the development of an optimized nutri bar. Different formulations containing 5%, 10%, 15% and 20% of chia seed flour were evaluated. The developed bar was analysed for its nutritional quality, sensory attributes, physiochemical properties and storage quality. The results revealed that the 10% incorporation of chia seed with protein content $3.72 \pm 0.11\%$, crude fibre $10.52 \pm 0.08\%$, ash $2.0\%$, crude fat $3.52 \pm 0.19\%$, carbohydrate $51.00 \pm 0.24\%$ and antioxidant activity $32.23 \pm 0.69\%$ inhibition was highly acceptable in terms of sensory attributes. The shelf life study revealed that the nutri bars when stored at a temperature of $10^\circ\text{C}$ sealed in low density polyethylene bags tend to be more shelf stable. The gross energy of the chia seed nutri bar was calculated to be $250.52 \pm 0.63\%$ kcal.

Keywords: Antioxidant, chlorogenic acid, diet supplement, energy, rapidly consumed.

CHIA (*Salvia hispanica* L.) is a herbaceous plant, cultivated annually, and indigenous to Southern Mexico and Northern Guatemala\(^1\). It has a high oil content (39%), is rich in polyunsaturated fatty acids, mainly omega-3 fatty acids (linolenic acid, 68) and omega-6 (linoleic acid, 12–21%) (ref. 2) and has protein content of 15–25% (ref. 3). Moreover, the fibres present, (18–30%) potentiate the use of chia in the production of functional foods\(^4\) and the dietary fibre (over 30% of the total weight), both soluble and insoluble, which are important components of the human diet; and proteins of high biological value around 19% of the total weight\(^5\). In addition, the seed contains natural antioxidants such as phenolic glycoside-Q and K, chlorogenic acid, caffeic acid, quercetin and kaempferol\(^6\) which protects consumers against adverse conditions, such as some cardiovascular diseases and few types of cancers; as well as supplies vitamins and minerals\(^3\).

A non-cooked bar is substantially non-perishable, readily portable and can be rapidly consumed. To meet nutritional needs, fortified non-cooked bars were introduced to provide a portion of the daily requirement of minerals and vitamins\(^4\). Consumers demand and desire health foods, which are portable, convenient and proportioned. Often, many options that are minimally processed, rich in nutrients and also taste good are not available. The food bars are snacks of good sensory and nutritional characteristics due to their high carbohydrate, protein, lipid and mineral contents\(^9\). This can be recommended to pregnant and lactating women or women of child bearing age it is rich in calcium and iron. Nutri bars are a diet supplement and are mostly consumed by people who want to maintain their calorific needs\(^10\). There are various bars available in the market such as energy bar, snack bar, granola bar, protein bar and other nutri bars. Nutri bars were initially marketed to athletes as a source of energy. However, the growing luxury groups and health-conscious consumers increased the sales performance of nutri bars. Due to the growing consumer demand for natural, convenient and nutritious food products, there is a need to modify, innovate and improve the nutritive composition of nutri bars for increased health benefits\(^11\).

Therefore, in the present study, chia seed flour has been incorporated in the formulation of the nutri bar with ingredients like dates and sesame seeds. The aim of the study was supplementing chia seeds for its high PUFA, protein and dietary fibre content in the nutri bar and assessment of its nutritive quality, antioxidant capacity, functional properties like water and oil absorption capacity, sensory acceptability and shelf life.

**Materials and methods**

**Materials**

Dates, sesame seeds and chia seeds used for the preparation, and LDPE bags and cardboard boxes used for packaging of nutri bars were purchased from the local market in Jalandhar, Punjab, India. The chia seed nutri bar was prepared by modifying the method described by Ateequddin and Ingle\(^9\) as shown in Figure 1. The different formulations of chia seed nutri bar were prepared as shown in Table 1.

