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ORIGINAL ARTICLE

Cereal bar functionalised with non-conventional alfalfa and dhaincha protein isolates: quality characteristics, nutritional composition and antioxidant activity

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Abstract The utilization of conventional protein sources like gluten, soy, dairy proteins, and nuts in the development of protein-enriched cereal bars presents a challenge for their consumption by the population suffering from celiac and other food protein allergies. In the present investigation, protein-rich cereal bars were developed using non-conventional protein isolates (alfalfa and dhaincha (API & DPI) and were evaluated for their quality attributes, nutritional composition, and bioactive potential. The incorporation of protein isolates increased the weight, density, and non-enzymatic browning and decreased the water activity in the bars. The hardness of the bar increased with the addition of protein isolates; however, reduced hardness was observed at 7.5 and 10% levels of API. Supplementation with protein isolates enhanced the protein content (7.83–16.71%), total phenols (1642–4956 GAE µg/g), total flavonoids (268–984 QE µg/g), DPPH radical scavenging activity (96.38–114.82 TEAC µmol/100 g) and reducing power (1926–3586 AAE µg/g) of the bars. Cereal bars maintained good sensory score and overall acceptability at 10 and 5% level of incorporation of API and DPI respectively.

Keywords Antioxidant activity · Alfalfa protein isolate · Cereal bar · Dhiancha protein isolate · Gluten free · Quality

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Introduction

The paradigm change in the lifestyle of the consumers has resulted in greater consumption of 'convenience foods' owing to the scarcity of time required for culinary preparations. Various Ready to Eat (RTE), Ready to Use (RTU), and Ready to Seve (RTS) foods and beverages are occupying huge market shelves and includes various products like cookies, carbonated and fruit beverages, and snack foods. However, many of the convenience food products have gained bad repo in the nutrition circles owing to their large sugar, salt, and fat content (particularly saturated and trans fats) (Dhir and Singla 2019). Cereal bars are considered a healthy alternative to conventional convenience foods as these can be tailored to suit the needs of the consumer and allow much flexibility in choosing the ingredients for its formulation (Sahni 2015). Growing demands of consumers for convenient and healthy food have perpetuated the development of a variety of cereal bars rich in protein, fibre, and bioactive constituents (Samuel and Peerkhan 2020; Rawat and Darappa 2015; Marques et al. 2015). These bars are positioned as an alternative healthy snack that allows its consumption as meal replacements or as healthy substitutes to usual snacking products. Due to their enhanced nutritive value and high amount of healthful ingredients; cereal bars have become an integral part of the diet of consumers aspiring for healthy and convenient food products (Dutcosky et al. 2006). Popped millets and brown rice are particularly a good choice for the development of cereal bars due to the presence of high amount of dietary fibre, vitamins, minerals, and bioactive constituents (Huang et al. 2018; Kaur et al. 2018; Sahni 2015).

Particularly, extensive research has been carried out for the development of cereal bars rich in proteins (Samuel and

Peerkhan 2020; Coelho Das Neves 2016; Rawat and Darappa 2015; Nadeem et al. 2012) due to its popularity among the youth, where it is utilized for its convenience in various weight loss and muscle gain regimes (Sahni et al. 2018). Besides enhancing the nutritional quality of cereal bars, added protein improves quality attributes of the bar by conferring it good binding, structure and strength, and enhancing water holding capacity and Maillard browning (Nadeem et al. 2012). A variety of ingredients like milk proteins, soy, nuts, and gluten have been utilized for protein-rich formulations (Samuel and Peerkhan 2020; Coelho Das Neves 2016; Rawat and Darappa 2015; Nadeem et al. 2012; Singh and Mohamed 2007). However, utilization of aforesaid ingredients presents a challenge for the development of cereal bars due to reluctance in the consumption of these ingredients by a broad spectrum of the population representing celiac patients and those intolerant to dairy products, soy, and nuts.

However, non-dairy, soy, nut, and gluten-free formulations present a challenge of low protein content. Therefore, it is essential to utilize alternative ingredients to develop nutritious formulations for the development of protein-rich cereal bars. The protein content of such formulations can be improved by adding non-conventional protein ingredients. Non-conventional protein ingredients have witnessed an increase in the market share due to increased veganism and high sustainability. Alternative proteins from plant sources have shown substantial growth in recent years. Alfalfa (*Medicago sativa*) and dhaincha (*Sesbania aculeata*) seeds have huge potential for their utilization as alternative non-conventional protein sources due to their high protein content, ease in cultivation, resistance to disease and pests (Sharma and Sahni 2021a, b; Sahni et al. 2021). Alfalfa protein isolate can be a good source for protein supplementation in cereal bars owing to its high protein and essential amino acid content, good techno-functionality, and associated bioactive potential (Sahni et al. 2020). Similarly, dhaincha protein isolate can be a good choice for protein-rich formulations due to its high protein and essential amino acid content, antioxidant capacity, and good hydration and gelation properties (Sahni 2020). Thus, the present investigation was carried out to develop protein-enriched cereal bars using popped millets and brown rice, cornflakes, and non-conventional (alfalfa and dhaincha) protein isolates and to evaluate the developed cereal bar for its quality attributes, nutritional composition, and bioactive potential.