**Methods**

Nutritional analysis of nutri bar

The nutri bar was analysed for its chemical composition (moisture, crude protein, crude fat, ash and crude fibre)
Table 1. Formulations of nutri bar incorporated with chia seeds powder

| Treatments           | Ingredients              | Control | CSN₁ (5%) | CSN₂ (10%) | CSN₃ (15%) | CSN₄ (20%) |
|----------------------|--------------------------|---------|-----------|------------|------------|------------|
| Dates (g)            | 100                      | 95      | 90        | 85         | 80         |
| Chia seed powder (g) | –                        | 5       | 10        | 15         | 20         |
| Sesame seeds (g)     | 5                        | 5       | 5         | 5          | 5          |

Figure 1. Process flow chart for the development of chia seed nutri bar.

using methods by AOAC. Oven dry method (AOAC method 977.11) was referred to calculate moisture content. Kjeldahl’s method (AOAC method 955.04) was referred to analyse crude protein. Soxhlet method (AOAC method 960.39) was used for crude fat determination. Dry ashing method (AOAC method 923.03) was used to determine ash content, and gravimetric method (AOAC method 991.43) was used to examine crude fibre and the carbohydrate content was determined by the difference method.

Gross energy value

The gross energy value of developed nutri bars were determined by using standard factors of 4, 9 and 3.75 kcal/g for protein, fat and carbohydrate respectively. The calculated energy values were summed up to give gross energy values.

Mineral analysis

For sodium and potassium estimation, an aliquot of ash (0.01 mg/ml) solution was diluted so that it contains less than 10 ppm of sodium and potassium. Sufficient HCl was added so that the concentration of the acid is same as that in the standard solution. The diluted extract was atomized in a calibrated flame photometer with the wavelength dial set at 589 nm and transmittance set at 100% for the top standard solution of sodium and at the 768 nm and transmittance set at 100% for the top standard solution of potassium. The concentrations were noted from the standard curve by applying the following formulae (1) and (2).

Physical properties of nutri bar

The water absorption capacity was determined by taking 1 g of sample mixed in 15 ml of distilled water. The mixture was transferred to a 25 ml centrifuge tube and agitated on a vortex mixer for 2 min and then centrifuged at 4000 rpm for 20 min. The supernatant was discarded and the tube was weighed and applied on eq. (3). Similarly, for oil absorption capacity, water was replaced by oil, the same procedure was followed and values were marked in eq. (4).

Free radical scavenging activity of nutri bars

Free radical scavenging activity (FRSA) was measured as per the method of 1 g of sample extracted with 5 ml of
and the following eq. (5) was used.

Shelf life analysis was performed on the most acceptable odourless plastic containers with a glass of water for rins-ly prepared nutri bars were served to the panellists in given instructions were given to the panel members for from extremely liked to extremely disliked, with the any diseases. A score card consisting of preference rating evaluated were collected to eliminate errors in the process. Sensory attributes included colour and appear-tions. The data regarding sensory panel screening and coded randomly from 101 to 106 to avoid any preassump-tions. Nutri bars were semi-trained panellists (25 males and 25 females of age 20–35 years) using 9 point hedonic scale. Nutri bars were evaluated using commercially available nutri bars containing peanuts, to accustom them to the food type. The scores received while organoleptic evaluation were statistically analysed by ANOVA18,19.

Results and discussion

Nutritional analysis

Nutritive composition of nutri bar incorporated by chia seeds is given in Table 2. It was observed that moisture content decreased with the increase in chia seeds composition21, owing to low moisture content of chia seeds, in-creasing trend of chia seed nutri bar is due to high mineral content in the chia seeds especially Mg, Ca, Fe, Zn, Mg, Co and Se (ref. 24). Although the fat content of dates is less (0.14 g/100 g)3, however, due to the high content of fat in chia seeds (24.83% per 100 g)22, the fat of nutri bar ranged from 2.0% to 4.5%. The fibre content varied from 10–5 mol/l DPPH (ethanolic) was added into the test tube with 0.1 ml of sample extract and the decrease in absorbance became steady. Ethanol was used as a blank17 and the following eq. (5) was used.

\[ \text{Antioxidant activity} = \frac{A_0 - A}{A_0} \times 100, \]  

where \( A_0 \) is the absorbance of DPPH as blank and \( A \) the absorbance of sample.

Organoleptic evaluation of nutri bar

Sensory characteristics of nutri bar samples were evaluated for different sensory attributes by a group of 50 semi-trained panellists (25 males and 25 females of age 20–35 years) using 9 point hedonic scale. Nutri bars were coded randomly from 101 to 106 to avoid any preassump-tions. The data regarding sensory panel screening and evaluation were collected to eliminate errors in the process. Sensory attributes included colour and appearance, taste, texture, mouth feel and overall acceptability. The panel members chosen were healthy and free from any diseases. A score card consisting of preference rating from extremely liked to extremely disliked, with the given instructions were given to the panel members for the assessment of their liking and preferences. The freshly prepared nutri bars were served to the panellists in odourless plastic containers with a glass of water for rinsing the mouth in between the samples. The area used for sensory analysis was isolated and aroma free. The sensory panel was trained using commercially available nutri bars containing peanuts, to accustom them to the food type. The scores received while organoleptic evaluation were statistically analysed by ANOVA18,19.

Shelf life analysis

Shelf life analysis was performed on the most acceptable treatment after organoleptic evaluation. Packed nutri bars fortified with chia seeds were stored at different temperatures for the shelf life study (10°C, 25°C and 37°C for 28 days). The samples were withdrawn at an interval of 7 days during storage and analysed for hydroxy methyl furfurall (HMF), thiobarbituric acid (TBA) and free fatty acid content (FFA) test as per the procedures used by Rasane et al.20.

Statistical analysis

All the tests were carried out in triplicates and the mean were presented along with standard deviation. The significant difference of mean values and product optimization was performed with Duncan’s multiple range test and post hoc test using SPSS 16.0 software (SPSS) Italia, Bologna, Italy at a significance level of 5%.
results were reported by Romankiewicz et al.\textsuperscript{25}, where they studied the effect of addition of chia seeds on quality and nutritional value of white bread. They observed that the dietary fibre content increases as high as 7.19\% with 8\% chia seed addition. The energy content correspondingly increased from 227.03 to 272.53 kcal with increasing concentration of chia seed, which shows that chia seeds, have high nutrient density.

\textit{Mineral analysis}

The concentrations of sodium and potassium in chia seeds were 71.9 and 635 mg/100 g respectively, which imparts the properties like lowering of blood pressure and maintaining the electrolytes\textsuperscript{26}. Table 3 shows the amount of sodium and potassium present in the nutri bars which are in agreement with the results given by Kibii et al.\textsuperscript{27}, where they have prepared yoghurt enriched with chia seeds.

\textit{Physico-chemical properties}

The functional properties play a vital role in the manufacturing of the product. The nutri bars were analysed for their functional properties including water absorption and oil absorption capacity (OAC and WAC) as shown in Figure 2. The OAC and WAC depend on the amino acid composition, surface polarity and protein conformation like intrinsic factors\textsuperscript{28}. The water absorption capacity increased significantly ($P < 0.05$), but not much difference was observed in between control and 5\% chia seed incorporation and in between 15\% and 20\% chia seed incorporation. The value ranged from 0.71 ± 0.71 (control) to 1.67 ± 0.15 (20\% incorporation) that signifies that nutri bars tend to absorb more water with increase in the concentration of chia seeds. This is due to the high water retention capacity and high fibre content of chia seeds. OAC is a highly essential characteristic for the development of food products and for a longer storage life. The decreasing trend observed for oil absorption capacity of nutri bars were in agreement with the results shown by Haripriya and Aparna\textsuperscript{28} where they studied the effect of roasting on chia seed-based instant soup mix.

\begin{table}[h]
\centering
\begin{tabular}{lcc}
\hline
Treatments & Sodium (ppm) & Potassium (ppm) \\
\hline
Control & 30.33 ± 0.58\textsuperscript{a} & 19.33 ± 0.18\textsuperscript{a} \\
5 \% & 36.00 ± 0.21\textsuperscript{b} & 30.67 ± 0.53\textsuperscript{b} \\
10\% & 42.33 ± 0.58\textsuperscript{c} & 37.33 ± 0.27\textsuperscript{c} \\
15\% & 51.67 ± 1.15\textsuperscript{d} & 43.33 ± 0.13\textsuperscript{d} \\
20\% & 65.33 ± 0.50\textsuperscript{e} & 48.00 ± 0.20\textsuperscript{e} \\
\hline
\end{tabular}
\caption{Mineral content of nutri bar}
\end{table}

The values are represented in mean ± standard deviation derived for triplicate experiments ($n = 3$). The values denoted with different superscripts differ significantly at $P < 0.05$ in a column.