Materials and methods

Material

Alfalfa (*Medicago sativa*) and dhaincha (*Sesbania aculeata*) seeds were obtained from Punjab Agricultural University, Ludhiana. Seeds were processed by wet heat processing (110 °C for 10 min) prior to milling (Sahni and Sharma 2020; Sahni et al. 2021) Processed seeds were milled and the flour was passed through 60 mesh sieve. Processing was carried out to improve the techno biofunctionality of the resultant protein isolates (Sahni et al. 2020). Sorghum, brown rice, finger millet, corn flakes, honey (Dabur) and cinnamon powder were procured from local market of Ludhiana.

Preparation of non-conventional (alfalfa and dhiancha) protein isolates (API and DPI)

Protein isolates were prepared by the pH-based solubilisation and precipitation method described by Sahni et al. (2020) by following the procedure of Ahmed et al. (2018) with some modifications. Alfalfa and dhaincha flour were dispersed in distilled water (1:20 flour to water ratio) and homogenized for 60 s (120 s for dhaincha) using a T-25 element of Ultra-Turrax homogenizer (IKA®-Werke GmbH & CO. KG, Staufen, Germany). The pH of the homogenate was adjusted to pH 10.0 using 2 M NaOH for solubilisation of protein, followed by its centrifugation at 5000 × g for 30 min at 4 °C to obtain supernatant. pH of the supernatant was adjusted to pH 4.0 using 2 M HCl and allowed to stand for 60 min (90 min for dhaincha) to induce precipitation of the protein, followed by its recovery by centrifugation at 5000 × g for 30 min at 4 °C. Precipitated protein was re-dispersed in deionised water and neutralised using 0.1 M NaOH, followed by freeze drying using a lyophilizer (Macro Scientific works, New Delhi, India). The prepared alfalfa and dhaincha protein isolates were having water absorption capacity 205 and 239% and least gelation concentration of 20 and 14% respectively.

Preparation of cereal bar

Sorghum, brown rice and finger millet were popped using black salt as conduction medium in an open iron pan (temperature of 170 ± 10 °C, 14% grain moisture content). Popped sorghum (35 g), brown rice (20 g), finger millet (10 g) and corn flakes (35 g) were coarsely crushed. Crushed grains were mixed with cinnamon powder (200 mg) and honey was used as a binding agent (60 g). The composite mixture was evenly filled into stainless steel moulds lined with butter paper and baked at 120 °C for

20 min, followed by cooling. Protein isolates were supplemented at 2.5, 5, 7.5 and 10% (w/w of grains).

Physical properties

Weight and volume of the cereal bar were recorded using digital weighing balance and vernier caliper respectively. Weight and the density of the cereal bar were expressed in g and g/cm^3 respectively. Water activity was determined using water activity meter (Thermoconstanter Novasina.TH200, Switzerland) at 28 °C. The non-enzymatic browning index was measured as per Hwang et al. (2001) with some modifications. 5 g sample was extracted with 50 mM $CaCl_2$/ 50 mM Tris buffer (pH 7.0), followed by centrifugation at 2000 × g for 15 min to obtain supernatant. The optical density of the supernatant was measured at wavelength 420 and 550 nm (LMSP-V325; Labman Scientific Instruments) which are corresponding to the formation of early and late Maillard reactions products respectively. Water was used as blank and the non-enzymatic browning index was calculated using the following formula.

$$\text{Non} - \text{enzymatic browning index} = \text{Absorbance}_{420\ \text{nm}} - \text{Absorbance}_{550\ \text{nm}}$$

Texture

The texture of the bar was evaluated using TA.HD*plus* Texture Analyzer (Stable Micro System Ltd.). Warner Bratzler Blade was used for measuring the hardness and the maximum force required to break the bar was determined using a single bite test with 20 mm/sec of pre-test and post-test speeds; and 75% compression (Sahni et al. 2019).