\textit{Free radical scavenging activity of nutri bars}

Antioxidant capacity is one that quantifies the ability of complex biological samples to quench DPPH free radicals\textsuperscript{29}. The antioxidant activity significantly ($P < 0.05$) increased by chia seed incorporation is shown in Figure 3. The results were in accordance with Costantini et al.\textsuperscript{30} in their study of incorporation of chia seed flour in white bread. They reported that tartary buckwheat flour and chia seed incorporation by 10\% increased the antioxidant activity by 75\% in the prepared bread.

\textit{Organoleptic evaluation of nutri bar}

Sensory acceptance of chia seed nutri bars were determined by the changes in terms of colour and appearance, taste, aroma, texture, mouthfeel and overall acceptability. Highest values were observed for the 10\% incorporated chia seed nutri bar and the values reported for colour and appearance, taste, aroma, texture and overall acceptability were 7.18 ± 0.76, 7.24 ± 0.58, 8.38 ± 0.82, 6.75 ± 0.43
Table 4. Shelf life analysis of nutri bar

| Parameters                        | Temperature (°C) | Packaging material used | 0th day       | 7th day       | 15th day      | 21st day      | 28th day      |
|-----------------------------------|-----------------|-------------------------|---------------|---------------|---------------|---------------|---------------|
| Moisture (%)                      | 10              | LDPE pouches            | 35.45 ± 0.24a | 36.93 ± 0.71b | 38.25 ± 0.21c | 39.89 ± 0.48e | 40.13 ± 0.63e |
|                                   |                 | Cardboard boxes         | 35.14 ± 0.12c | 36.33 ± 0.73c | 37.55 ± 0.67d | 39.84 ± 0.12d | 41.49 ± 0.36e |
|                                   | 25              | LDPE pouches            | 39.77 ± 0.62a | 40.39 ± 0.37b | 42.79 ± 0.69b | 45.15 ± 0.77c | 45.98 ± 0.87e |
|                                   |                 | Cardboard boxes         | 39.20 ± 0.61d | 40.89 ± 0.47b | 41.22 ± 0.33c | 42.47 ± 0.54d | 46.89 ± 0.17c |
|                                   | 37              | LDPE pouches            | 37.27 ± 0.15a | 39.44 ± 0.67b | 41.83 ± 0.33c | 42.15 ± 0.31c | 43.19 ± 0.34d |
|                                   |                 | Cardboard boxes         | 38.13 ± 0.54a | 38.93 ± 0.14c | 40.13 ± 0.37d | 41.87 ± 0.94c | 44.65 ± 0.92d |
| Hydroxyl methyl furfural (HMF; μmol/g) | 10              | LDPE pouches            | 4.91 ± 0.09a  | 8.20 ± 0.11d  | 10.60 ± 0.09f | 13.22 ± 0.04e | 14.86 ± 0.04f |
|                                   |                 | Cardboard boxes         | 4.37 ± 0.22c  | 6.40 ± 0.14f  | 8.44 ± 0.27g  | 11.23 ± 0.16e | 14.75 ± 0.24f |
|                                   | 25              | LDPE pouches            | 3.12 ± 0.06a  | 7.06 ± 0.11c  | 11.43 ± 0.02d | 15.60 ± 0.07b | 19.79 ± 0.15b |
|                                   |                 | Cardboard boxes         | 4.32 ± 0.14b  | 8.73 ± 0.09d  | 12.47 ± 0.02c | 15.82 ± 0.04b | 19.19 ± 0.09c |
|                                   | 37              | LDPE pouches            | 2.59 ± 0.09a  | 6.53 ± 0.04e  | 10.73 ± 0.02e | 14.98 ± 0.13e | 19.24 ± 0.52c |
|                                   |                 | Cardboard boxes         | 1.86 ± 0.14c  | 5.82 ± 0.68e  | 10.20 ± 0.08g | 15.49 ± 0.90b | 20.84 ± 1.83c |
| Thiobarbituric acid test (TBA) (mg malenaldehyde/kg) | 10              | LDPE pouches            | 4.10 ± 0.01a  | 7.61 ± 0.11c  | 11.12 ± 0.21c | 13.40 ± 0.05d | 14.54 ± 0.11c |
|                                   |                 | Cardboard boxes         | 4.37 ± 0.52c  | 6.40 ± 0.14a  | 8.44 ± 0.27c  | 11.23 ± 0.37e | 15.23 ± 0.16c |
|                                   | 25              | LDPE pouches            | 1.74 ± 0.41a  | 3.64 ± 0.32a  | 5.53 ± 0.23c  | 7.40 ± 0.12c  | 9.28 ± 0.34c  |
|                                   |                 | Cardboard boxes         | 1.36 ± 0.00a  | 5.74 ± 0.05c  | 10.12 ± 0.10d | 12.79 ± 0.02c | 15.38 ± 0.02a |
|                                   | 37              | LDPE pouches            | 1.80 ± 0.12a  | 3.69 ± 0.08c  | 4.58 ± 0.65c  | 7.49 ± 0.41c  | 10.33 ± 0.33c |
|                                   |                 | Cardboard boxes         | 0.44 ± 0.32c  | 1.74 ± 0.62c  | 4.33 ± 0.23c  | 5.75 ± 0.17c  | 9.33 ± 0.67c  |
| Free fatty acid (FFA) (% oleic acid) | 10              | LDPE pouches            | 1.35 ± 0.17a  | 1.42 ± 0.23c  | 1.50 ± 0.47c  | 1.72 ± 0.55e  | 1.87 ± 0.79c  |
|                                   |                 | Cardboard boxes         | 1.12 ± 0.03a  | 1.42 ± 0.14a  | 1.57 ± 0.19c  | 2.15 ± 0.63e  | 2.93 ± 0.24c  |
|                                   | 25              | LDPE pouches            | 0.97 ± 0.13a  | 1.35 ± 0.39c  | 1.50 ± 0.27c  | 1.80 ± 0.39c  | 2.27 ± 0.43c  |
|                                   |                 | Cardboard boxes         | 0.97 ± 0.13a  | 1.35 ± 0.44c  | 2.43 ± 0.29c  | 5.50 ± 0.63d  | 6.72 ± 0.44c  |
|                                   | 37              | LDPE pouches            | 1.35 ± 0.17e  | 1.42 ± 0.23c  | 1.72 ± 0.71c  | 1.80 ± 0.35d  | 1.94 ± 0.63c  |
|                                   |                 | Cardboard boxes         | 0.82 ± 0.13d  | 1.12 ± 0.30c  | 1.27 ± 0.53c  | 1.57 ± 0.20d  | 1.80 ± 1.13c  |

Figure 4. Graphical representation of sensory analysis of chia seed nutri bar.

and 6.35 ± 0.55, respectively as shown in Figure 4. However, the lowest score was observed for 20% incorporation of chia seeds, i.e. 6.35 ± 0.55. The overall scores increased significantly ($P < 0.05$) from 7.18 ± 0.76 to 8.38 ± 0.82 in case of control, 5% and 10% incorporation and started decreasing from 30% to 40% incorporation with the values of 6.75 ± 0.43 and 6.35 ± 0.55 respectively. These results are close to that obtained from Zaki$^{31}$ who studied the effect of adding chia seeds in camburger. He reported that 3% chia seed addition in the camburger received the highest scores compared to other samples.
Shelf life evaluation