Colour measurement

Colour was determined as L*, a*, and b* values using Hunter lab colorimeter (CR-300 Minolta Camera, Japan). L* value represented lightness (ranging from 0 to 100 for lightness to darkness), a* value represented redness ' + a' to greenness '-a' and b* value represented ' + b' yellowness to '-b' blueness.

Nutritional composition

Moisture, crude protein (using the factor 6.25 × N), crude fat, crude fibre and ash were evaluated using standard procedures (AACC, 2000). Nitrogen Free Extract was estimated by subtracting the sum of moisture, crude protein, crude fat, crude fibre, and ash from 100 (Hossain and Becker 2001) as per the following equation. Values were expressed on a dry-matter basis.

$$\text{Nitrogen Free Extract: } 100 - \%(\text{Moisture} + \text{crude protein} + \text{crude lipid} + \text{crude fibre} + \text{ash})$$

Bioactive constituents and antioxidant activity

1 g sample was extracted with 80% (v/v) methanol. Total phenolic content was estimated colorimetrically by Folin–Ciocalteu assay (Flores et al. 2014). 0.5 mL of methanolic extract was mixed with 0.5 mL deionised water, followed by the addition of 5 mL of 10% (v/v) Folin–Ciocalteu reagent. After 5 min, 4 mL of saturated sodium carbonate was added to it and allowed to stand in dark for 15 min. Absorbance was measured at 765 nm using a spectrophotometer and expressed as gallic acid equivalent (GAE µg/g). Total flavonoids and DPPH radical scavenging activity was evaluated as per Kiranmai et al. (2011). For the estimation of total flavonoids to 1 mL methanolic extract added 1.5 mL pure methanol, 0.1 mL 10% aluminium chloride, 0.1 mL potassium acetate solution, and 2.8 mL deionised water and vortex well to allow proper mixing. Absorbance was measured at 415 nm using spectrophotometer and expressed as quercetin equivalent (QE µg/g). For the estimation of DPPH radical scavenging activity, 1 mL methanolic extract was added in a test tube and added 1 mL tris buffer, followed by the addition of 2 mL DPPH (2,2-diphenyl-1- picryl hydrazyl). Test tubes were incubated for 30 min in dark. Absorbance was measured at 517 nm using a spectrophotometer. Deionised water was used as control and values were expressed as Trolox equivalent antioxidant capacity (TEAC µmol/100 g). Reducing power was estimated as described by Sharma and Sahni (2021a). For determining reducing power, to 1 mL extract added 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, followed by incubation for 20 min. 2.5 mL of 10% trichloroacetic acid was added to it and centrifuged at 3000 × g for 20 min. 2.5 mL of the supernatant obtained was mixed with 2.5 mL water and 0.5 mL $FeCl_3$ (0.1%). Absorbance was measured at 700 nm using spectrophotometer and expressed as ascorbic acid equivalent (AEE µg/g). All the results were expressed on dry matter basis.

Sensory evaluation

Samples coded with random three digit numbers were evaluated for sensory attributes (colour and appearance, texture, taste, flavour, and overall acceptability) using 9 point hedonic rating by 30 semi-trained panellists (15 males and 15 females, 23–57 years old). The evaluation

was carried out at 27 ± 2 °C and panellists were provided with drinking water in between the samples for cleansing the mouth.

Statistical analysis

Five replications were taken for the evaluation of physical and textural attributes. Ten replications were taken for colour values whereas triplicate values were taken for the rest of the analysis. The data were analyzed statistically using SPSS software (Version 22.0, IBM Corporation) to determine statistical significance at $p < 0.05$. ANOVA was performed and means were compared by post-hoc Tukey's test. Values are represented as mean ± standard deviation Sensory data were subjected to analysis by Friedman bilateral variance rank analysis. Principle component analysis (PCA) was done using Statistica v.12.

Results and discussion

Physical properties

The weight of the cereal bar increased significantly ($p < 0.05$) with the increased level of supplementation of protein isolates (Table 1). An increase in the weight of the bar can be ascribed to the concurrent increase in the moisture content of the bar with the incorporation of protein isolates (Table 2). A higher increase in the weight was manifested with the same level of supplementation for DPI in comparison to API due to the higher water absorption capacity of DPI. A contrary trend was observed for the water activity, where water activity of the bar decreased with the increase in the level of protein isolate. The reduction in the water activity of the bar can be ascribed to the high water binding capacity of proteins (Sahni et al. 2018). In addition, lower water activity was manifested with bars supplemented with DPI in comparison to API. Lower water activity values for DPI supplemented bars can be justified based on the better gelation behaviour of DPI (14% LGC) that resulted in increased binding of water to denatured proteins at the same level of supplementation. Singh and Mohamed (2007) observed a similar trend of decrease in the water activity of protein-rich cookies with enhancement in the level of soy protein. The low water activity of protein-enriched bars exhibited quality improvement of the cereal bar by virtue of the manifestation of low water activity with increased shelf-life stability of the product.