Food packaging has a vital role in analysing the shelf life of foods as they inhibit the entrance of oxygen and loss and gain of moisture in foods which shows that selection of suitable packaging material is important to ensure the better keeping quality of the product during storage to prevent oxidation of lipids. Lipid oxidation and browning are the important phenomenon occurring in food systems that depicts the food deterioration during storage and processing. In the present study, the lipid oxidation of optimized nutri chia seed bars was studied by tracking the changes that took place in free fatty acids (FFA), Thiobarbituric acid (TBA) and browning was studied by analysing hydroxyl methyl furfuryl (HMF) in the most acceptable treatment, i.e. 10% chia seed incorporated nutri bar on the basis of organoleptic evaluation. The result shown in Table 4, stated the change in moisture content, HMF, TBA and FFA values after 28 days of storage for 10% added chia seed nutri bar. The moisture content increased significantly at all the temperatures in a span of 28 days in both ziplock pouches and cardboard boxes. The highest moisture content of 46.89 ± 0.17 was observed in the sample stored in cardboard boxes at 25°C after the 28th day of storage. The results were in accordance with Padamashree et al.33 who developed choco quinoa nutri bar and observed significant increase ($P < 0.05$) in moisture content in the storage period of 9 months. The increase in moisture content (measured by oven drying procedure) of the nutri bar was observed from 7.33 to 8.21, 7.70 and 7.51 packaged in polypropylene, metalized polyester with vacuum and without vacuum respectively.

The HMF is the product formed as an intermediate of the Maillard reaction or caramelization of sugar at high temperature. The HMF values showed significant increase ($P < 0.05$) in the values at 37°C, 25°C and 10°C in the nutri bar stored in ziplock pouches as well as cardboard boxes. The highest value of 20.84 ± 1.83 was observed in the samples stored in cardboard boxes at 37°C. Similar result has been reported by Mesias et al.35 in their work on chia seed biscuits. Their results showed a significant increase from 22 mg/kg (0% chia flour) to 71 mg/kg in case of highest chia seed incorporation (20%) in wheat flour biscuits. They suggested the maximum incorporation of chia seed by 10%, as more incorporation beyond this level led to the formation of acrylamide, HMF and furfural.

High TBA values are associated with lipid oxidation due to processing conditions, packaging and storage and also because of high fat content. Similarly, the TBA values increased significantly ($P < 0.05$) from the 0th to 28th day at different temperatures. The nutri bar stored at 25°C showed that the highest values were recorded at 25°C stored at 13.40 ± 0.05 mg malenaldehyde/kg. The results were in agreement with Scapin et al.35 in their study of effect of chia seed addition in pork sausage. They stated the increase in TBARS value from 0.61 ± 0.02 to 1.12 ± 0.03 in the highest incorporation of chia seed extract (2%) in the storage period of 28 days.

For FFA values, a significant increase ($P < 0.05$) was observed at all the storage temperatures in both ziplock as well as cardboard boxes. The lowest degradation was observed in cardboard boxes stored at 37°C by a value of 1.80 ± 0.13 and the highest was observed in bars stored in cardboard boxes at 25°C by a value of 6.72 ± 0.44. Ryavanki and Hemalatha38 showed a similar trend in the shelf life study of low glycemic index snack bar prepared from red sorghum flakes. They reported significant increase in the free fatty acid (oleic acid) values from 1.36 ± 0.42% to 6.16 ± 0.45% at high temperature, and at ambient temperature the values ranged from 1.36 ± 0.42% to 2.37 ± 0.25%.

Conclusion

The study demonstrated that date and chia seeds can be used to formulate chia-date bar which improves protein, carbohydrate and crude fibre content. The developed bar was assessed properties for nutritional quality, sensory attributes, physicochemical and shelf life analysis. The optimized product 10% (dates 90 g and chia seed flour 10 g) revealed that the nutritional content of the nutri bar had protein content (3.72%), crude fibre content (10.52%), potassium (37.33 ppm), ash content (2.0%), crude fat (3.52%) and antioxidant (32.23 ± 0.69%). The organoleptic evaluation results showed the highest scores of 8.38 ± 0.82 for 10% chia seed flour incorporation in the nutri bar which was further carried for shelf life analysis. The results of shelf life analysis showed the significant degradation in the span of 28 days by significant increase ($P < 0.05$) in moisture, TBA, HMF and FFA values at different temperatures and packaging material. The suitable temperature for the storage of nutri bar is 10°C in LDPE pouches.
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