A concurrent increase in the density of the bar was noted with the increase in the level of incorporation of protein isolates and was found to be higher in cereal bars with DPI in comparison to API at the same level of supplementation. The higher density of DPI supplemented bars can be ascribed to an increase in the weight of bar as well as replacement of coarse particles of grains with finely powdered protein isolate that allowed compact filling of bar mixture in the mould. The non-enzymatic browning index increased concurrently with the supplementation of protein isolates and higher browning was manifested with the incorporation of DPI in comparison to API. Coelho Das

Table 1 Physical and textural characteristics of cereal bars

Supplementation (%)	Physical characteristics				Hardness (N)
	Weight (g)	Density (g/cm^3)	Non-enzymatic browning index (OD/5 g sample)	Water activity	
Control	25.698 ± 0.17^{i}	3.62 ± 0.02^{i}	0.410 ± 0.01^{i}	0.351 ± 0.0^{a}	162.45 ± 4.29^{e}
API					
2.5	27.876 ± 0.20^{h}	3.93 ± 0.02^{h}	0.425 ± 0.02^{h}	0.351 ± 0.0^{a}	168.34 ± 3.26^{de}
5	30.149 ± 0.19^{f}	4.25 ± 0.02^{f}	0.435 ± 0.01 ^{g}	0.322 ± 0.0^{b}	182.39 ± 5.48^{c}
7.5	31.546 ± 0.20^{d}	4.45 ± 0.02^{d}	0.470 ± 0.01^{e}	0.320 ± 0.0^{c}	148.58 ± 4.36^{f}
10	32.436 ± 0.17^{b}	4.57 ± 0.02^{b}	0.485 ± 0.02^{d}	0.312 ± 0.0^{e}	129.12 ± 4.44^{g}
DPI					
2.5	28.443 ± 0.14 ^{g}	4.01 ± 0.01 ^{g}	0.450 ± 0.02^{f}	0.322 ± 0.0^{b}	172.30 ± 3.63^{d}
5	31.236 ± 0.18^{e}	4.40 ± 0.02^{e}	0.530 ± 0.03^{c}	0.315 ± 0.0^{d}	183.42 ± 3.92^{c}
7.5	31.944 ± 0.12^{c}	4.50 ± 0.01^{c}	0.585 ± 0.02^{b}	0.315 ± 0.0^{d}	204.86 ± 4.56^{b}
10	32.860 ± 0.14^{a}	4.64 ± 0.01^{a}	0.683 ± 0.02^{a}	0.310 ± 0.0^{f}	284.54 ± 8.74^{a}

API Alfalfa protein isolate; *DPI* Dhaincha protein isolate

Values are expressed as mean ± standard deviation ($n = 5$). The means within column followed by different superscripts are significantly different ($p < 0.05$)

Table 2 Nutritional composition of cereal bars

Supplementation (%)	Moisture (g%)	Crude protein (g%)	Crude fat (g%)	Crude fibre (g%)	Ash (g%)	NFE (g%)○
Control	7.15 ± 0.06 ^{g}	7.83 ± 0.12^{e}	1.72 ± 0.04^{a}	1.92 ± 0.02^{a}	2.09 ± 0.01^{a}	79.31
API						
2.5	7.34 ± 0.07^{f}	10.02 ± 0.08^{d}	1.74 ± 0.06^{a}	1.86 ± 0.02^{b}	2.01 ± 0.01^{b}	77.03
5	7.84 ± 0.09^{d}	12.26 ± 0.26^{c}	1.68 ± 0.04^{a}	1.74 ± 0.02^{c}	1.93 ± 0.02^{c}	74.55
7.5	8.26 ± 0.11^{d}	14.44 ± 0.13^{b}	1.49 ± 0.04^{b}	1.65 ± 0.01^{d}	1.72 ± 0.01^{d}	72.44
10	8.57 ± 0.06^{c}	16.71 ± 0.14^{a}	1.32 ± 0.02^{c}	1.51 ± 0.03^{e}	1.64 ± 0.02^{e}	70.25
DPI						
2.5	7.68 ± 0.09^{e}	10.05 ± 0.14^{d}	1.69 ± 0.03^{a}	1.83 ± 0.01^{b}	2.03 ± 0.02^{b}	76.72
5	7.94 ± 0.09^{d}	12.11 ± 0.11^{c}	1.71 ± 0.04^{a}	1.74 ± 0.01^{c}	1.91 ± 0.01^{c}	74.59
7.5	8.78 ± 0.08^{b}	14.28 ± 0.16^{b}	1.53 ± 0.07^{b}	1.64 ± 0.02^{d}	1.72 ± 0.01^{d}	72.05
10	9.06 ± 0.05^{a}	16.51 ± 0.32^{a}	1.41 ± 0.05^{c}	1.54 ± 0.02^{e}	1.67 ± 0.01^{e}	69.81

API Alfalfa protein isolate; *DPI* Dhaincha protein isolate. Nitrogen Free Extract: 100—% (Moisture + crude protein + crude lipid + crude fibre + ash)

Values are expressed as mean ± standard deviation (n = 3) on dry-matter basis. The means within column followed by different superscripts are significantly different ($p < 0.05$) calculated by difference

Neves (2016) observed the increase in the browning index of bar formulation with high protein content.

Textural characteristics

The incorporation of API caused the increase in the hardness of the bar at 5% level of incorporation, however, further addition of API resulted in a decrease in the hardness value (Table 1). However, the addition of DPI resulted in a concurrent increase in the hardness of the bar, and particularly very high value of hardness was observed with 10% level of supplementation. Proteins from different sources behave differently in protein-rich bar formulations depending on the innate nature of the protein, its interaction with other ingredients, and the resultant physico-chemical characteristics of the bar like moisture content and water activity (Nadeem et al. 2012). The increased hardness of the bar with the incorporation of protein isolates can be manifested with the protein–protein association (Sahni et al. 2018). Though, reduced hardness at 7.5 and 10% API can be due to the increased moisture content of the bar. However, high values of hardness for the bars with DPI in comparison to API supplemented bars can be attributed to better gelation properties (LGC 14%) of the DPI resulting in strong protein–protein interaction. Rawat and Darappa (2015) observed linear increase in the hardness of baked energy bars with the increase in the proportion of protein-rich ingredients in the formulation.

Colour characteristics

L* value of the bar decreased whereas a* value increased with the increase in the protein isolate supplementation (Fig. 1). However, the effect on L* and a* value was more pronounced in bars supplemented with DPI. However, no change in b* value was observed with the supplementation of API whereas incorporation of DPI resulted in the reduction of b* value. The decrease in lightness (L*) and increase in the redness (a*) of bars can be ascribed to the development of Maillard browning products (Sahni et al. 2018). Moreover, the pronounced change in the colour values of DPI supplemented bars in comparison to bars supplemented with API was also concomitant with higher values of the non-enzymatic browning index of DPI as compared to API (Table 1). Coelho Das Neves (2016) also reported similar colour changes in protein bars due to non-enzymatic browning. Overall, bars supplemented with DPI exhibited more pronounced change in the colour in comparison to bars supplemented with API.

Nutritional composition

A linear increase in the moisture content was observed with the increase in the level of supplementation of protein isolates (Table 2). The moisture content of the bar is based on the cumulative effect of affinity of different ingredients of the bar towards binding water and its loss during baking. In addition, heat-induced gelation of proteins during baking and their consequent improved water binding capacity plays important role in dictating the moisture content of the bar (Sahni et al. 2018). Bars supplemented with DPI

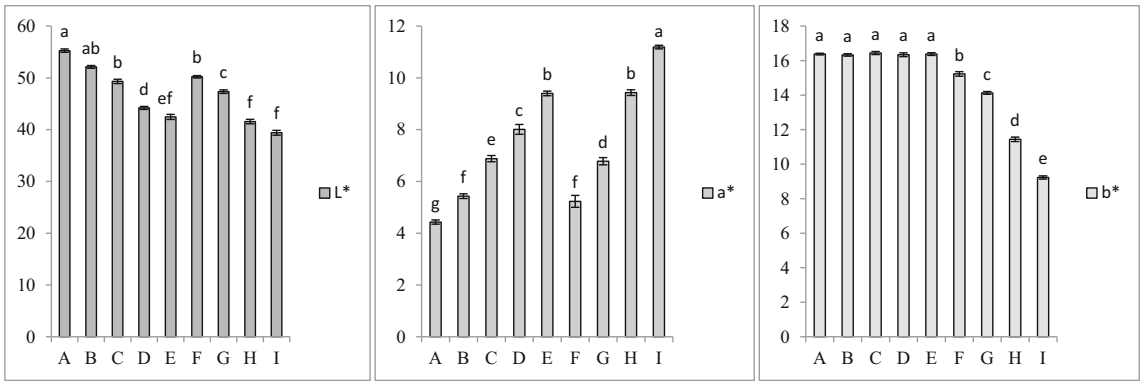

Fig. 1 Colour characteristics of cereal bars. A (Control), B (AP1 2.5%), C (AP15%), D (AP1 7.5%), E (AP1 10%), F (DP1 2.5%), G (DP15%), H (DP1 7.5%), I (DP1 10%).Values are expressed as mean and error bars represent standard deviation (n = 10). The means with different superscripts are significantly different ($p < 0.05$). API: Alfalfa protein isolate; DPI: Dhaincha protein isolate

exhibited higher moisture content as compared to bars with API due to better water binding capacity and gelation of DPI. A marked increase in the protein content was observed with the incorporation of protein isolates, justifying the utilization of API and DPI for the development of protein-enriched cereal bars. However, no significant ($p < 0.05$) variation was observed in the protein content of the bar at the same level of supplementation of API and DPI. Ash, fibre, and NFE of bars slightly decreased with the incorporation of protein isolates. Protein isolates majorly contain protein in contrast to grains that have other major constitutes like fibre, minerals, and carbohydrates and therefore can justify aforesaid trend of slight reduction of ash, fibre, and NFE. Rawat and Darappa (2015) reported a similar trend of increase in the moisture and protein content with the addition of protein-rich ingredients in baked energy bars.

Bioactive constituents and antioxidant activity

Table 3 highlights the bioactive potential of developed cereal bars. Cereal bars exhibited a good amount of bioactive constituents and high antioxidant activity attributed to the presence of whole grains. Supplementation of API and DPI further enhanced ($p < 0.05$) the total phenols, flavonoids, DPPH radical scavenging activity, and reducing power of the bars. An increase in the total phenol content was higher in bars with DPI (1642–4956 GAE µg/g) in comparison to API (1642–4080 GAE µg/g) whereas bars supplemented with API (268–984 QE µg/g) exhibited higher level of flavonoids in comparison to DPI 268–549 QE µg/g). The increase in the bioactive constituents also discerned its effect as a resultant increase in the antioxidant activity of the bars. However, pronounced effect was observed on the reducing power in comparison to DPPH radical scavenging activity. Pronounced effect on reducing power can be attributed to the higher correlation of phenolics and flavonoids with reducing power in contrast to DPPH radical scavenging whereas the increase in the DPPH radical scavenging has been manifested with non-phenolic antioxidants (Al-Laith et al. 2019; Liu et al. 2014; Sharma and Sahni 2021a, b). Cereal bars with DPI exhibited higher increase in the reducing power whereas API supplemented bars showed higher increase in the DPPH radical scavenging activity. Studies have reported the increase in the bioactive constituents and antioxidant potential of cereal bars incorporated with fruit powders (Silva et al. 2016; Marques et al. 2015). The incorporation of API and DPI also exhibited similar enhancement in the bioactive potential of the cereal bars in addition to enhancing the protein content (Table 2).

Sensory characteristics

Incorporation of API exhibited no pronounced effect on the colour and appearance, taste, and flavour score of the bar (Fig. 2a) However, improvement in the texture score was manifested with the 7.5 and 10% level of addition of API with consequent improvement in the overall acceptability of the bar (Fig. 2a). The improvement in the texture score of bars at 7.5 and 10% API can be manifested with the reduced hardness of the bar (Table 1). The incorporation of protein isolates at 7.5 and 10% API improved the bite of the cereal bars. The incorporation of API improved the overall acceptability of the bar, exhibiting high acceptability at 10% API. Contrary to the aforesaid trend, the DPI supplemented bars maintained good sensory quality up to 5% level of incorporation. Marked reduction in all the sensory attributes and low overall acceptability were observed at 7.5 and 10% levels of incorporation of DPI. Particularly, taste and flavour scores exhibited a pronounced decline in the scores due to the manifestation of the typically strong taste and odour of DPI. However, the

Table 3 Bioactive constituents and antioxidant activity of cereal bars

Supplementation (%)	Bioactive constituents		Antioxidant activity	
	Total phenols (GAE μg/g)	Total flavonoids (QE μg/g)	*DPPH•* radical scavenging activity (TEAC μmol/100 g)	Reducing power (AAE μg/g)
Control	1642 ± 10.3^{i}	268 ± 7.2^{i}	96.38 ± 0.18^{g}	1926 ± 8.4^{h}
API				
2.5	2167 ± 7.4^{h}	444 ± 5.8^{f}	101.11 ± 0.16^{e}	2311 ± 12.4 ^{g}
5	2770 ± 15.2^{f}	632 ± 11.1^{c}	105.45 ± 0.07^{c}	2785 ± 5.7^{e}
7.5	3438 ± 8.2^{d}	801 ± 9.3^{b}	109.72 ± 0.11^{b}	3115 ± 9.5^{c}
10	4080 ± 12.1^{b}	984 ± 8.1^{a}	114.82 ± 0.09^{a}	3464 ± 8.9^{b}
DPI				
2.5	2387 ± 21.7^{g}	340 ± 12.1^{h}	99.71 ± 0.15^{f}	2405 ± 11.2^{f}
5	3111 ± 13.6^{e}	417 ± 5.3 ^{g}	101.31 ± 0.08^{e}	2865 ± 9.2^{d}
7.5	3900 ± 12.2^{c}	480 ± 8.2^{e}	103.08 ± 0.16^{d}	3216 ± 9.4^{b}
10	4956 ± 19.1^{a}	549 ± 11.2^{d}	105.39 ± 0.12^{c}	3586 ± 14.3^{a}

API Alfalfa protein isolate; *DPI* Dhaincha protein isolate

Values are expressed as mean ± standard deviation (n = 3) on dry-matter basis. The means within column followed by different superscripts are significantly different ($p < 0.05$)

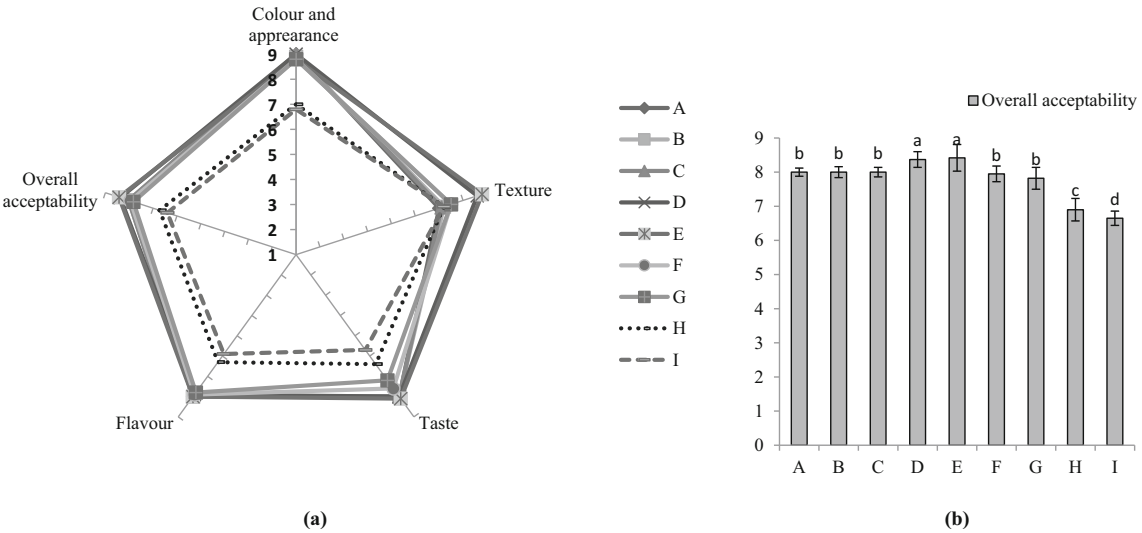

Fig. 2 (**a**) Quantitative descriptive profile of sensory characteristics of cereal bars (**b**) Overall acceptability of cereal bars. A (Control), B (AP1 2.5%), C (AP15%), D (AP1 7.5%), E (AP1 10%), F (DP1 2.5%), G (DP15%), H (DP1 7.5%), I (DP1 10%).Values are expressed as mean and error bars represent standard deviation (n = 30). The means with different superscripts are significantly different ($p < 0.05$). API: Alfalfa protein isolate; DPI: Dhaincha protein isolate

decline in the texture score of the bar can be correlated with the development of excessive hardness (Table 1).

Principle component analysis (PCA)

PCA loading plot (Fig. 3a) depicts the relationship between the different properties of the cereal bars, where properties that lie in the same quadrant are positively correlated whereas properties that lie in the opposite quadrant are negatively correlated to each other. It can be clearly observed that non-enzymatic browning (NEB) and hardness (H) exhibited a negative effect on the overall acceptability (OA) of the cereal bar. It can be discerned from the loading plot that DPPH radical scavenging activity (DPPH RSA) and total flavonoids (TF) are positively correlated whereas reducing power (RP) and total phenols (TP) were positively correlated in cereal bar formulations. PCA score plot (Fig. 3b) reflects the variability in the samples as a function of the distance between different points. It can be observed that samples A, B, F lie in

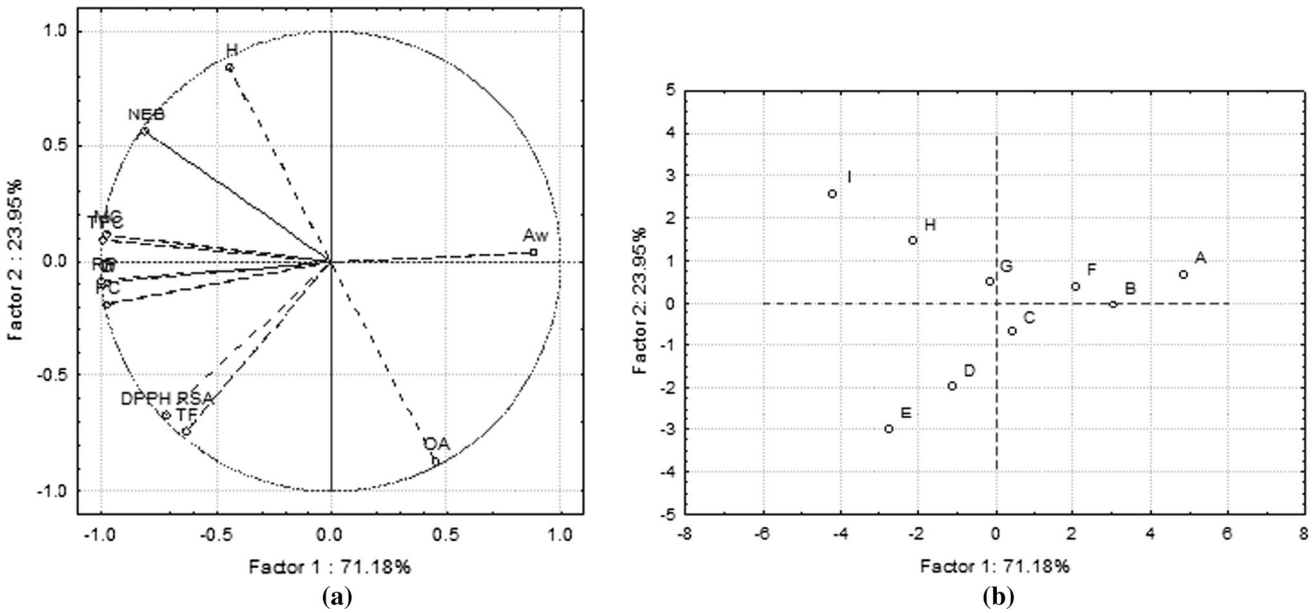

Fig. 3 Principle component analysis (PCA) showing loading (**a**) and score plot (**b**) for cereal bars. H: Hardness, NEB: Non-enzymatic browning, TPC: Total phenols, TF: Total Flavonoids: RP: Reducing Power, DPPH RSA: DPPH Radical Scavenging Activity, MC: Moisture, PC: Protein, Aw: Water Activity, OA: Overall Acceptability, A (Control), B (AP1 2.5%), C (AP15%), D (AP1 7.5%), E (AP1 10%), F (DP1 2.5%), G (DP1 5%), H (DP1 7.5%), I (DP1 10%). API: Alfalfa protein isolate; DPI: Dhaincha protein isolate

the same quadrant depicting the similarity of 2.5% API and DPI formulation with the control bar. However, the score plot further validates the quality enhancement of protein-enriched bars with 10% API (E) due to high total flavonoids (TF) and DPPH radical scavenging activity (DPPH RSA) without much increase in non-enzymatic browning (NEB) and hardness (H) whereas bar with 5% DPI (G) exhibited a balance of overall acceptability as well as bioactive potential.

Conclusion

Alfalfa and dhaincha protein isolates can be successfully utilized as an alternative non-conventional protein ingredient for the development of protein-enriched non-dairy, gluten, soy, and nut free cereal bars. The addition of protein isolates exhibited improvement in the quality attributes of the cereal bar. The addition of API and DPI not only markedly increased the protein content but also pronouncedly improved the bioactive constituents and antioxidant activity of the bars. Cereal bars with 10% API and 5% DPI maintained good sensory attributes and overall acceptability. Utilization of non-conventional protein isolates for the formulation bars with millets, brown rice, and corn flakes as major ingredient presents an innovative approach to deliver a product with enhanced nutritive value, nutraceutical potential, and suitability for consumption by a broad spectrum of population intolerant to gluten, soy, nuts, and dairy proteins.

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Declarations

Conflict of interest Authors declare no conflict of interest.

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Consent to participate Not applicable.

Consent for publication Not applicable.

Data availability All data generated or analysed during this study are included in this article.

Code availability Not applicable.

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